Characterization of preimaginal developmental stages of two cryptic South African species of the *Merodon planifacies* complex (Diptera: Syrphidae: Eristalinae: Merodontini), with differentiation through morphometry analysis

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ABSTRACT

Merodon capi Vujić et Radenković, 2020 and *Merodon roni* Radenković et Vujić, 2020 are two cryptic species belonging to the *Merodon planifacies* Bezzi, 1915 species complex that have recently been described, with the differences between them being revealed by molecular and geometric morphometry studies of adult specimens. In the present study, the preimaginal morphology of both species is described and displayed, with the material used corresponding to samples taken from bulbs of *Merwilla plumbea* (Lindl.) Speta plants at two locations in KwaZulu-Natal province (South Africa). The preimaginal stages were compared, looking for morphological evidence supporting species divergence. Linear and geometric morphometric analyses of the larval posterior respiratory process were conducted for the first time and the results of these analyses support the separation of the two taxa. Information about the life cycle and the relation with the host plant is also provided. In addition, we carried out a preliminary study of the adult feeding requirements of the species, based on the description of the mouthparts and an analysis of the pollen present in the gut and on the integument of adults. The results of the study supports a close relationship between the preimaginal stages and plant species of the Hyacinthaceae family.

1. Introduction

Syrphidae, commonly known as hoverflies, are one of the most diverse and species-rich families among dipterans. It is comprised of more than 250 genera and 6600 species, divided into four subfamilies: Eristalinae, Microdontinae, Pipizinae and Syrphinae (Thompson and Rotheray, 1998; Ssysmank, 2001; Mengual et al., 2015). They are present in all the biogeographic areas except the Antarctic (Speight, 2020) and can be found in a wide range of habitats and ecosystems. Most of the adults in this family are flower visitors, feeding on pollen and nectar (Tooker et al., 2006). Nevertheless, larval feeding habits are highly diverse, exhibiting an extensive range of feeding modes including saprophagy, zoophagy, mycophagy or phytophagy, among others. Phytophagous larvae are present in some species of Syrphinae, but this habit is mainly present in Eristalinae (Rotheray and Gilbert, 1999; Van Zuijen and Nishida, 2010). They feed on plant tissues: stems, roots, leaves, fruits and underground storage organs such as bulbs. The close connection that phytophagous larvae and plants present makes these species good bioindicators of habitat quality (Sommaggio, 1999; Rotheray and Gilbert, 2011; Popov et al., 2017).

One of the main representative phytophagous genera is the genus *Merodon* Meigen, 1803 (Eristalinae, Merodontini). This is one of the richest genera of hoverflies, being predominant in the

Palearctic region (181 species) but also present in the Afrotropical region (14 species) (Vujić et al., 2021a).

Despite this richness that Syrphidae present, most of the knowledge about them is based on adults; little is known about the preimaginal stages. This is more problematic with phytophagous species because there is very scarce information about which plants the larvae feed on, and this hinders the process of finding larvae to describe (Preradović et al., 2018). In the case of the genus *Merodon*, until now only six species have larval descriptions and only eleven species have had their pupae described (Hodson, 1932; Heiss, 1938; Stuckenberg, 1956; Dixon, 1960; Hartley, 1961; Ricarte et al., 2008, 2017; Andrić et al., 2014; Preradović et al., 2018; Vujić et al., 2020, 2021b).

Frequently, hoverfly species that are in the same taxonomic category also share larval feeding habits. According to Rotheray and Gilbert (1999), most larval morphological changes are related to feeding habits: modifications of the mouth hooks, thorax, locomotory organs, respiratory spiracles, etc. Therefore, the preimaginal-stage biology, functional morphology or even ecology can shed light on different aspects of the whole family, including taxonomy and evolution. The paucity of information about larval biology and ecology greatly impedes the understanding of evolutionary and phylogenetic relationships between species, not only within the family, but also in relation to the host species. In the particular case of in *Merodon*, in recent decades there has been a lot of interest in its systematics (Mengual et al., 2006; Marcos-García et al., 2007, 2011; Ståhls et al., 2009; Vujić et al., 2011, 2012, 2021a; Radenković et al., 2018) and one of the problems that researchers have found during their studies is the extreme lack of information on the preimaginal stages of the genus.

The information obtained so far about *Merodon* larvae indicates their phytophagous feeding habits. More precisely they feed on the underground storage organs of plants of different species belonging to the Hyacinthaceae family. As an example, *Merodon equestris (Fabricius, 1794)* and *M. geniculatus Strobl, 1909* have been reared on *Narcissus* L. bulbs (Ricarte et al., 2017), and *M. hurkmansi Marcos-García, Vujić and Mengual in* Marcos - García et al. (2007) and *M. luteihumerus Marcos-García, Vujić and Mengual in* Marcos - García et al. (2007) have been reported feeding on *Leopoldia comosa* (L.) Parl. and *Drimia maritima* (L.) Stearn, respectively (Ricarte et al., 2008). A close relationship between the host plant and each species of the genus *Merodon* is presumed to exist, including aspects such as adult oviposition and feeding among others (Ricarte et al., 2017; Speight, 2020).

The *Merodon planifacies* subgroup belongs to the *Merodon melanocerus* group, which is an Afrotropical branch of the monophyletic *M. desuturinus* lineage. This subgroup is composed of three species: *M. planifacies* Bezzi (1915), *Merodon capi* Vujić et Radenković in Vujić et al. (2020) and *Merodon roni* Radenković et Vujić in Vujić et al. (2020). The latter two taxa, which belong to the *capi* complex, are cryptic species that can only be distinguished according to molecular data and wing morphometric evidence that have only recently been revealed (Djan et al., 2020; Vujić et al., 2021a).

The main objective of the present paper is to study the preimaginal stages of both these cryptic species, focusing on the study of their morphology and ecology and searching for morphological diagnostic characters that allow us to distinguish between *M. capi* and *M. roni* larvae.

Furthermore, we combined field observations and pollen analysis to analyze feeding requirement and compare them in the *M. desuturinus* lineage (i.e., *Merodon drakonis* Vujić et Radenković in Radenković et al., 2018) in order to establish the ecology and feeding habits of the species.

2. Methodology

2.1. Collecting site and adult identification

Samples of the early stages of *M. capi* and *M. roni* were collected from inside bulbs of the eastern South African *Merwilla plumbea* (Lindl.) Speta (subfamily Scilloideae of Asparagaceae, also treated as the family Hyacinthaceae). In a previous paper the name *Merwilla natalensis* (Planch.) Speta was used for the host plant of *M. capi* and *M. roni* (Djan et al., 2020). However, the concept applied here to *M. plumbea* (= *M. natalensis*) differs from the one adopted by Jessop (1970), which was linked to South-West South African plants later recognized as the monotypic genus *Spetaea* Wetschnig and Pfosser (2003). The larvae were reared in captivity, feeding on the bulb in which they were found until pupation and adult emergence. The material was sampled at two different locations in KwaZulu-Natal province, in the north-west of South Africa, specifically in Cathedral Peak valley (*M. capi*) and Royal Natal National Park (*M. roni*) during spring of 2015 and summer of 2016. The adults were killed by freezing and pinned for their preservation and taxonomic identification using the keys, descriptions and illustrations of Bezzi (1915); Curran (1939); Hull (1944) and Djan et al. (2020).

2.2. Morphological studies

The terminology used for preimaginal descriptions follows Rotheray (1991, 1993), the head skeleton description was made following Courtney et al. (2000), Rotheray and Gilbert (2008) and Rotheray (2019), and the description of adult's mouthparts follows Schiemenz (1957) and Krenn et al. (2005). Four third instar larvae (L3) from each location (Royal Natal and Cathedral Peak) were preserved by immersion in cold water to facilitate body extension, and then slowly heated for about 4 min to kill them. The larval integument was thickly coated in dry decaying bulb tissue. To remove this layer, the larvae were immersed in water for 8 h. After this period, the larvae were cleaned using brushes of different stiffness and thickness under the stereomicroscope, and subsequently, washed and preserved in 70% ethanol.

The micromorphology of these third-instar larvae was studied using a cryo-scanning technique coupled to Scanning Electron Microscopy (cryo-SEM). The larvae were fixed on a holder with a layer of O.C.T. compound (Tissue-Tek O.C.T. Compound, Sakura Finetek) and then frozen rapidly in liquid nitrogen for 2 min. Afterwards, the specimen holder was transferred to a system for cryo-SEM (Oxford CTI500). The sample was freeze-etched, maintained under vacuum conditions, increasing the temperature from $-150\,°C$ to $-90\,°C$ for about 2 min, to eliminate contamination by frost, and then a thin layer of gold was "sputtered" onto the material for 3 min. Finally, the sample was transferred to the cold stage of the SEM (S3000N Hitachi), kept at about $-150\,°C$, and secondary electron images were observed and recorded at an accelerating voltage of 10 kV. This procedure was conducted using the technical services of the Technical University of Valencia (UPV, Spain).

The debris adhering to the puparium integument was removed by placing the specimens in an ultrasonic cleaner for a few minutes. The cleaned specimens were mounted on stubs and examined under a scanning electron microscope (S3000N Hitachi) using variable pressure (or low vacuum) mode at 20 kV. This technique allows a direct evaluation of the specimens without coating the samples with gold. This procedure was conducted using the technical research services of the University of Alicante (UA, Spain).

The head skeletons were extracted from the puparia and third-instar larvae and cleared by an immersion in hot 10% KOH for 15 min. The resulting suspension was neutralized by immersing the

structure in 98% CH₃COOH. After the clearing process, the structures were preserved in glycerin.

The mouthparts of the adults were described for *M. capi, M. roni* and for *M. drakonis* (used as comparison). Before the extraction of the mouthparts, pictures were taken using a stereomicroscope to show their natural position on the adult. For dissection, the adults were placed in a humidity chamber for 24 h. After this period, and with the help of an entomological needle, the mouthparts were extracted. A clearing process was performed by immersion in hot 10% KOH for 5 min. The resulting suspension was neutralized by immersing the structure in 98% CH₃COOH. After the clearing process, the structures were preserved in glycerol.

The morphology of the third-instar larvae, puparia, head skeleton and adult mouthparts was analyzed using a stereomicroscope (Leica M205C), and pictures were taken using a camera that was attached to it (Leica DFC450). The dimensions of preserved specimens were measured using the ImageJ informatics tool (v 1.52) (Schneider et al., 2012) on the basis of pictures previously obtained.

2.3. Morphometric analysis

The analysis was conducted on the PRP (Posterior Respiratory Process) of the pupae of both cryptic taxa: *M. capi* (N = 12) and *M. roni* (N = 27). High-resolution pictures were obtained using Scanning Electron Microscopy (s3000 N Hitachi), and the linear morphometry analysis was performed using the ratios between six linear measurements of the PRP: Angles (R/L), Height Plate/Width Plate (HP/WP), Length/Width Plate (L/WP), Length/Height plate (L/HP), Width Apex/Length (WA/L) (Supplementary material A: Fig. A.1). These linear measurements were obtained using ImageJ, and then the ratios between them were calculated.

Outline geometric morphometry was used to quantify the shape variability of the curvature of the second spiracular opening on the left side of the spiracular plate on the PRP (Supplementary material A: Fig. A1). Eleven semi-landmarks were digitized using the 'resample curve by length' option in the TpsDig 2.05 software (Rohlf, 2006). CoordGen 7.14 with an integrated Semi-land module was used for semi-landmark superimposition using a distance-minimizing protocol, which minimizes the shape differences due to the arbitrary nature of semi-landmark positions along the curve (Bookstein, 1997; Zelditch et al., 2004).

Both linear and geometric morphometric measurements were obtained three times to estimate the measurement error, and the average measurement for each trait was used in the analysis (Arnqvist and Mårtensson, 1998). The measurement error was found to be negligible.

The differences in the PRP traits (both linear and outline) between *M. capi* and *M. roni* were inferred based on Principal Component Analysis (PCA) and stepwise Discriminant Analysis (DA), followed by a succeeding Canonical Variates Analysis (CVA). PCA was used to summarize variation, while CVA and DA were used to test differences in PRP traits between species. All the statistical analyses were performed in Statistica for Windows (Dell Statistica, 2015).

2.4. Adult feeding requirements analysis

A qualitative analysis of pollen was performed to reveal the specific feeding behavior of the adults. In order to get pollen samples from the body surface, each specimen was thoroughly rinsed in water. To ensure all the pollen grains were detached in the liquid, the samples were introduced into an ultrasonic cleaner for 10 min. After visual confirmation that no pollen remained on the body using a stereomicroscope, each specimen was dissected, and the gut content was immersed in hot 10% KOH for 5 min. The

resulting suspension was neutralized by immersing the structure in 98% CH₃COOH.

The pollen grains were extracted from the liquid suspension by centrifugation at 3000 rpm, and all the sediment was transferred to a microscopic slide and sealed using fuchsine-stained glycerin jelly. All the pollen present on the slide was counted at 200x magnification. The qualitative pollen analysis was performed on a sub-sample of 500 pollen grains (or on the total quantity of pollen if it was below 500 grains) using 400x or 600x magnification. The pollen identification to the highest possible taxon or morphological type was supported by pollen atlases (Moore and Webb, 1978; Reille, 1995, 1998, 1999a, 1999b; Bucher and Kofler, 2004) and plants collected from the region where the insects were captured.

The number of available samples was not large enough for a comprehensive statistical evaluation, so here we provide qualitative descriptions. For each pollen type the relative frequency was calculated and for further interpretation sporadic pollen was excluded (<3%) and the focus was placed on very frequent (>45%) and frequent (16−45%) pollen types, while rare pollen (3−15%) was also taken into consideration, as it could have indicated specificity in feeding behaviors. Such information is commonly used in melissopalynology (Vergeron, 1964; Louveaux et al., 1978; Behm et al., 1996).

3. Results

A total of 26 third-instar larvae were collected (18 specimens in the Royal Natal National Park and 8 specimens from Cathedral Peak valley) in September and November 2015 and February 2016. Four specimens of each species were preserved for morphological studies, while fourteen specimens of *M. roni* and four specimens of *M. capi* were left to develop and pupate. In addition, empty puparia were found inside the *M. plumbea* bulbs, making a total of 27 puparia of *M. roni* and 12 of *M. capi* available for the study. A total of 12 *M. roni* (6 females and 6 males) and 3 males of *M. capi* emerged successfully.

3.1. Preimaginal morphology description

As with the adults, the morphology of the two species studied were identical to the naked eye. To avoid unnecessary repetition, the morphology of the preimaginal stages will be described once, referring to both species.

Overall characters: Length 13.61 ± 0.29 mm (N = 8), height 4.27 ± 0.19 mm (N = 8), and greatest width 4.56 ± 0.19 mm (N = 8). Mostly light brown in color, becoming darker at the posterior end. Sub-circular in cross section and slightly tapered anteriorly. As in other *Merodon* species, mouth hooks external and sclerotized. Integumental vesture well developed with short, rounded, blunt and slightly sclerotized spicules, smaller on the ventral surface. Ventral spicules surrounding PRP sharper than on the rest of the surface. Segmental sensilla not very pronounced, bearing very short setae and lacking fleshy papillae at the base. Anal segment with one pair of lappets, located ventrally, below the PRP and with the fleshy projection poorly developed (Fig. 1).

Head skeleton: about two times longer than broad formed, from anterior to posterior part, by mouth hooks, intermediate and basal sclerite. Mouth hooks are external, fused centrally to each other, dark and heavily sclerotized. Mouthhooks thick and rounded at the upper apex, the distance in the apex equal to the basal width. Mandibular lobes very sclerotized and fused to the mouthhooks, forming a robust structure. Mouthhooks worn down and a scratched apex present due to the use and friction with the feeding tissues, therefore the presence of accessory teeth is doubtful. On the posterior part of the mouth hooks, the apodemes of mandibular

adductor and abductor muscles situated at the base and apex, respectively. Also, on the posterior part, the labial sclerite very sclerotized, thickened and fused to the intermediate sclerites. Intermediate sclerites located between mouth hooks and basal sclerite, with the salivary duct present in this section. Basal sclerite formed by dorsal and ventral cornu. The dorsal cornu well sclerotized, narrowed towards the apex and covering the whole length of the ventral cornu, giving the structure a pear-shape appearance. Dorsal cornu fused to ventral cornu by the vertical plate, sclerotizing all the central area. Ventral cornu long, slightly curved, and thin in profile view, with the cibarium at the base, transversal ridges barely developed. Posterior part of the ventral cornu heavily sclerotized, forming the grinding mill structure: with the mortar, a heavily sclerotized structure at the base and the pestle, a mobile structure acting as a roof (Fig. 2). *Pseudocephalon and thorax:* Dorsal lip smooth and lacking any ornamentation. Lateral lips barely developed, bearing thick and elongated setae, bifurcated at the apex (Figs. 3A and C). Ventral lip covered by short rounded and slightly sclerotized spicules with 1−3 short projections on top (Figs. 3A and D). Dorsal surface of the prothorax with three longitudinal grooves. Antenno-maxillary organs well-developed, situated between the mouthhooks and the dorsal surface of the prothorax; consisting of two pairs of cylindrical-shaped structures situated on top of two fleshy and lumpy cushions. Antenna and maxillary palpus clearly identifiable, several satellite sensilla present on top (Fig. 3B). Anterior fold of the prothorax with the same vestiture as the rest of the dorsal surface but less sclerotized, reaching up to third sensilla of prothorax. Anterior larval spiracles are a pair of rounded and small spiracles located on the dorsal surface of the prothorax (Fig. 4A). They present three or four elongated spiracular openings facing the inner part of the prothorax, and a big scar at the base, orientated to the margins of the pupae (Figs. 4B and C). Mesothoracic prolegs and feeding channel absent (Fig. 3A).

Abdomen: Primordia of pupal spiracles present on the dorsal surface of the first abdominal segment, indicating third instar (Fig. 1A). Absence of prolegs, pairs of raised and more sclerotized domes on 1−7th abdominal segments as locomotory organs, lacking crochets. Dorsally, 1−6th abdominal segments bearing three folds each, 1st and 2nd segmental sensilla present on the second fold and 3rd and 4th segmental sensilla slightly posterior; on the 7th abdominal segment, three folds present, bearing first segmental sensilla on the second fold and 2nd and 3rd on the third fold. On the anal segment, three very thin folds appear, only 4th and 5th sensilla located on a fleshy projection not well developed, ventrally located, which can be considered as a lappet. 2nd and 3rd sensilla do not present attached basal projection (Fig. 1B).

Posterior Respiratory Process (PRP): Blackish-brown, sub-elliptical in cross section, almost as long as wide (visible from dorsal view), 0.64 ± 0.09 mm long, 0.61 ± 0.08 mm the thinnest part and 0.78 ± 0.06 mm the widest (N = 39). Annular groove present on the basal quarter of the surface, granular surface up to the groove and smooth upstream of the groove up to the apex, with thin vertical wrinkles and dents (Fig. 6E). The spiracular plate is 1.6 times wider than long, slightly narrowed in central area, four pairs of sinuous spiracular openings around two central scars; spiracular openings clearly separated from each other; first pair significantly shorter and less convoluted than the rest. Four pairs of inter-spiracular setae emerge from the spiracular plate, and close to the second inter-spiracular setae is a small hole without setae, presumably acting as a sensorial organ (Fig. 6D).

Chaetotaxy: Prothorax (P) with ten pairs of sensilla, both mesothorax (Ms) and metathorax (Mt) with eight pairs of sensilla. 1−7th abdominal segments with ten pairs of sensilla (9th sensilla only visible through SEM), anal segment (A8) with eight pairs of sensilla and one pair of lappets (Fig. 5).

Puparium overall description: Sub-circular in cross-section with the anterior end truncated, tapered posteriorly, and flattened
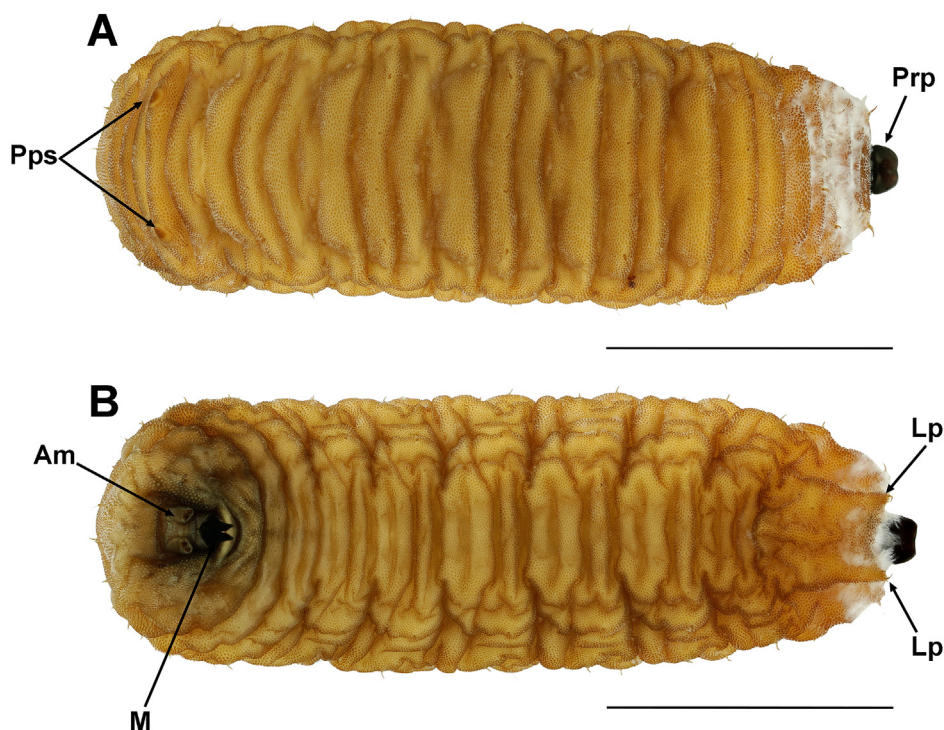

Fig. 1. *Merodon roni* third instar larvae. **A:** Dorsal view. **B:** Ventral view. Abbreviations: Am - Antenno-maxillary organs; Lp − Lappet; M− Mouthhooks; Pps − Primordia of pupal spiracles; Prp − Posterior Respiratory Process. Scale bars: 5 mm (**A** and **B**).
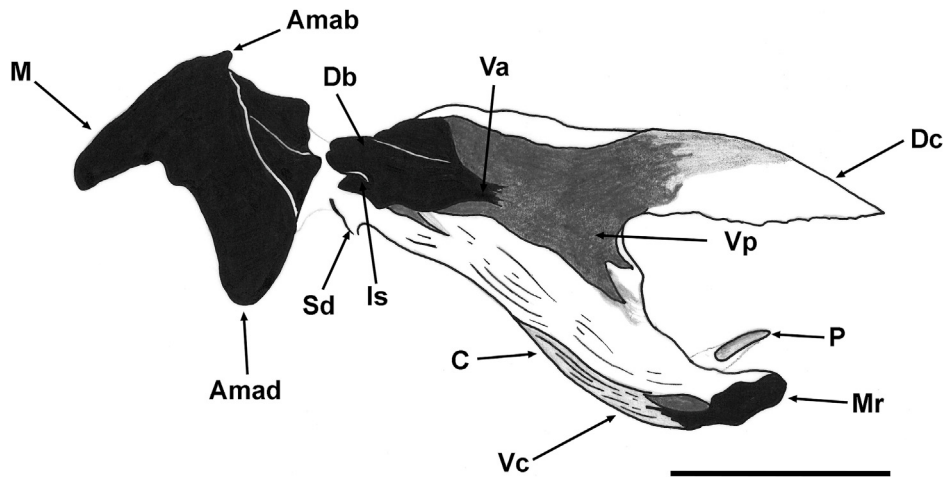
Fig. 2. Head skeleton of third instar larvae of *Merodon roni*. Abbreviations: Amab − Apodeme for mandibular abductor; Amad − Apodeme for mandibular adductor; C − Cibarium; Db − Dorsal bridge; Dc − Dorsal cornu; Is − Intermediate sclerite; M− Mouthhooks; Mr − Mortar; P − Pestle; Sd − Salivary duct; Va − Ventral arm; Vc − Ventral cornu; Vp − Vertical plate. Scale bar: 500 μm.
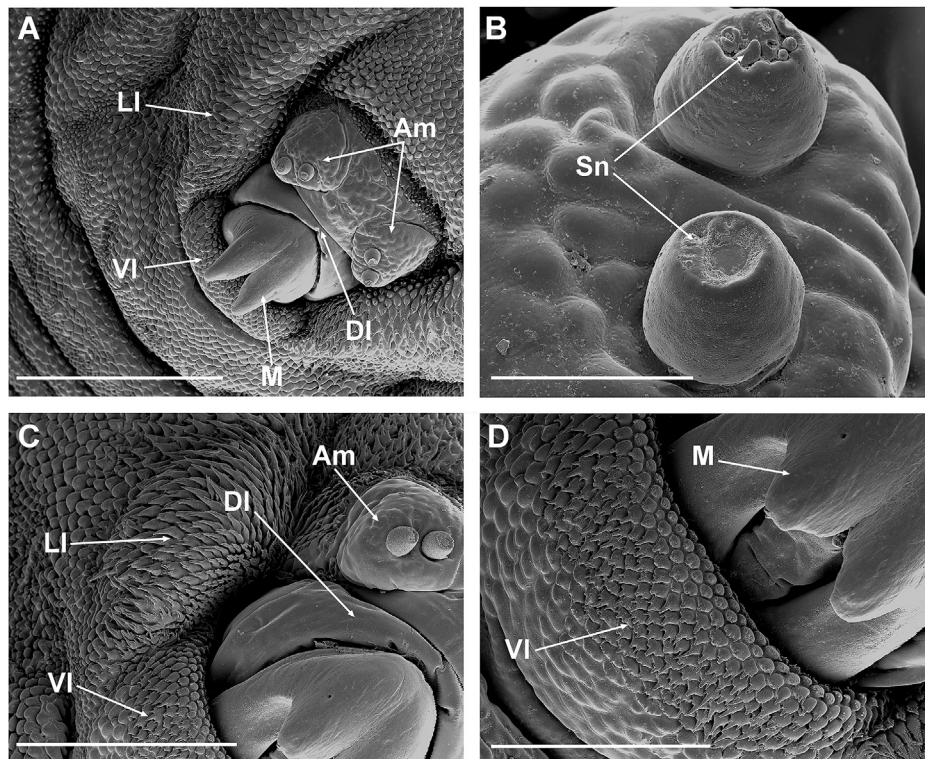

Fig. 3. Third instar larvae of *Merodon capi*. **A:** Prothorax, ventral view. **B:** Antenno-maxillary organ. **C:** Detail of the lips. **D:** Ventral lip. Abbreviations: Am - Antenno-maxillary organs; Dl − Dorsal lip; Ll − Lateral lip; M− Mouthhooks; Sn − Sensillae; Vl − Ventral lip. Scale bars: 1 mm (**A**), 600 μm (**C**), 400 μm (**B**) and 100 μm (**D**).

ventrally. Light brown in color (Fig. 6A). The length including PRP 14.5 mm ± 0.36 mm, maximum width 5.5 ± 0.15 mm and maximum height 5.3 ± 0.24 mm (N = 18). Pupal spiracles projected from middle of the upper part of the operculum, separated by a distance about three times the length of one spiracle.

Pupal spiracles: Cylindrical structures about 1.1 ± 0.07 mm (N = 7) in length (length - breadth ratio of spiracle 3−1) slightly tapered and with a rounded prominence at the apex, separated around three times its length. Dark brown color at the base, with a bright brown apical part. Two thirds of the dorsal surface covered in densely distributed and irregularly spaced tubercles, facing towards the

margins of the puparium; but also present on lateral surfaces and at the apex of the ventral surface (Fig. 6B). Tubercles slightly domed and circular, composed of multiple layers, with 5−8 radially arranged tear-shaped spiracular openings. Surface between tubercles smooth or slightly rough at the apex; surface of the basal part and the rounded prominence of the apex granulated (Fig. 6C).

3.2. Morphometric analysis

Linear measurements: Principal component analysis of the linear measurements produced five principal components (PCs), of which
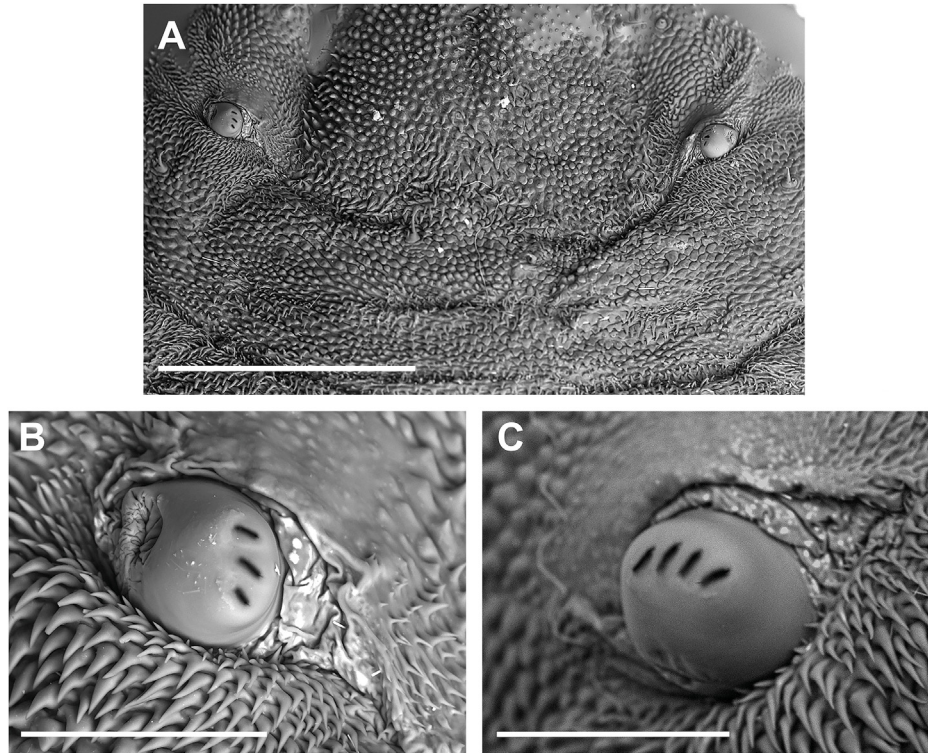
Fig. 4. Puparium of *Merodon capi.* **A:** Dorsal view of prothorax with the larval spiracles. **B:** Larval spiracle in puparium (three openings). **C:** Larval spiracle in puparium (four openings). Scale bars: 1 mm (**A**) and 200 μm (**B** and **C**).
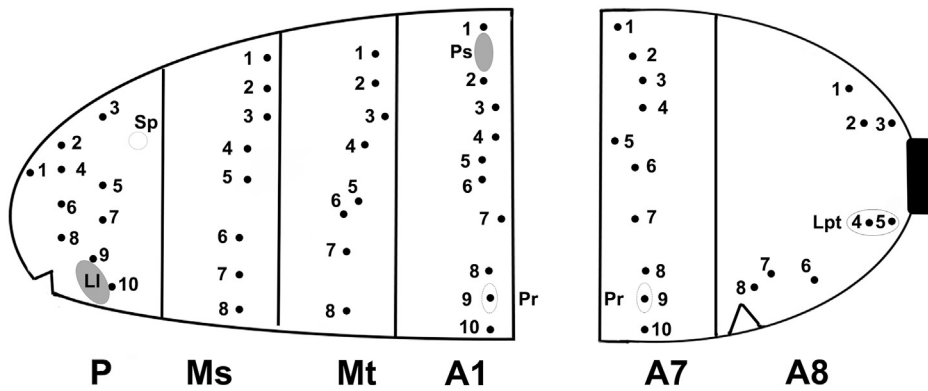

Fig. 5. Map of the chaetotaxy of the third instar larva of *Merodon capi* in lateral view showing the positions of the sensilla group. P — Prothorax; Ms — Mesothorax; Mt — Metathorax; A1 and A7 — Abdominal segments; A8 — Anal segment; Ll — Lateral lip; Lpt — Lappet; Pr- Proleg; Ps — Primordia of pupal spiracles; Sp — Anterior spiracle.

the first two were meaningfully correlated with variables and used in further analysis. The first PC explained most of the variation (49.44%) and was positively correlated with Length vs. Height of Plate (L/HP) and Length vs. Width of Plate (L/WP) ratios, and negatively correlated with ratio of right vs. left angles of dorsal view of PRP (R/L). The results imply that *M. capi* has a shorter PRP (lower values of ratios L/WP and L/HP), compared with *M. roni* which has a longer one (higher ratios of L/WP and L/HP). The second PC was positively correlated with Width of Apex vs. Length (WA/L) and negatively correlated with Height of Plate vs. Width of Plate (HP/WP) ratios. PC2 accounted for 28.07% of the total variation and indicted intraspecific variability in Width of Apex vs. Length (WA/L) and Height of Plate vs. Width of Plate (HP/WP) ratios, which were slightly greater in *M. roni* (Fig. 7A). Additionally, DA applied on PCs evidenced significant divergence between *M. capi* and *M. roni*

($F5,19 = 8.646084$ p = 0.000208). The overall classification success of the DA was 91.66%, with only two specimens (one from each species) being misclassified. The succeeding CVA was produced on a highly significant canonical axis related with differences in linear measurements between the species (CV1: Wilks' Lambda = 0.293972; $\chi 2 = 23.8733$; P-value < 0.000230).

Geometric morphometric: The PCA carried out on the shape variables produced 18 PCs. Stepwise discriminant analysis conducted on the PCs revealed that *M. capi* and *M. roni* differed highly significantly in the curvature of the spiracular opening ($F10,12 = 3.728837$ p = 0.00173), with an overall classification success of 95.45%. Of the whole sample, only one specimen of *M. capi* was misclassified. Canonical variate analysis gave one significant axis which confirmed the shape differences of the PRP spiracular opening between species (Fig. 7B). Furthermore,
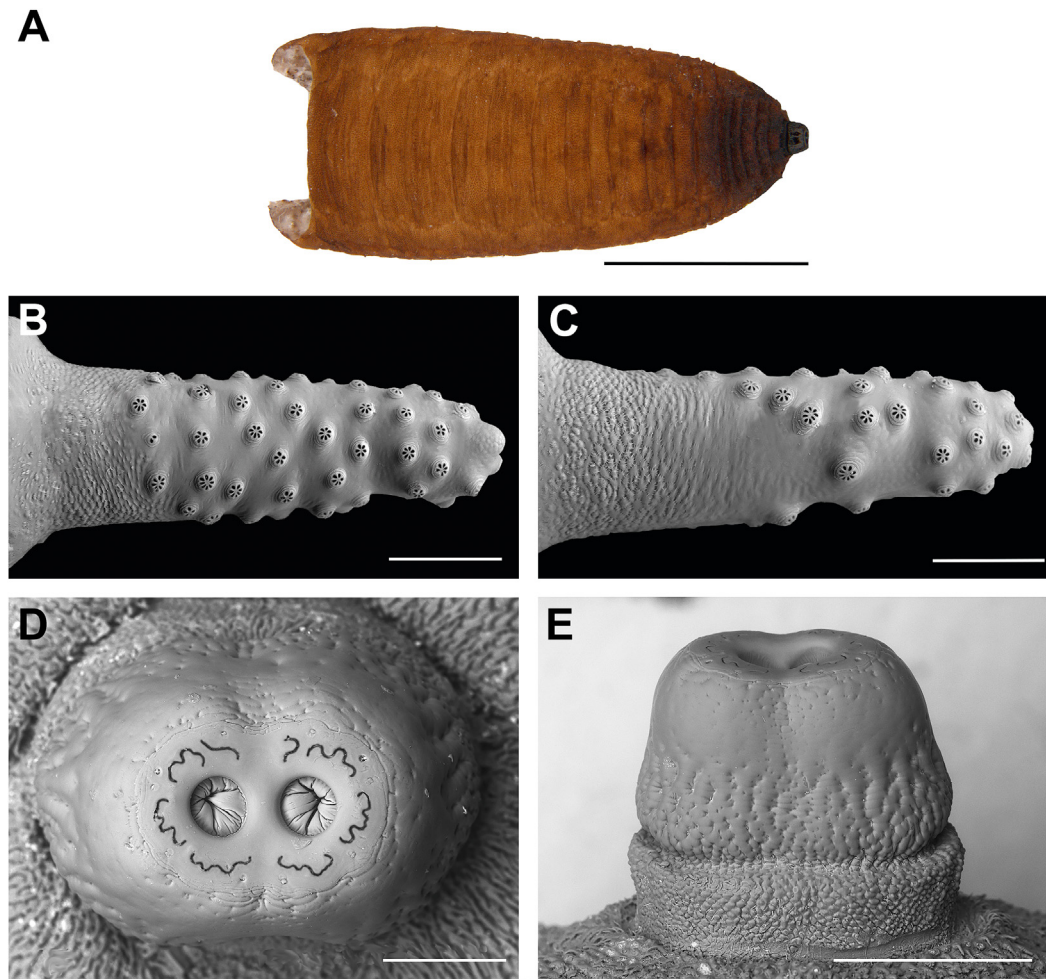
Fig. 6. Pupae of *Merodon capi*. **A:** Dorsal view of open puparium. **B:** Dorsal view of pupal spiracle. **C:** Ventral view of pupal spiracle. **D:** Vertical view of Posterior Respiratory Process. **E:** Dorsal view of Posterior Respiratory Process. Scale bars: 5 mm (**A**), 500 μm (**E**), 250 μm (**B, C, D**).

superimposed outline drawings showed that the major differences between the openings were in the curvature of the central area, with this being more concave in *M. capi* and less pronounced in *M. roni*.

3.3. Adult morphology and behavior

3.3.1. Mouthpart description

The three species studied share a very similar general morphology. To avoid unnecessary repetition, the morphology of the mouthparts will be described by mentioning the main characters that differ between the species.

Regarding the rostrum, it is two times longer than wide in *M. capi* and *M. roni* and almost three times longer than wide in *M. drakonis*. Clypeal sclerites, sclerotized and well developed, cover one quarter of the structure in *M. capi* and *M. roni* but one half of the structure in *M. drakonis*. The frontoclypeal plate, located on top of clypeus, is well sclerotized in the three species, but thin and elongated in *M. capi* and *M. roni*, while robust and rounded in *M. drakonis*. The fulcral plate is markedly curved in the three species, being more pronounced in *M. capi* and *M. roni*, and presenting a broader curve on top of the rostrum. At the opposite site to the clypeal sclerite, the fulcral apophysis is well developed in the three species, being more curved and sclerotized in *M. drakonis* (Figs. 8A and C). The haustellum is elongated, with the sucking tube covering the same length as the rostrum in *M. drakonis* and around 80% of the rostrum length in *M. capi* and *M. roni*. The maxillary palp of *M. capi* and *M. roni* is short and rounded, densely covered in short setae and bears long setae at the apex, while in *M. drakonis* it is long and thin, and only bears a few long setae (Figs. 8B and D). The labellum is well developed, the paraphysis bars are robust and sclerotized, with a hook-shaped end at the apex and they reach up to the end of the prementum in *M. drakonis*. There is a lanceolated and sclerotized structure on the outer part of the furca membrane, covering almost all of it in *M. drakonis* and half of it in *M. capi* and *M. roni* (Figs. 9A and B). On the posterior apex of the labellum, covering half of the inner part of the labella, and standing out from the discal sclerite, there is a series (14 in *M. capi* and *M. roni* and 20 in *M. drakonis*) of digital prolongations forming the pseudotrachea (Figs. 9C and D).

M. capi and *M. roni* measurements. Rostrum, length:1.063 ± 0.057 mm, width: 0.45 ± 0.02 mm. Sucking tube, length: 0.87 ± 0.065 mm, width: 0.12 ± 0.04 mm. Labella, length: 1.09 ± 0.24 mm, width: 0.26 ± 0.03 mm. Length of labella (pseudotracheal area): 0.51 ± 0.06 mm (N = 4). There are 14 projections in the pseudotrachea. Ratio: 27 pseudotrachea/mm (Figs. 8 and 9).

M. drakonis measurements. Rostrum, length:1.26 ± 0.067 mm, width: 0.49 ± 0.07 mm. Sucking tube, length: 1.23 ± 0.05 mm, width: 0.22 ± 0.03 mm. Labella, length: 1.51 ± 0.08 mm, width: 0.31 ± 0.05 mm. Length of labella (pseudotracheal area): 0.62 ± 0.06 mm (N = 3). There are 20 projections in the pseudotrachea. Ratio: 32 pseudotrachea/mm (Figs. 8 and 9).
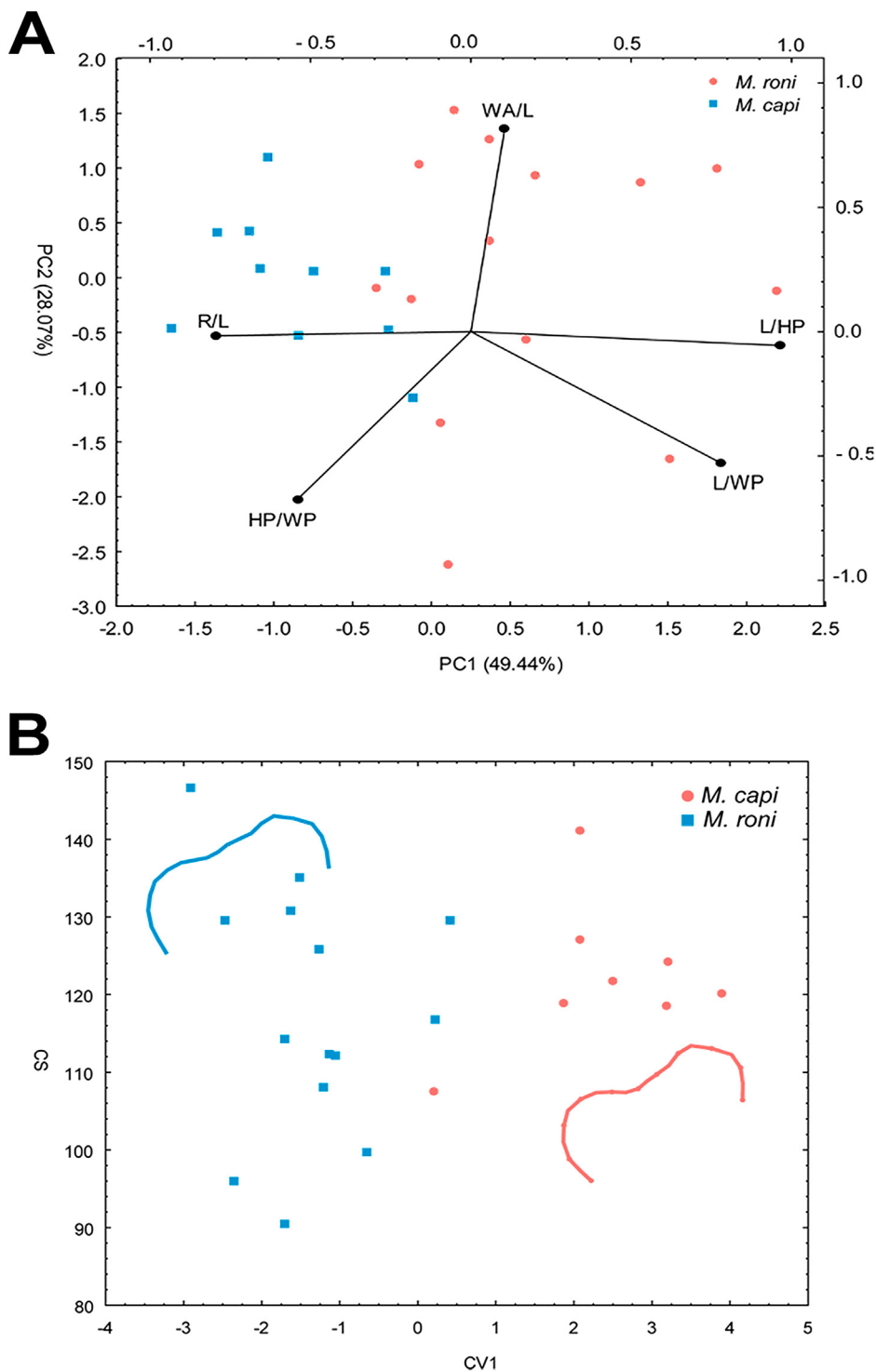
Fig. 7. Morphometric analysis graphs. **A:** Principal component analysis (PCA) biplot of linear morphometric PRP traits of *Merodon capi* and *Merodon roni*. Axis: Upper X and right Y - correlation coefficient between variables and first and second PCs, respectively; Lower X and left Y - factor scores of observations for first and second PCs, respectively. **B:** Scatter plot of CV1 scores against centroid size (CS) with superimposed outline drawings of average shape for *Merodon capi* and *Merodon roni*. Differences were magnified twice to make them more visible.

3.3.2. Pollination visits

Pollen grains were extracted from the integument and guts of five *M. roni* (2 male and 3 female), four *M. capi* (2 male and 2 female) and nine *M. drakonis* (4 male and 5 female) adults. A notably smaller amount of pollen was extracted from *M. capi* and *M. roni*, in particular from the gut, compared with *M. drakonis* (Fig. 10). There

was no major difference regarding the quantity of pollen present on the integument and in the gut for the two cryptic species (*M. capi* and *M. roni*).

A total of 36 pollen types were determined from samples, but 11 stood out for their quantity (Fig. 11). In order to overcome the very low quantity of pollen extracted from the *M. capi* and *M. roni*
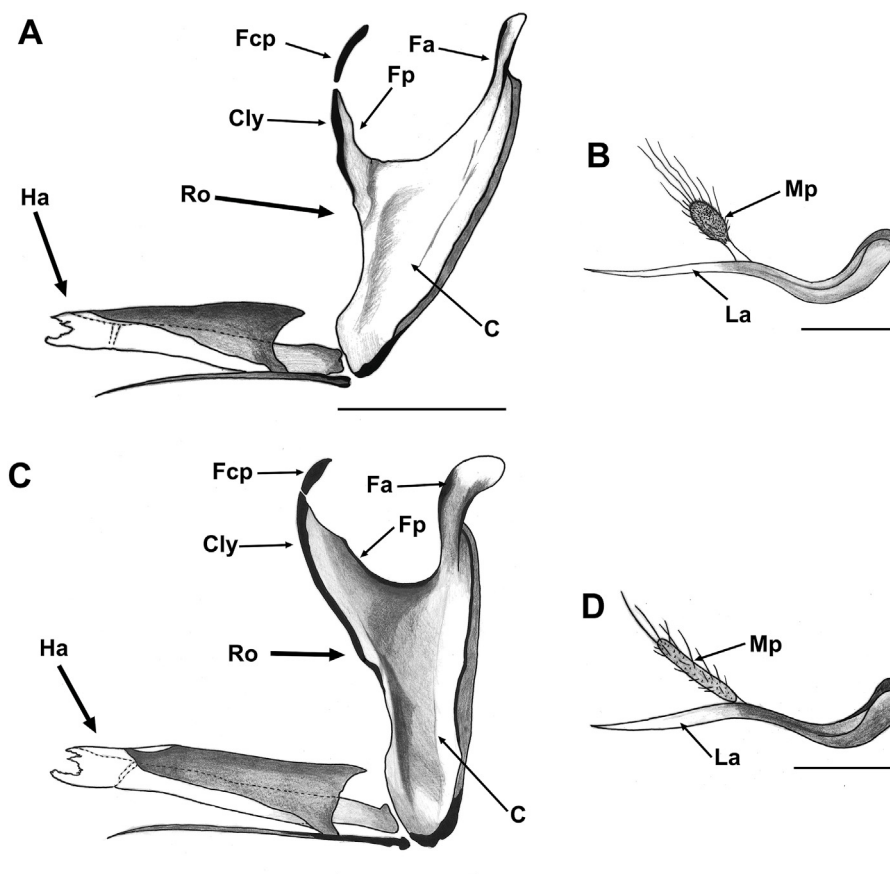
Fig. 8. Scheme of adult mouthparts of *Merodon drakonis, Merodon capi* and *Merodon roni*. **A:** Rostrum and haustellum of *Merodon capi* and *Merodon roni*. **B:** Maxilla of *Merodon capi* and *Merodon roni*. **C:** Rostrum and haustellum of *Merodon drakonis*. **D:** Maxilla of *Merodon drakonis*. Abbreviations: C − Cibarium; Cly − Clypeal sclerite; Fa − Fulcral apophysis; Fcp − Frontoclypeal plate; Fp − Fulcral plate; Ha − Haustellum; La − Lacinia; Mp − Maxillary palp; Ro - Rostrum. Scale bars: 500 μm (**A** and **C**) and 250 μm (**B** and **D**).

specimens, the qualitative analysis was performed by grouping data obtained from each specimen, thus making it suitable for comparison between the three species. Senecio-type plants (Punt and Hoen, 2009), Hyacynthaceae/Liliaceae, Euphorbiaceae and *Myrica* were frequently identified in the three species, while more diverse pollen types were identified in *M. capi* and *M. roni*. In addition, a notable amount of Apiaceae pollen type 1 (Cerceau-Larrival, 1962) was characteristic for *M. capi* and *M. roni*, while Geraniaceae pollen type was characteristic for *M. drakonis* (Fig. 11). Fern spores were recovered from most of the specimens analyzed both on the integument and in the gut. The analysis did not identify *M. plumbea* pollen in any of the species analyzed.

3.4. Life cycle

Although no direct oviposition of any female was observed, a large number of females seen and collected were lying on the surface of the bulb, on the basal part, leading to the assumption that eggs are laid on the epigeal part of the bulb, which the larvae enter once the eggs hatch. The larvae feed on bulb flesh tissue, creating tunnels and developing inside; and then the third instar larvae heads from the inner parts of the bulb to the external area by tunneling through the tissue. Pupation occurs on the outer scales of the bulb, mostly on the basal part, in order to the protect pupae and ease the exit of the imago after emergence. Fresh pupae and empty puparia were found on the outer leaves of the bulbs (Fig. 12).

With the exception of one case with two larvae, most of the bulbs collected had only one larva inside. All the larvae collected

presumably had the same level of development and age. This indicates that females probably only lay eggs once per season. Adults were observed in spring and summer, with the specimens mostly being collected in October, November and December.

4. Discussion

4.1. Differentiation between species

As occurs with the adults, the preimaginal morphology of the two species is indistinguishable using traditional techniques of study. Nevertheless, both morphometric analyses revealed evidence supporting the separation of the two taxa. Previous studies have proven the usefulness of morphometric analysis in species differentiation in both adult and preimaginal stages (Nuñez and Liria, 2016; Sontigun et al., 2017). With regards to syrphid taxonomy, geometric morphometric analysis has been used, in combination with other taxonomical methods, in the description and identification of species and groups (Sasić et al., 2016; Sasic et al., 2018; Arok et al., 2019; Djan et al., 2020). However, previous studies have always been focused on landmark-based methods for the wings and genitalia of adult specimens. The present study is a novel approach as these methodologies are being used in the preimaginal stages, and specifically in the analysis of the PRP which is one of the larval characters of greatest taxonomic importance.

Both Principal Component Analysis and Discriminant Analysis indicate significant differences between the two species with respect to linear and geometric morphometry measurements.
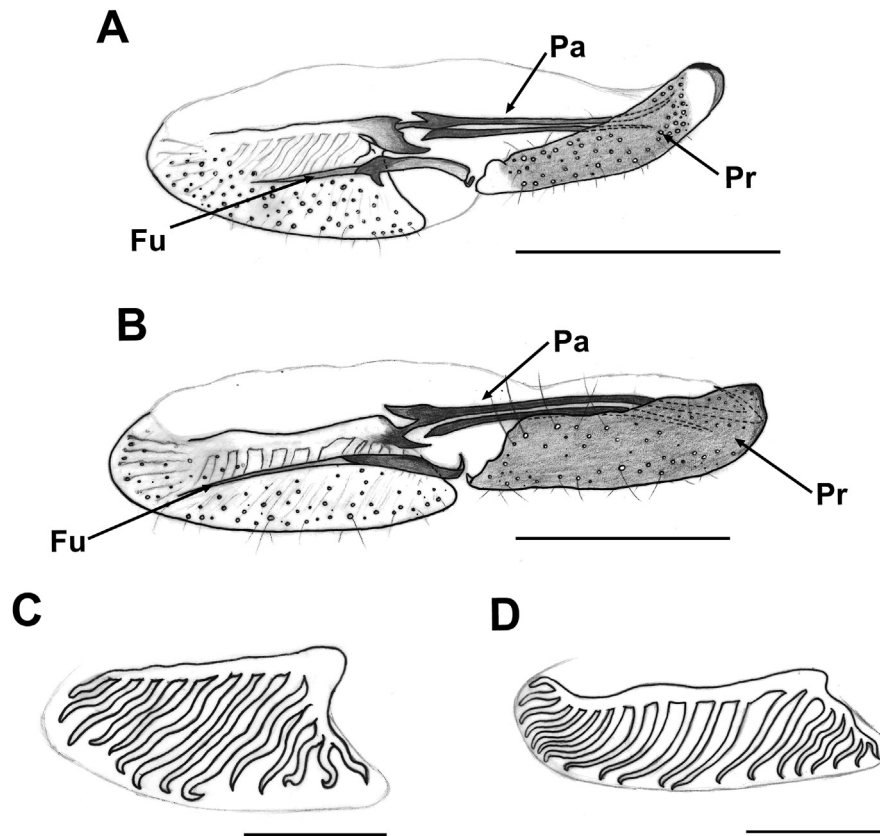
Fig. 9. Scheme of adult mouthparts of *Merodon drakonis, Merodon capi* and *Merodon roni*. **A:** Labellum of *Merodon capi* and *Merodon roni*. **B:** Labellum of *Merodon drakonis*. **C:** Pseudotrachea of labellum of *Merodon capi* and *Merodon roni*. **D:** Pseudotrachea of labellum of *Merodon drakonis*. Abbreviations: Fu − Furca; Pa − Paraphysis; Pr - Prementum. Scale bars: 500 μm (**A** and **B**) and 250 μm (**C** and **D**).

These results are in line with the differentiation established by Djan et al. (2020) based on the molecular and morphometric analysis of adults. The present results not only support the differentiation of the two species by Djan et al. (2020), but also prove that the morphometric analysis of the preimaginal stages is a useful tool for integrative taxonomy, in determining and supporting species differentiation when traditional taxonomy is ineffective.

4.2. Larval taxonomy of the genus

Until now, including the descriptions made in this paper, data on the preimaginal morphology (larva/puparium) of only 14 species of the genus *Merodon* has been published (see summary in Table B.1 of the Supplementary material B).

Considering the characters shared by all known species of the genus *Merodon* (see Ricarte et al., 2008), there are two main characters present in the species analyzed here that are not present in the others. The first is related to the shape of the anterior respiratory processes, which Ricarte et al. (2008) describe as a cylindrical structure, while the larvae observed in this study present a small and rounded structure, more similar to a semicircle than a cylinder. According to the summary table, it seems to be one of the possible diagnostic characters for these two species, as in all the other descriptions these processes have a cylindrical appearance (even mentioned in *Merodon avidus* (Rossi, 1790) as twice as long as broad). The number of spiracular openings present in the anterior respiratory process is a character that varies between the species too, with most of the species having two openings (*M. avidus, M. equestris, M. geniculatus, M. hurkmansi, M. opacus* Vujić, Likov and Radenković in Vujić et al., 2020, *Merodon makrisi* Vujić,

Radenkovi and Tot in Vujić et al., 2021b, *M. pulveris* Vujić et Radenković in Radenković et al., 2011, *Merodon natans* (Fabricius, 1794)) but between four and five openings are described in *M. luteihumerus* (Marcos-García et al., 2007) and between three and four openings have been observed in *M. capi* and *M. roni*.

The second character that differs from the description made by Ricarte et al. (2008) is related to the number of lappets present on the anal segment. Ricarte et al. (2008) indicated that all larvae of the genus have four pairs of lappets, highlighting that the middle pair is divided into two proximal ones. However, in *M. capi* and *M. roni* only one pair is present, and they are not well developed. Moreover, Andrić et al. (2014) described only two pairs of lappets for *M. avidus* and highlighted the ventral ones, the same as for *M. opacus* (Vujić et al., 2020), while *M. makrisi, M. pulveris* and *M. natans* (Vujić et al., 2021b), in *M. equestris* only one pair is described by Heiss (1938) and Hartley (1961); even Ricarte et al. (2017) mentioned only three pairs in *M. geniculatus*. This scenario seems to suggest that the number of lappets is not a character shared by all the preimaginal stages of the genus and that there is a high degree of variability in the presence of fleshy projections bearing sensilla on the anal segment of the larvae. However, this could also mean that lappet is not a well-defined term, or that the concept of lappet has not been internalized by the researchers and needs to be clarified and delimitated in order to be able to make comparisons. When looking at the available pictures attached to the descriptions, the latter hypothesis is more feasible, given that most of the anal segments of the species look similar to each other, having the same number of projections and organization of segmental sensilla, according to the criteria of the present authors.

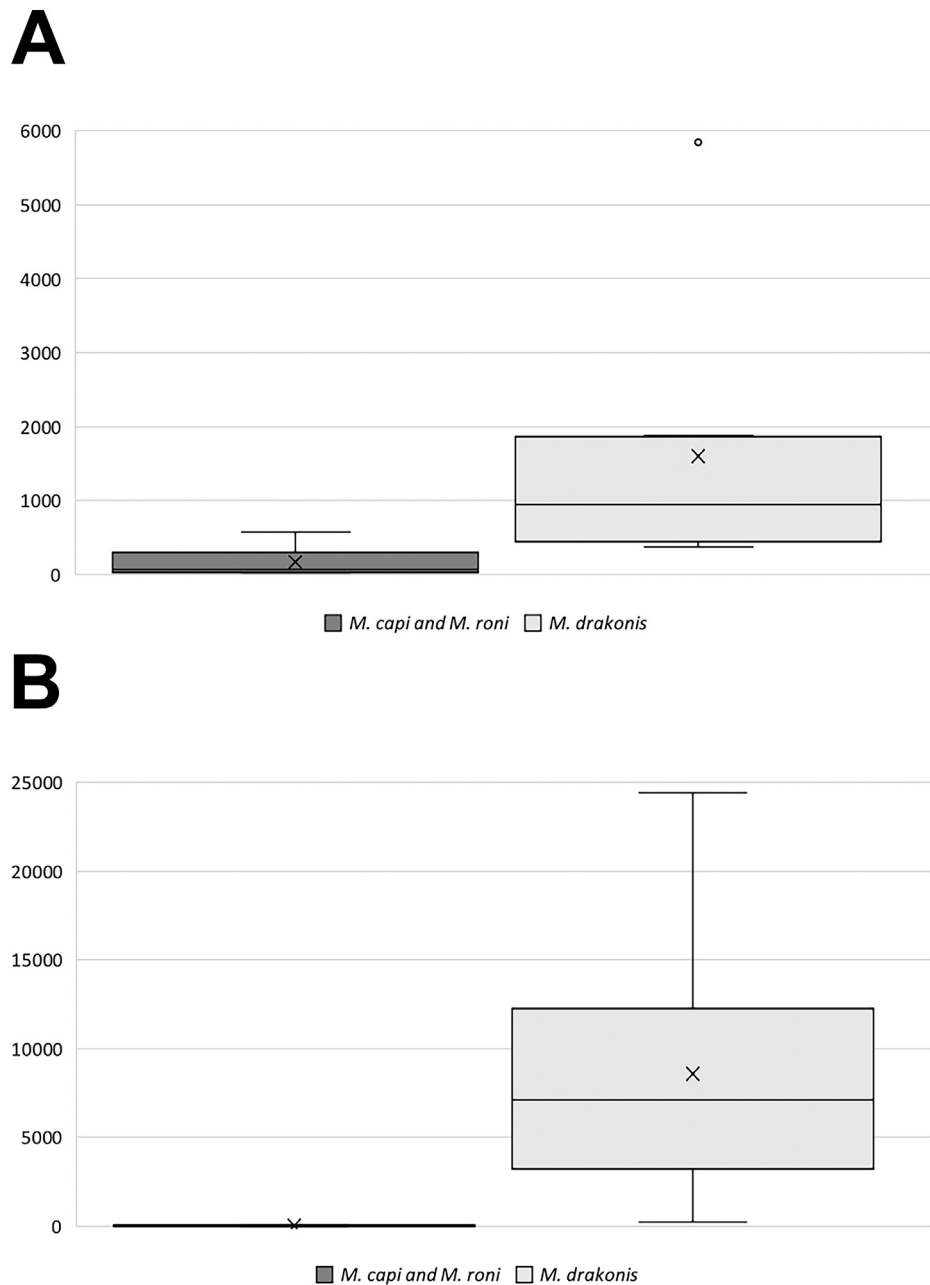To the list of characters common to the genus made by Ricarte

A

B

Fig. 10. Box plots depicting the quantity of pollen extracted from integument (**A**) and gut (**B**) (black line indicates median value, asterisk indicates mean, circle is outlier).

et al. (2008), considering the review carried out in the present work of all the descriptions available, some additional characters should be mentioned as common to the genus. The integument of *Merodon* larvae remains constant among all the species, consisting of small, blunt spinules and segmental sensilla bearing long setae. The differences between species are mostly related to the level of sclerotization, the density of spinules on the body and, above all, the way in which each author describes it. Another noteworthy common character is the presence of four spiracular openings in the PRP and its shape, which is sinuous to convoluted, with the degree of sinuosity varying among the species. The last character to add to the common features of the genus is the general shape of the pupal spiracles, as all the spiracles studied are basically cylindrical, tapered and with a rounded protuberance at the apex, and separated from each other by a distance three to five times their width

and with the surface covered in tubercles with a number of linear-to-oval radially-arranged openings. However, there are some specific characteristics of the pupal spiracles that present interspecific variability, such as the way the tubercles are distributed on the spiracle, and the surface ornamentation: smooth in *M. calidus* Fabricius, 1805 and *Merodon bombiformis* (Hull, 1944), coriaceous in *M. luteihumerus*, polygonal in *M. rufus Meigen, 1838, M. opacus, M. makrisi* and *M. avidus,* reticulate in *M. geniculatus* and granulated at the base and the apex and smooth between domes in *M. capi* and *M. roni*. The number of spiracular openings also varies, not only interspecifically but intraspecifically as well.

When analyzing the morphology of the mouthhooks, all the descriptions agree that they are sclerotized and conspicuously projected outwards; this structure is designed for scratching the plant tissue which the larvae feed on. A character that differs
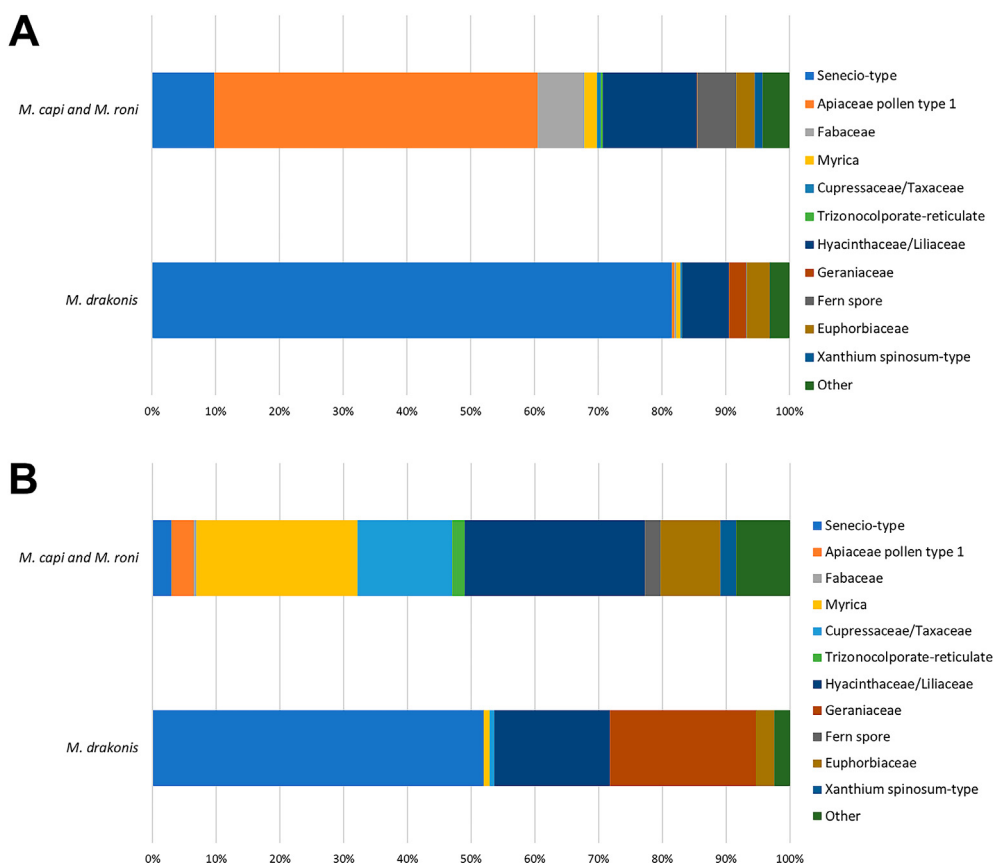
Fig. 11. Diversity of dominant pollen types identified from analyzed specimens of *Merodon drakonis*, *Merodon capi* and *Merodon roni*: integument (**A**) and gut (**B**).
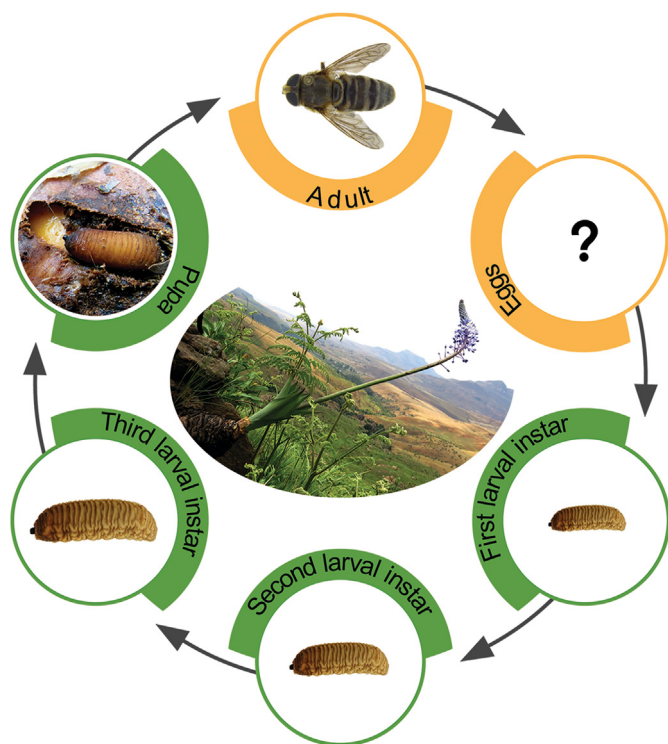

Fig. 12. Life cycle of *Merodon capi* and *Merodon roni*. Green circles indicating stages occurring inside the bulb, yellow area indicating stages occurring outside the bulb. L1, L2 and L3: First, second and third larval instar, respectively. Question marks indicates lack of information regarding egg morphology.

between the species is the distance between mouthhooks on the apex in relation to their basal width. It is generally the same distance (*M. calidus*, *M. avidus*, *M. capi*, *M. roni* and *M. hurkmansi*), except for *M. equestris* in which the distance is longer and *M. luteihumerus* in which it is shorter. The presence or absence of accessory teeth has been considered as a character with taxonomical information in the genus *Merodon* in the last few years: *M. equestris* presented accessory teeth in the mouthhooks together with *M. avidus* according to Andrić et al. (2014). Nevertheless, Preradović et al. (2018) not only indicate the absence of accessory teeth in *M. avidus* (in the head skeleton from the puparium) but point out a considerable difference in length among the three mouthhooks analyzed, caused by the wearing of the mouthhooks due to their use throughout the development of the larvae. In this study, a total of eight larval mouthhooks have been analyzed, together with some head skeletons extracted from pupae. The same variation due to wearing has been detected in the larvae and pupae studied, as some mouthhooks were longer and sharper, while others were shorter and with the base scratched; in the same way, reminiscences of accessory teeth were identified in some of the larvae. The genus *Merodon*, as phytophagous larvae, has a very active feeding mode, and the mouthhooks are sclerotized and robust to be able to scratch the vegetal tissue. During this process they undergo wear, becoming smaller and changing in shape, just as has been described in the teeth of mammal herbivores. The variation in the length and shape not only affects the presence or absence of the accessory teeth, but may also affect the distance of the apex with respect to the basal width. Therefore, we consider that the mouthhooks of the genus *Merodon* are very variable during the development of the larvae, and therefore these characters are

not reliable interspecific diagnostic characters.

The PRP of the genus *Merodon* is a feature that is very variable within the genus. With the information available until now, this variation appears to follow a pattern and is partially related to the phylogenetic lineages. The *avidus - nigritarsis* lineage (*M. avidus* and *M. opacus*) presents an extremely short PRP that is wider than long (button-shaped), not noticeable from the dorsal view, and with the absence of ornamentation and grooves (Andrić et al., 2014; Vujić et al., 2020). On the contrary, the *aureus* lineage (*M. bombiformis* and *M. calidus*) presents a barrel-shaped PRP, that is rounded at the apex and base and has two grooves, one at the base and one constraining the apex, right before the spiracular plate (Stuckenberg, 1956; Preradović et al., 2018). However, differences between the *natans* lineage (*M. makrisi, M. pulveris* and *M. natans*), the *desuturinus* lineage (*M. capi, M. roni)* and the *albifrons* lineage *(M. geniculatus, M. luteihumerus, M. hurkmansi* and *M. equestris*) are not so obvious, as all have a long PRP, that is noticeable from the dorsal view, with the presence of a groove at the base or close to the base. Apparently, the *natans* lineage presents a PRP that is more like a truncated cone, being wider at the base than at the apex (Vujić et al., 2021b), while the *desuturinus* lineage presents a PRP with a similar length and width, and which is rectangular to square in shape. Nevertheless, this difference is very subtle and not even applicable to all the species. It needs to be highlighted that there is an exception to this shape, namely in the form of *M. rufus* (belonging to the *desuturinus* lineage), which has a PRP that is much wider than long, noticeable from the dorsal view, and a base that is much wider than the apex, and which narrows abruptly in the apical third (Preradović et al., 2018).

The shape of the spiracular plate derives from the shape of the PRP and therefore also appears to be partially related to the division of the lineages. The *Avidus - nigritarsis* lineage is characterized by a smooth spiracular plate (Andrić et al., 2014; Vujić et al., 2020), while the *aureus* lineage presents a very irregular shape, with marked indentations where the interspiracular setae are present (Stuckenberg, 1956; Preradović et al., 2018). The *natans, albifrons* and *desuturinus* lineages have a smooth or slightly irregular plate, which has small indentations in *natans* and is slightly narrowed in the central area in most of the species of the *desuturinus* lineage. Again, *M. rufus* presents a different shape within its lineage, and has a smooth plate (Preradović et al., 2018).

Considering these two aspects of PRP shape in all the species, it cannot be stated that there is a clear PRP shape distinction for each of the lineages, but what can be affirmed is that, with the information available until now, PRP shape is a good character for identifying species belonging to the *aureus* and *avidus - nigritarsis* lineages. Further research is needed to confirm this character in more species of all the lineages, and to look for some diagnostic characters for the *natans, albifrons* and *desuturinus* lineages.

As has been mentioned above, all the species present four spiracular openings in the spiracular plate, but the shape of the openings differs slightly between the species. Some of them are characterized by highly convoluted openings (*M. avidus, M. hurkmansi* and *M. equestris*), while the rest have slightly curved or sinuous ones. On the spiracular plate of all of them four pairs of inter-spiracular setae with different degrees of branching are described. Small nodules distributed near the spiracular openings have been described for the first time by Vujić et al. (2020, 2021b) in *M. opacus, M. natans* and *M. makrisi*. In the present study, a small hole without setae close to the second inter-spiracular setae in *M. capi* and *M. roni* is described, which apparently has not been described previously, but looking at the images of the description of other species, this hole is also present in *M. calidus, M. rufus, M. luteihumerus, M. geniculatus, M. makrisi, M. natans, M. opacus* and *M. bombiformis*.

4.3. Host plant and adult ecology

Third-instar larvae and pupae of *M. capi* and *M. roni* were found inside epigeal bulbs of *M. plumbea* (*M. natalensis* as synonym). The bulbs of this plant are characterized for being big (around 15 cm high), completely epigeal, resting above the ground with only the roots anchoring them and covered by hard brown scales. The plant is perennial and deciduous. The leaves appear during the spring, and are light green, long and tapered, and form a rosette on the top of the bulb. It can be found in groups or as a solitary individual. The inflorescence occurs during early summer from the center of this rosette, reaching the height of almost one meter. It has a thin and violet-blue raceme composed of many flowers. The plant is native to South Africa, and its distribution is restricted to the eastern subtropical areas of the country. It can be found growing on slopes, hills, and cliffs near watercourses (Notten, 2001; Sparg et al., 2015). In the localities where the plant species were sampled, the bulbs were in steep valleys among rocks and within montane *Themeda* Forsskål and some scattered *Protea* L. trees.

Until now, of the 14 species of *Merodon* whose preimaginal stages have been described, there is information about the host plant for 11 of them. *M. makrisi, M. natans* and *M. pulveris* were found feeding on *Prospero autumnale* (L.) Speta (Vujić et al., 2021b), *M. avidus* is associated with *Ornithogalum umbellatum* L. (Preradović et al., 2018), *M. bombiformis* was found in some species of *Gladiolus* L. (Stuckenberg, 1956), *M. equestris* and *M. geniculatus* were reared from species of *Narcissus* bulbs (Ricarte et al., 2017), *M. hurkmansi* was reported on *L. comosa, M. luteihumerus* larvae were found feeding on *D. maritima* (Ricarte et al., 2008), and *M. capi* and *M. roni* on *M. plumbea* as has been described in this study. As there are not many preimaginal stages described, more evidence is needed to describe and properly state the degree of host plant specificity for the larvae of this genus. Nevertheless, as all the plant species mentioned above belong to the Asparagales order, and most of them to the Hyacinthaceae family, the connection of the preimaginal stages of the *Merodon* genus with the plant family stated by Djan et al. (2020) cannot be contradicted.

Regarding the adults, the mouthparts of the three analyzed species have a very similar morphology. There are two informative morphological aspects in relation to the feeding activity of the species that differ between the species. According to Gilbert (1981), the percentage of pollen intake in the diet is correlated with different morphological aspects and the proportions of the labella: these being the ratio between the length of the labellum and the prementum and the ratio between the number of pseudotracheae per mm of labellum. In the studied species, *M. drakonis* presents a ratio between the labellum and the haustellum of approximately one, while in *M. capi* and *M. roni* it is about 0.87. The number of pseudotrachea in the labella of *M. drakonis* is 20, with a ratio of 32 per mm of labella; in *M. capi* and *M. roni* the number of pseudotrachea is 14 and the ratio is 27 per mm. In both cases *M. drakonis* presents higher values, indicating that its labellum is broader and more adapted to pollen feeding, and therefore, the pollen intake of this species would be higher than in *M. capi* and *M. roni*. Moreover, the mouthparts of *M. drakonis* are more robust and sclerotized than the ones of *M. capi* and *M. roni*. The higher degree of sclerotization present in *M. drakonis* also supports the idea of greater pollen feeding activity.

The role of the pseudotracheae in pollen feeding is not known for certain, but they are key structure in this process; Schuhmacher and Hoffman (1982) described them as food-tubes, between which pollen grains are conducted into the sucking tube. Pollen grains have been found in the pseudotracheal channels (food-tubes); however, they cannot act as a filter because pollen grains are far bigger than the width of these channels (Gilbert, 1981).

When analyzing the amount of pollen present in the gut and on the body, notably less pollen was retrieved from *M. capi* and *M. roni* compared with *M. drakonis;* these results support the description given for mouthpart morphology. The field observation of *M. capi* and *M. roni* stated that, despite several plants flowering in the sampling location, none of the specimens was captured on the flowers but rather on the bare soil and leaves (Djan et al., 2020). Therefore, presumably the adults either take scattered pollen grains from the leaves and ground instead of from, flowers or the sampling did not coincide with the adults' feeding activities. On the other hand, *M. drakonis* was only collected from flowers, thus having a more direct source of pollen.

When focusing on the diversity of pollen grains, the dominance of *Senecio*-type in the analyzed samples was expected, bearing in mind their abundance in the region and flowering being concomitant with the occurrence of adults (Pooley, 1998). However, the percentage of this pollen type on the integument and in the gut of *M. drakonis* is much higher than in *M. capi* and *M. roni*. In general, *M. drakonis* had higher percentages of certain pollen types, while the other two species presented low percentages of all the pollen types. This, together with the fact that they were not seen on flowers, could indicate that *M. drakonis* has a more active and specific feeding habit than *M. capi* and *M. roni*, which feed in a more wandering and passive way, which could be supported also by the lower degree of sclerotization and development observed in the mouthparts.

Taking all this information into account, in the present study the relation between adults of *M. capi* and *M. roni* and the host plant cannot be confirmed. The adults were always seen flying around the plant populations, and no adults were sampled more than 20 m from them; however, none of the specimens was captured on the flowers but on bare soil. Moreover, the pollen of *M. plumbea* was not found in neither the gut or on the integument of any of the studied species. The information is still too scarce to affirm the presence of a close relationship between the adults and the host plant in these species, or indeed in most of the species of the *Merodon* genus studied so far; such a relationship was stated by Ricarte et al. (2008), between *M. luteihumerus* and *Urginea maritima* but further research is needed to define this aspect of adult ecology of the genus.

4.4. Conclusions

Regarding the methodological approach, the results of this paper show that the morphometric analysis of the preimaginal stages can be useful in integrative taxonomy, when traditional taxonomy techniques are not sufficient. On the other hand, with regards to the larval morphology of the genus *Merodon*, it needs to be highlighted that the larval morphology is quite similar among the species, which have a lot of characters in common. The most variable character is the shape of the Posterior Respiratory Process, which is partially related to the taxonomic lineages of the genus. Finally, when focusing on the biology and ecology of the studied species, this study supports the, already noted, close relationship between the preimaginal stages of the *Merodon* species and plant species of the Hyacinthaceae family, but further research is necessary to establish the connection degree of adults with the host plant.

Author statement

Methodology: A.A., C.P.B., J.A., B.S., P.R.; Investigation: A.A., J.A., A.C., M.M., C.P.B., B.S., P.R.; Resources: S.R., A.V.; Data curation: A.A.; Writing − original draft: A.A.; Writing − review & editing: M.M., J.A., A.C., A.V; Visualization: S.R., S.R., A.V.; Funding acquisition: S.R., S.R., M.M. All authors revised the manuscript and approved its final

version. Revision of manuscript and response of reviewers and editor comments: A.A.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.asd.2022.101187.

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