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Laboratory Biology, Immature and Adult Morphology of *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae), Parasitoids of *Drosophila* Flies

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ABSTRACT

The life history and immature stages of development of the wasp *Trichopria drosophilae* (Hymenoptera: Diapriidae), a biocontrol agent of drosophilid flies (Diptera: Drosophilidae), including the invasive *Drosophila suzukii*, were investigated. *T. drosophilae* develops as a solitary endoparasitoid from egg to adult in 22–26 days under laboratory conditions at room temperature. The egg of *T. drosophilae* is broad ovate, with a short petiole when freshly laid and is subsequently getting more elongate. There are three larval instars in *T. drosophilae*. The freshly hatched first instar larva has a narrow caudal part with a bifurcated indentated abdominal appendage, enlarged thoracic segments and a head with sclerotized sharp mandibles and large labial palpi. The second instar is poorly sclerotized with peculiar everted (exodont) mandibles. The third instar is grub-like, with three pairs of thoracic spiracles and stylet-shaped mandibles. The pupa of *T. drosophilae* is semitransparent and surrounded by numerous meconium pellets within the host puparium. Competition between conspecific rivals is observed. The siblicide is conducted using both the sharp mandibles and the small labrum and labium, which function like a sucker enabling emptying the rival's body. In the case of superparasitism, the first-instar larvae of the parasitoid are encapsulated within serosa membranes. Also, the peculiarities of adult morphology of *T. drosophilae*, as well as a comparison to similar European *Trichopria* species, are provided.

1 | Introduction

The Southeast Asian spotted-wing Drosophila, *Drosophila suzukii* Matsumura, has undergone rapid geographic expansion across North and South America, where it has been established on the U.S. mainland since 2008, as well as across Europe and Africa during the last decade (Hauser 2011; Calabria et al. 2012; Asplen et al. 2015; Garcia et al. 2022; Boughdad et al. 2021). This fact evoked special attention of a broad range of practical entomologists to drosophilids and their parasitoids. Several prospective biological control agents native to the invaded areas, were tested for the biological control of *D. suzukii* (e.g., Chabert et al. 2012; Knoll et al. 2017). Among the evaluated biological control agents, the endoparasitoid *Trichopria drosophilae* (Perkins) (Diapriidae), a parasitoid of drosophilid puparia (Figure 1A–C), has emerged as one of the most promising species, showing efficient parasitism under field conditions in at least some contexts (Rossi Stacconi et al. 2019).

Trichopria is the largest and most commonly collected genus of Diapriidae in Europe (Nixon 1980). The same author did not report *T. drosophilae* in Europe, but mentioned that “I do not claim to have worked out the species as satisfactorily as I should have liked” and that “the same species are likely to appear under different names”. This emphasizes the imperfect situation with species identification of the species of the

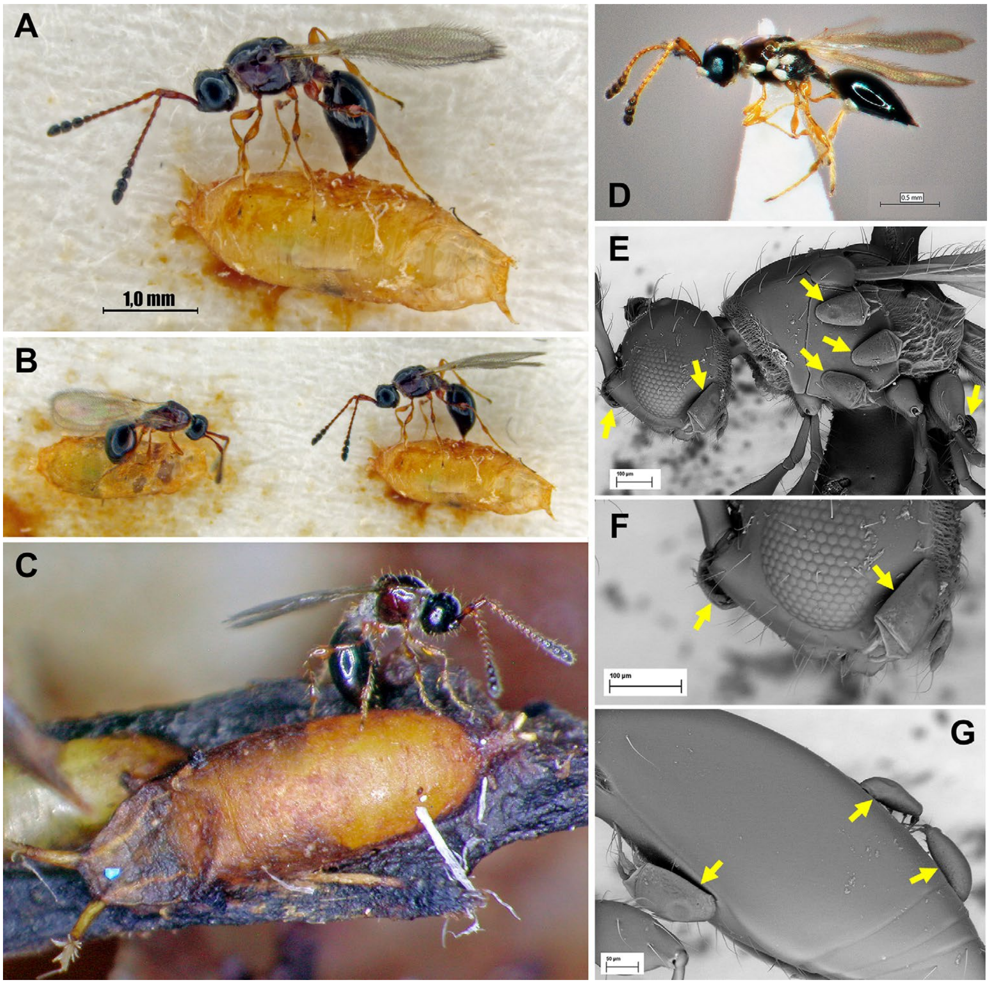

FIGURE 1 | Female of *Trichopria drosophilae* (light microscopy and SEM): (A–C) Female ovipositing, (D) Female bearing phoretic mites from laboratory culture (Italy, Lombardy, Arcagna; NHMUK collection), (E–G) Phoretic mites (yellow arrowed) attached to the parasitoid body (same locality and collection, SEM).

genus, at least in Europe, but likely in the entire world. *Diapria drosophilae* was described by Perkins (1910) from Hawaii; however, this species is believed to be of nearly cosmopolitan distribution now (Yi et al. 2020). Although different biological peculiarities of this parasitoid attracted the attention of various authors (e.g., Romani et al. 2002; Wang et al. 2016; Boycheva Woltering et al. 2019), features of its immature morphology and competitive interactions remain poorly studied. Furthermore, Abram et al. (2022) highlighted that the taxonomy of *Trichopria* is complex and in need of comprehensive revision. Species concepts within the genus may have been misinterpreted for years; for example, *T. drosophilae* and *T. virginica* (Ashmead) are nearly indistinguishable and may, in fact, be conspecific (Abram et al. 2022).

Although many biological control-related studies on estimation of biocontrol efficacy of *T. drosophilae* against *D. suzukii* were conducted (e.g., Rossi Stacconi et al. 2019; Gonzalez-Cabrera et al. 2019; Baser et al. 2025; Bin et al. 2026), too many of them have never deposited insect vouchers associated with their studies and have never involved taxonomic experts to confirm species identity (Abram et al. 2022). Therefore, it is necessary to describe in detail not only biological, but also morphological characteristics of the studied laboratory strains of *T. drosophilae*.

This paper is aimed to shed light on the hitherto unknown details of immature development and adult morphology of *Trichopria drosophilae*. The described data are expected to facilitate established and initiate further research programs on drosophilid parasitoids.

2 | Materials and Methods

2.1 | Insects

Two stock cultures of *Trichopria drosophilae* were used for the experiments: one was received from Agroscope (Zürich, Switzerland) in 2021 and another from Bioplanet (Cesena, Italy) in 2024. A stock colony of drosophilids was created by trapping local drosophilids by fruit baits in the vicinity of Hlobyne (Ukraine) and in Stuttgart (Germany). The drosophilid hosts were not identified to species, as all proposed puparia were accepted by the parasitoids, and because the morphology of immature stages is generally considered a constant trait determined primarily by genetics rather than host physiology. The cultures were subsequently maintained on fruit mush: chiefly made from apples (*Malus domestica*). Then the new stock cultures were maintained in cages (90×90×35cm) separately at room temperature (T° about 19°C–22°C) with humidity of 60%–70% throughout the year. The "Drosophila-stock" colonies (originated from a mixture of local drosophilids) and the "parasitoid-stock" colonies (parasitoids supplied with host puparia) were maintained separately. The Drosophila colony was represented by two cages where trays (30×15×6 cm) with fruit mash were exposed initially to about 100 adult drosophilids, and then the trays were changed about every 2 weeks, as long as the number of maggots and puparia increased. The parasitoid colonies were supplied by transferring the trays with unparasitized late-instar fly maggots and puparia from the "Drosophila-stock" to the "parasitoid-stock" colonies. Thus, the parasitoids were provided with constant access to hosts and completed their entire life cycle inside the cage. Fifteen parasitized puparia were kept in a thermostat at approximately 10°C to measure development time under colder conditions.

When insects were needed for experiments, fresh host puparia and living parasitoid females were taken from corresponding breeding cages. The experiments were conducted inside Falcon tubes (50 mL volume) sealed either with their original lids or with cotton plugs. The unparasitized fly puparia were glued to a rectangular piece of carton paper using fruit sap or honey and arranged into rows (up to 10 rows with about 6 puparia per row). Generally, about 10 females of *T. drosophilae* were supplied to each tube. Due to the descriptive nature of this study, the number of parasitoid females varied, but when superparasitism was invoked, this number was purposefully increased up to 30 per tube.

2.2 | Adult and Larval Morphology

Voucher specimens of adults were either air-dried or preserved in 70% ethanol and deposited in the collection of the I.I. Schmalhausen Institute of Zoology NAS of Ukraine (SIZK, Kyiv, Ukraine) and the State Museum of Natural History in Stuttgart (SMNS, Germany).

More than 300 parasitoid oviposition episodes were observed under a stereo microscope and host puparia were dissected at designated time intervals: immediately after oviposition, second day, fourth day, 1 week, 10, 15 and 20 days after oviposition. Dissections were conducted at room temperature, with at least 10 dissections and observations performed during each of the abovementioned time periods. The host puparia were dissected with entomological pins and sharp-point forceps. The isolated immature stages were either imaged alive in saline solution or fixed in 70% ethanol with further placement to absolute ethanol for further Critical Point Drying (CPD). Scanning electron microscopy (SEM) imaging of adult and immature specimens was carried out using a Zeiss EVO LS 15 (SMNS) microscope, which also allowed examination of uncoated specimens at 10–20 kV. The tiny CPD-dried specimens of immature stages (about 300 μm) were transferred onto SEM stubs by insect pins and using their static electricity. Then the stubs were coated with gold–palladium in the sputter Leica EM ACE200 (SMNS).

Color imaging of adult insects was conducted using a Leica Z16 APO microscope equipped with a Leica DFC 450 camera, and image processing with LAS Core software, with the same lighting (SIZK) and a Keyence VHX-5000 photomicroscope (SMNS). The abbreviations F1–12 are used for flagellar segments.

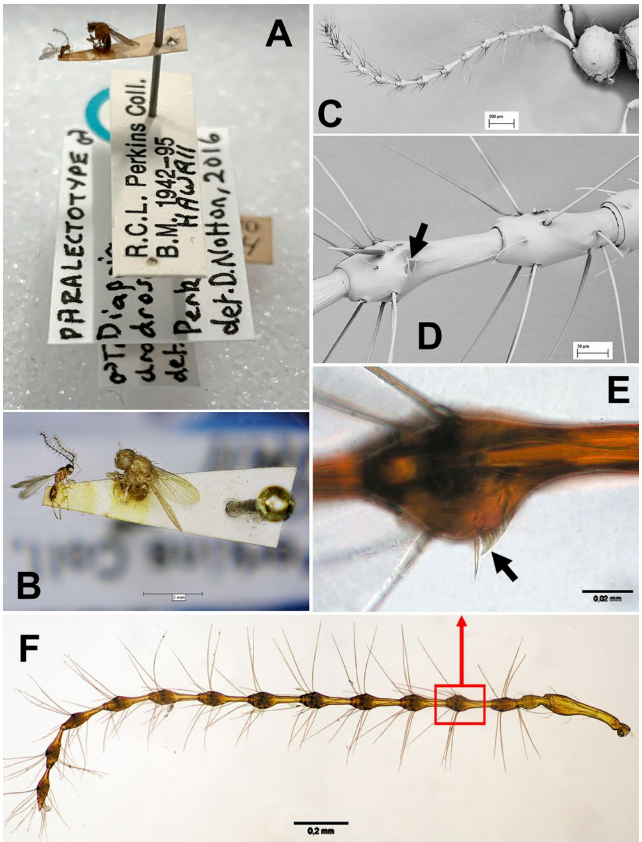

FIGURE 2 | Male of *Trichopria drosophilae* (light microscopy and SEM): (A, B) Paralectotype (NHMUK), (C–F) Head and antenna of specimen from laboratory culture (Italy, Lombardy, Arcagna; NHMUK collection), (E) F2 with small sclerotized tooth (= ventrolateral carina, black arrowed) shown as a framed inset of entire antenna in F.

2.3 | Parasitoid Identification

To ensure correct identification of the laboratory culture specimens, comparative materials of *T. drosophilae* were studied. These included the paralectotype (Notton 2014; a male, Hawaii, Honolulu, Oahu, 6–19–05; R.C.L. Perkins coll. B.M. 1942–95, paralectotype designated by D. Notton; Figure 2A,B) and materials from laboratory cultures (4 females, 4 males, Italy, Lombardy, Arcagna, southeast of Milan, 11.ix.2013, laboratory reared on *Drosophila* sp. on banana substrate: N. Amiresmaeili, 2014–93, Figure 1D–G) from the collection of the Natural History Museum, London (NHMUK). The comparison of *T. drosophilae* with other species of *Trichopria* was based on the collection of Diapriidae of SIZK.

3 | Results

3.1 | Adult Morphology of *T. drosophilae*

Below we provide a short morphological survey of *T. drosophilae* to locate it at least within the European species of the genus *Trichopria*. The female of *T. drosophilae* may be characterized by the combination of the following characters: the antennal club distinctly 3-segmented, flagellar segments longer than wide (Figure 3B), malar space about as long as ½ of eye width,

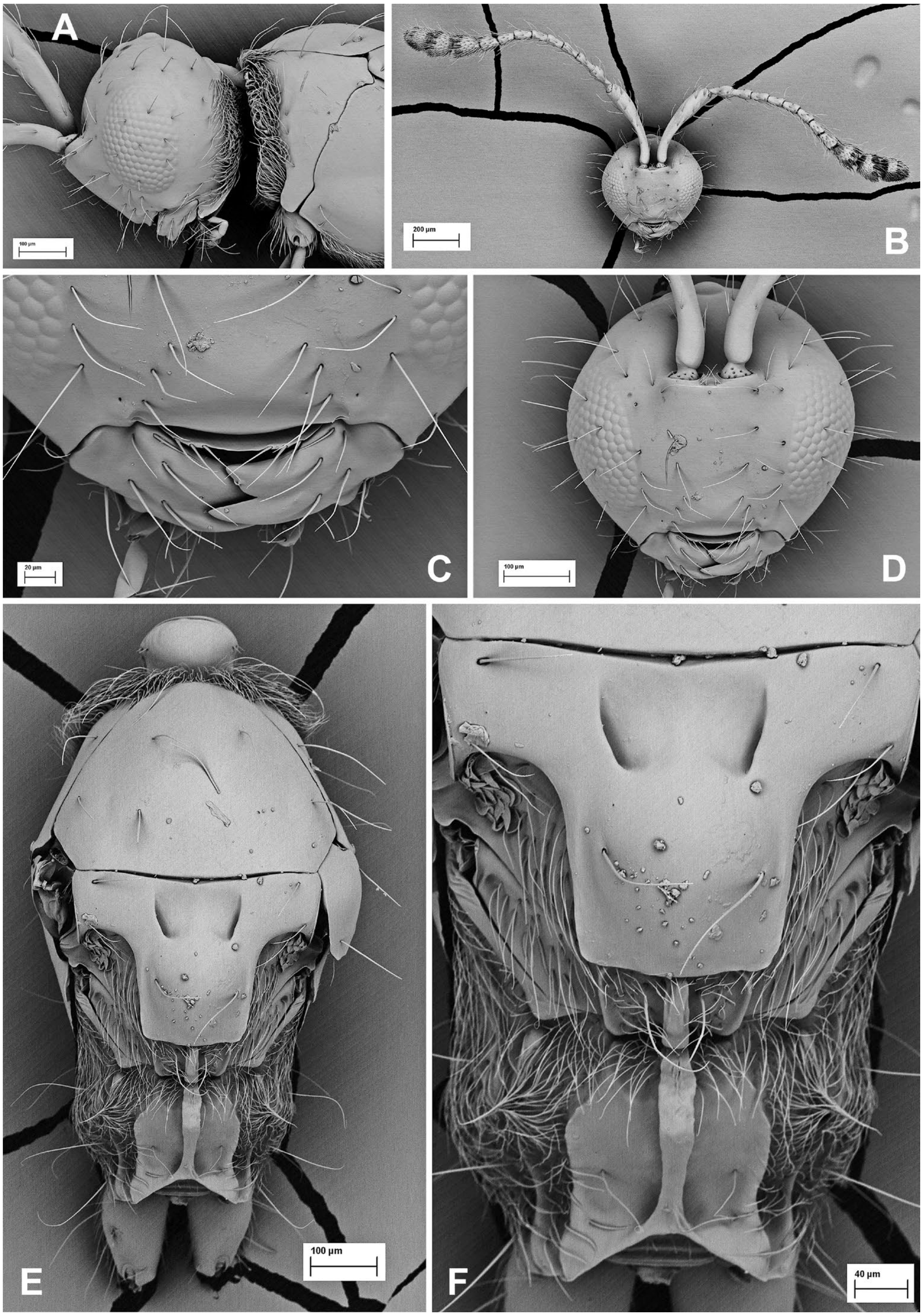

FIGURE 3 | Morphology of the female of *Trichopria drosophilae* (SEM): (A) Head and anterior part of mesosoma, lateral view, (B) Head, frontal view, (C) Lower face, (D) Face, (E) Mesosoma, (F) Scutellum and propodeum.

sometimes slightly less (0.4×) or slightly more (0.6×) than ½ of eye width, but never as long as or longer than the entire eye width (Figure 3C,D); pronotum and propleuron smooth, covered with a cushion of pubescence on the anterior margin of the pronotum (Figure 3A); mesoscutum weakly convex, smooth, almost bare, with three pairs of bristles; mesoscutellum as long as broad, without a keel or even a trace of it; anterior mesoscutellar pits fissure-like; metanotum pubescent throughout, with a median longitudinal keel; propodeum pubescent laterally, dorsally with a median keel, its posterior margin notched medially (Figure 3E,F); fore wing fully developed, hyaline, uniformly micropubescent, bordered with long setae along anterior, apical and posterior margins, with venation typical for the genus; petiole about 1.3× as long as broad; gaster long-ovate, nearly 2.0× as long as broad (Figure 4C). The female of *Trichopria drosophilae* is habitually similar to the females of *T. aequata* (Thomson) and *T. nigricornis* (Marshall), but differs in the laterally flattened mesoscutellum not bearing a keel (Figure 3E,F). The female of *T. drosophilae* may run to *T. evanescens* Kieffer if Nixon's (1980) key is followed. However, F3–F6 are notably longer than wide in *T. drosophilae* (F3–F6 are almost rounded in *T. evanescens*), the base of the first sternite is without thick pubescence right across

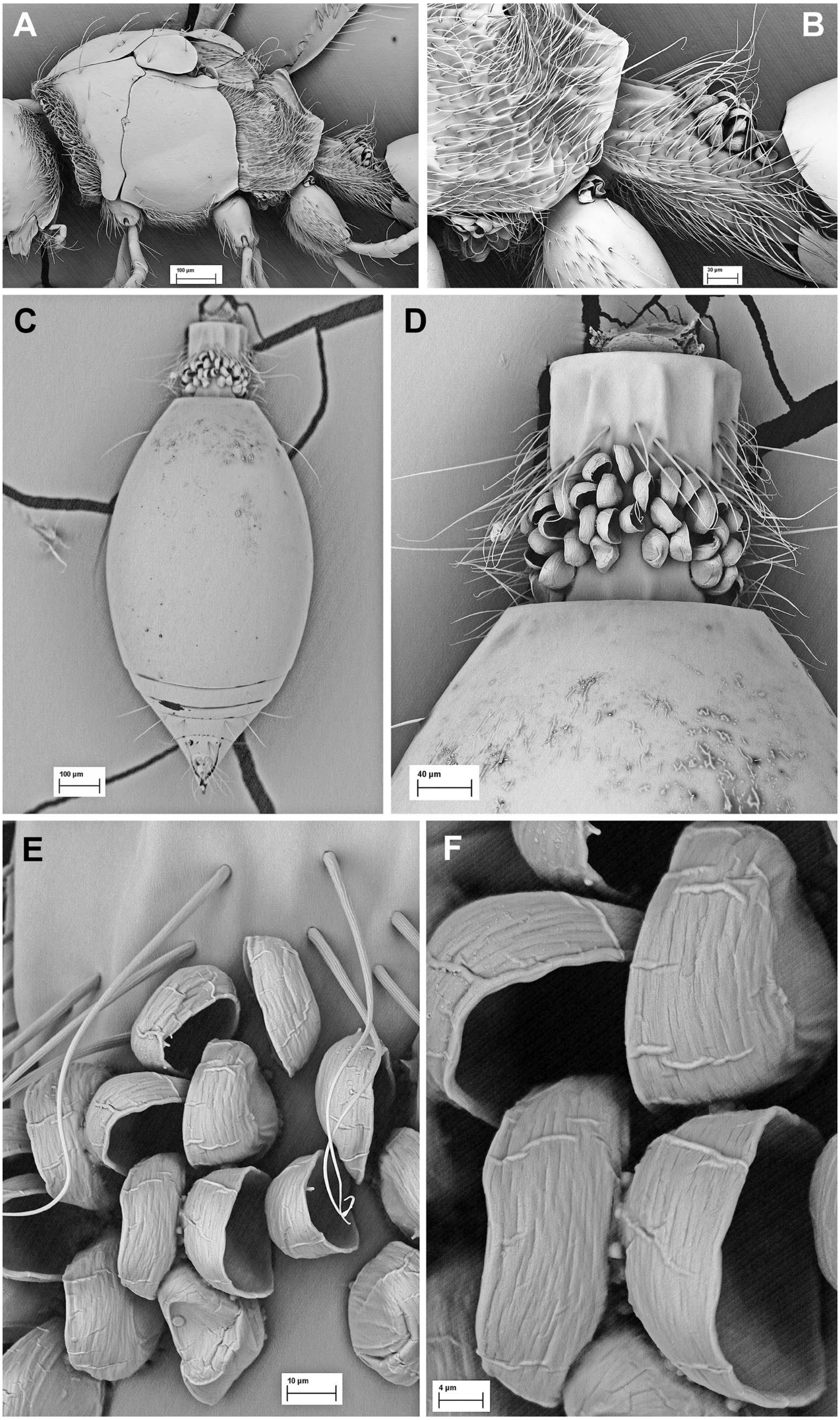

FIGURE 4 | Female of *Trichopria drosophilae*, morphological peculiarities of meso- and metasoma (SEM): (A) Mesosoma, (B) Posterior part of mesosoma and anterior part of metasoma, (C) Metasoma, (D) Petiole, (E, F) Foamy structures consisting of hyaline scales.

(with such pubescence in *T. evanescens*), and the visible part of the petiole is about 1.3× as long as wide (not longer than wide in *T. evanescens*).

Morphological peculiarities of *T. drosophilae* males concern mainly the antennal structure. The males of only a few European *Trichopria* species have antennae similar to *T. drosophilae* (Figure 2C–F): with F3–F9 elongate, narrowly fusiform or petiolate, with very long bristle-like hairs forming whorls and arising from the thickest part of their segments; with these hairs being virtually absent from the proximal third of segments, except for F1 (Nixon 1980). The antennal structure of *T. drosophilae* males is most similar to that of *T. verticillata* (Latreille), but the emargination on F2 is very weak in *T. drosophilae* (Figure 2E,F). The males of *T. aequata* and *T. nigricornis* have antennae similar to *T. drosophilae*; however, the emargination of F2 is ended by a distinct sclerotized tooth in these species, whereas such a tooth is missing in *T. drosophilae*. There is a tiny, poorly sclerotized and hardly visible tooth there instead (Figure 2E), reported as the ventrolateral carina by Romani et al. (2008) and Sacchetti et al. (1999), and it is not accompanied by strong emargination. This structure is associated with epidermal glands and plays an important role in the courtship pheromone transfer from male to female antennae during mating (Sacchetti et al. 1999; Romani et al. 2008).

The mesosoma of *Trichopria* species is covered with a dense group (collar or cushion) of setae, in particular on the pronotum, mesepisternum, metanotum, propodeum, and petiole (Figures 3A,E,F and 4A,B). Apart from these very densely haired areas, large hyaline scales (with the appearance of foam; Figure 4E,F) are present on the posterior half of the petiole (Figure 4D) and on the underside of the metepisternum (Figure 4A,B), as well as a small group on the upper sector of the hind coxa (Figure 4B) and the axilla at the place of contact with the metepisternum (Figure 3F).

The presence of these scales in *T. drosophilae* was one of the characters used by Kim et al. (2016) to synonymize the genera *Trichopria* Ashmead and *Alareka* Rajmohana & Narendran; the scales are extremely developed in *T. keralensis* Rajmohana & Narendran.

The biological function of these structures may vary, but some observations of the authors suggest their role in defensive behavior. Adults of *T. drosophilae* operate in a highly infested environment, often covered with mold and inhabited by nematodes and mites. Occasionally, nematodes from the surrounding substrate also accumulate on adult individuals of *T. drosophilae*. Eventually, most of them aggregate around the insect's head, the posterior part of the mesosoma, around the coxae, and the metasomal petiole. These areas are either covered with dense setae (borders of head and pronotum, posterior part of mesosoma) or bear both dense setae and hyaline scales (areas around hind coxa and petiole; Figure 4B). Often, adult parasitoids manage to scratch the nematodes off during grooming (personal observations). It is highly likely that these structures facilitate such grooming by preventing tighter contact between nematodes and the insect body. Also, the examined NHMUK specimens show that adults of both sexes occasionally bear phoretic mites, which are tightly attached to non-haired areas of the head and mesosoma and remain attached even after death of the parasitoid (Figure 1D–G). Thus, adults of *T. drosophilae* face numerous challenges from potentially unwanted phoretic associates, and the functional significance of at least some of their morphological features may be related to detaching from these organisms.

3.2 | Life History and Ovipositing Behavior of *T. drosophilae*

Our laboratory observations show that *T. drosophilae* is a hygrophilic species that can cope with humid surroundings and excessive moisture. Some other congeneric species are associated with semi-aquatic hosts and also partly submerge in water while attacking their hosts (e.g., *T. columbiana*; Coon et al. 2014). The females can oviposit into host puparia submerged in liquid substrate. Adult insects that come into contact with liquid, generally cope with subsequent drying by grooming.

Females of *T. drosophilae* are attracted to the smell of fresh or rotting fruits, even in the absence of dipteran immatures (Wolf et al. 2020). In our experiments, once a female reaches the fruits, she starts examining the substrate by drumming it with her antennae. As soon as a puparium is located, the parasitoid female drums its surface with the antennae and soon afterward bends its gaster and starts ovipositing (Figure 1A–C). Oviposition lasts several minutes, during which the female stands nearly still and only slightly moves her antennae. As the oviposition episode approaches its end, the female withdraws the ovipositor and retracts it into the gaster. Occasionally, the host puparium bears dark, melanized dots that are highly likely to represent immune responses resulting from penetration of the puparium by the parasitoid ovipositor. These dots, reported as “oviposition scars” for *T. anastrephae* Lima by Krüger et al. (2019), appear soon after oviposition and are therefore most likely associated with ovipositor penetration rather than with the development of parasitoid instars.

The laid egg (Figure 5A,B) develops for 3–4 days, after which the first instar larva hatches (Figures 5C,D and 6). In cases of superparasitism, the first instars become involved in siblicide (Figure 7A,B,I,J). Apparently, the age of the larva does not determine survival: occasionally, larger and fed first instars are found dead, whereas the “winner” has not yet started active feeding. When a single puparium is offered to a female of *T. drosophilae* for 2–3 h, only one parasitoid instar is found within the host puparium, and no superparasitism occurs, despite the female continuing to observe and probe the puparium. In our experiments, superparasitism occurred only under stimulated conditions, when a limited number of puparia was offered to an excessive number of females. When the ratio was 3–4 puparia per female over 2–3 days, siblicide was observed in all puparia, but without encapsulation. When an excessive number of females (more than 20 per 3–4 puparia) was used, dissected puparia revealed encapsulated parasitoid larvae (Figure 8A,B). In this case, each *T. drosophilae* larva was surrounded by a capsule, and several capsules were attached to one another (Figure 8A–C). Interestingly, most larvae were dead and only one was alive (the “winner”; Figure 8C,D, red arrow). Thus, encapsulation provided neither protection from

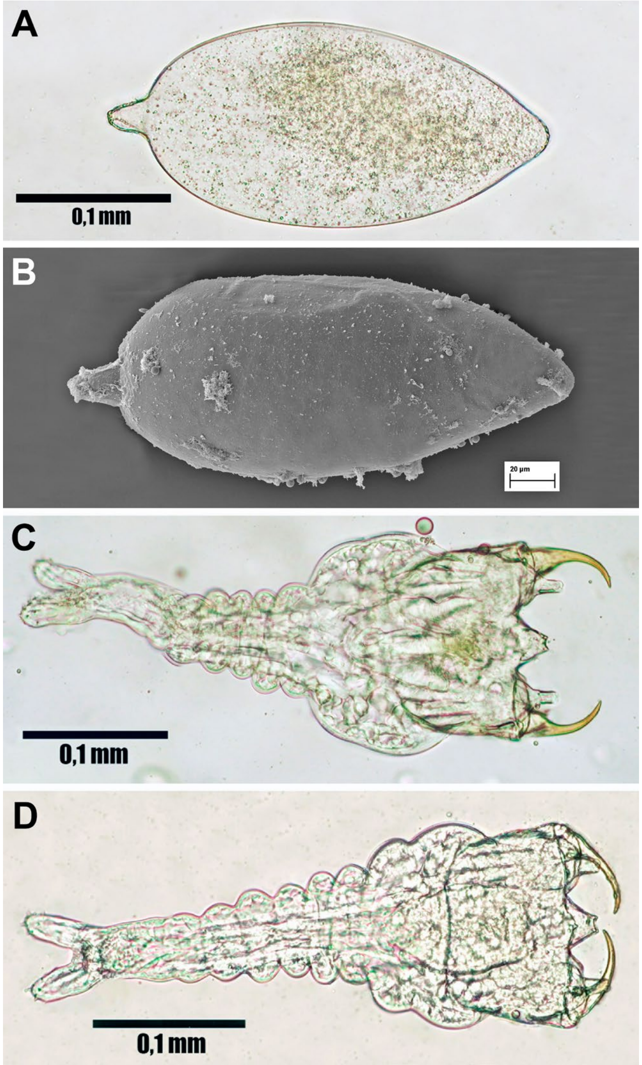

FIGURE 5 | *Trichopria drosophilae*, egg and first instar (light microscopy and SEM): (A, B) Egg, (C, D) Young (recently hatched) first-instar larva.

siblicide nor elimination of all parasitoid larvae. It also remains questionable whether the encapsulation is generated by the host or by the parasitoid. Although in most cases encapsulation is considered to result from the host immune response, involving the aggregation of host hemocytes around parasitoid eggs or larvae, other evidence supports the idea that the serosa (and associated cells) surrounding parasitoid embryos or larvae is derived from the parasitoid itself rather than from the host. These cells, often referred to as serosal cells or teratocytes, originate from the extraembryonic membranes of the parasitoid egg and persist around the developing larva or dissociate after egg hatching and are released into the host hemocoel, where they may contribute to host regulation and immune modulation (Smith 1991; Pennacchio et al. 2000; Pennacchio and Strand 2006; Falabella et al. 2005).

The sharp mandibles of the first instar (Figure 7C) likely play a key role in grabbing the competitor (Figure 7I,J), but final elimination is probably achieved by sucking off the rival (Figure 7A,B,E–H). The suction force of the mouth opening (Figure 7D) is strong enough to keep the two fighting larvae together even after ethanol fixation and critical point drying (Figure 7).

The first instar generally begins feeding on the 7th–9th day after oviposition, showing a notable increase in size (Figure 9). Depending on temperature and likely other factors (e.g., host condition), the second instar (Figure 10) may be found within the host around the 10th day after oviposition at room temperature (but sometimes earlier or later). It is easily discernible by its milky body and non-sclerotized cranium. Feeding by the second instar is likely the most destructive, as the host puparium already appears partially (one third to half) empty around the 14th day, when the next, young final instar is present. The final, third instar (Figures 11 and 12A,B) feeds for 3–5 days or longer, depending on host condition, and eventually voids the meconium and pupates shortly afterward.

The pupal stage (Figure 12C,D) lasts 5–7 days or more. The newly formed adult chews its way out of the puparium, leaving its exuviae and meconial pellets behind. Under laboratory conditions (approximately 19°C–22°C), total development time from egg to adult emergence was 22–26 days, with the first males eclosing one to 3 days before females; under colder conditions (around 10°C), development lasted up to 115 days. Larval development is asynchronous: host puparia parasitized nearly simultaneously may contain parasitoid larvae of different instars.

3.3 | Egg (Figure 5A,B)

T. drosophilae is a proovigenic species, as the female ecloses from the pupa with a load of about 40–50 mature eggs (Wang et al. 2016). c (Yi et al. 2020). The freshly laid egg (Figure 5A,B) is broad ovate, with a short petiole, about 260 μm long (excluding the petiole). The petiole is located on the thicker, blunter end, opposite the narrower tip. The freshly laid egg contains traceable yolk granules. About 24 h after oviposition, cell proliferation is observed at the periphery of the egg, and after 48 h, more cells are visible inside (in the stroma) instead of yolk granules. Later, the egg becomes more elongate in shape.

3.4 | Larvae

Three larval instars are observed.

3.5 | First Instar Larva (Figures 5–9)

The freshly hatched first instar (Figures 5C,D and 6C,D) is about 350–400 μm long, with a narrow caudal part and enlarged thoracic segments and head capsule (cranium). Its body possesses two discernible thoracic segments (without distinct subdivision between them) and seven abdominal segments, the terminal (seventh) of which is elongate and ends in a two-lobed appendage (Figures 5C,D and 6C,D). Each lobe of the appendage bears about 10 small teeth (Figure 6C) and can move independently. The other segments do not carry any teeth or denticles. No spiracles are present on the body of the larva (apneustic).

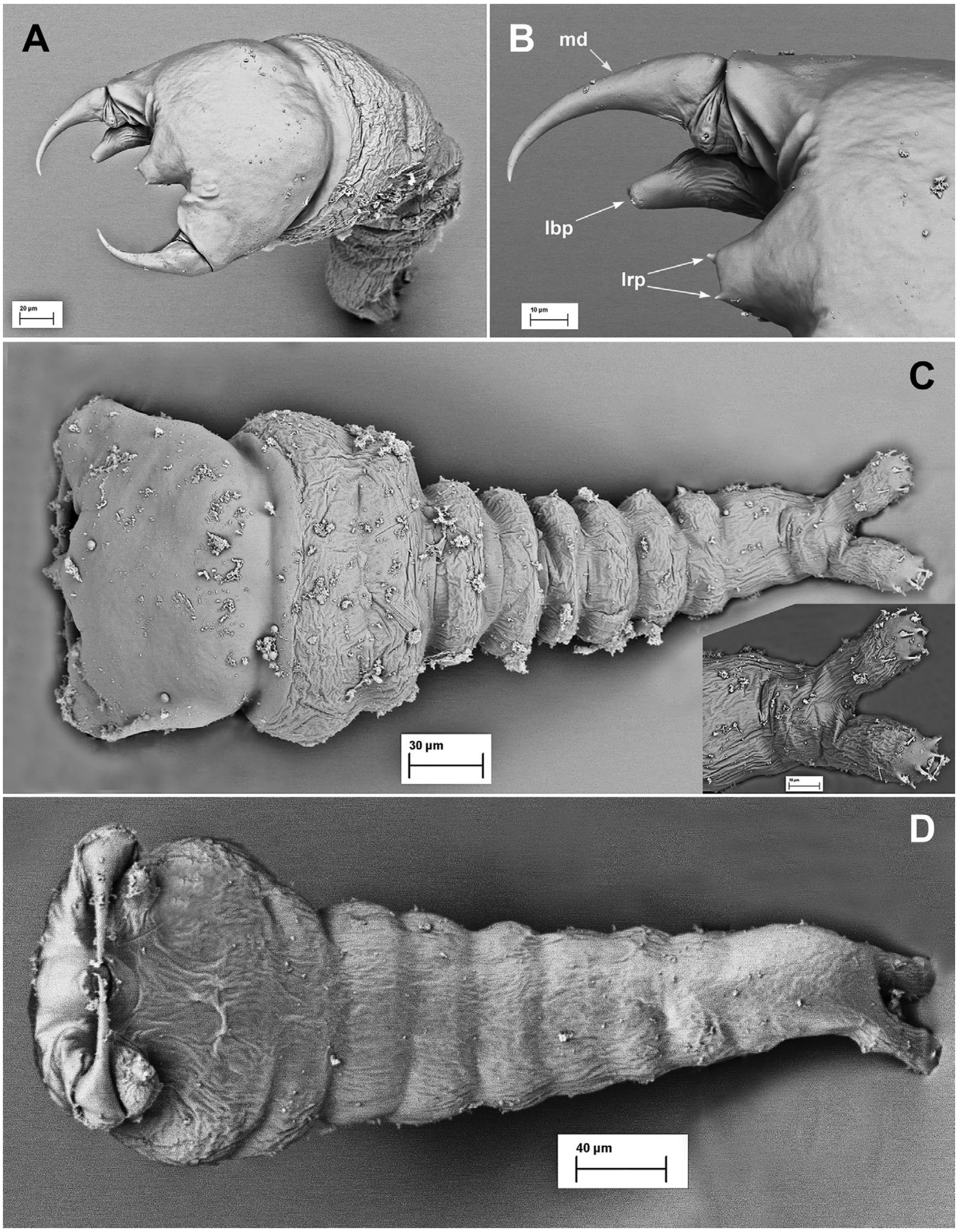

FIGURE 6 | *Trichopria drosophilae*, young first-instar larva (SEM): (A, C, D) Habitus, (B) Mouthparts. Caudal appendage shown in another viewpoint in inset in (C): md—mandible, lbp—labial palpus, lrp—labral palpi.

In young first instars, subdivision into thorax and abdomen is obvious (Figures 5C,D and 6C), whereas this subdivision nearly disappears later, when the larva is fully fed (Figure 9C,D).

The cranium bears sickle-like, sharp, large mandibles (Figures 6A,B and 7C), a pronounced labrum, and huge labial palpi (Figures 6A,B and 7C,D). Antennae are not traceable. The labrum ends with two palpi in the shape of small peaks and each large labial palpus possesses small sensoria at its tip (Figures 6B and 7C). The mouth opening is surrounded above by the labrum and below by the labium armed with a series of tiny teeth or palpi (Figure 7D).

The larva starts growing when dark content appears in its midgut 9 days after oviposition (Figure 9A,C). About at 10–14 day after oviposition, the larva is twice as large as the newly hatched larva: it is swollen and about 800 μm long and slightly more than 200 μm wide (Figure 9A–C). Despite the habitual difference, this is the same first instar, which can be identified by the same morphology of the cranium (Figure 9C,D) and caudal appendages (Figure 9A,B). These body parts do not undergo deformation, whereas the cuticle of the rest of the body swells due to the swollen midgut contents full of consumed host tissues.

3.6 | Second Instar Larva (Figure 10)

About 10–14 days after oviposition (but occasionally as early as 6 days if development is faster), the second instar may be observed

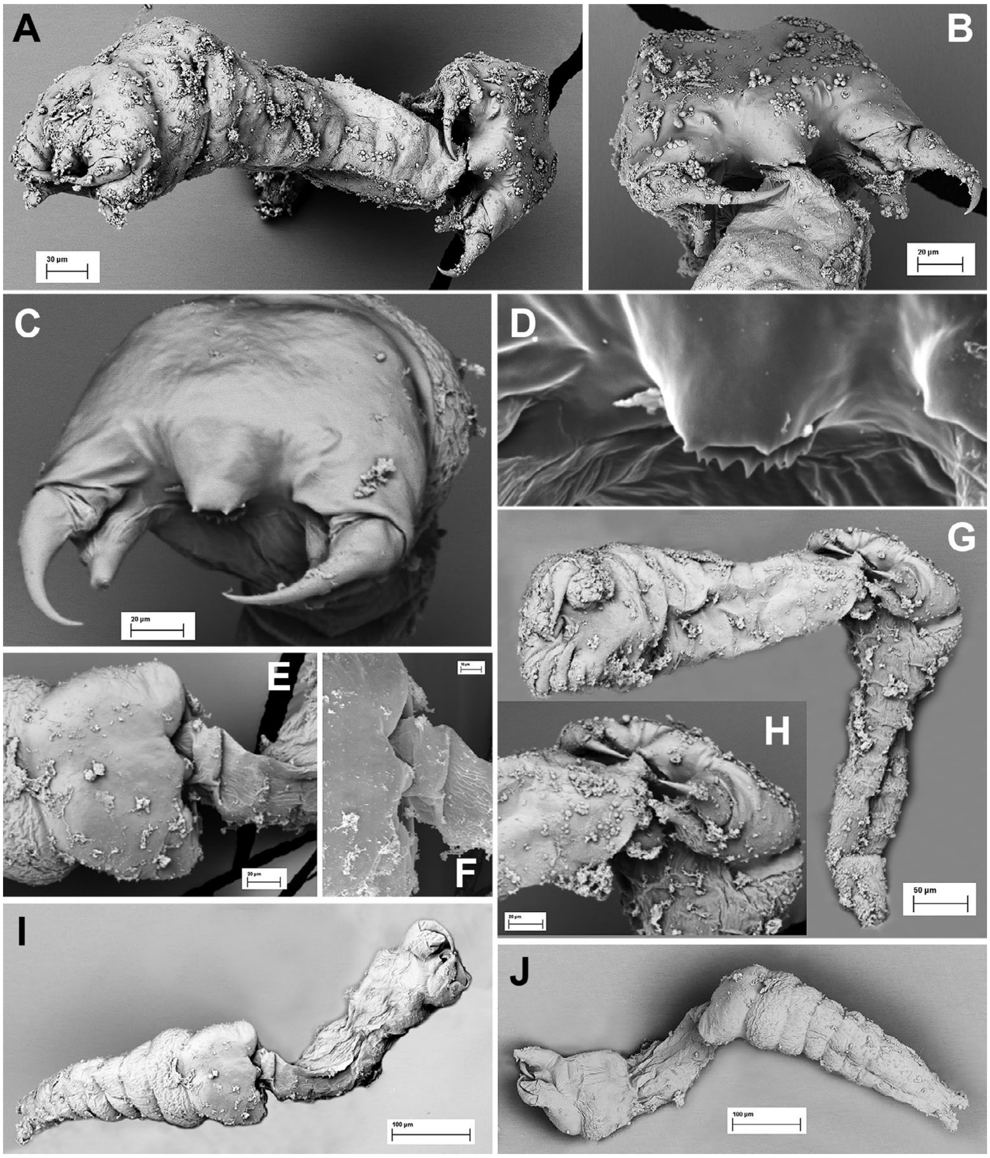

FIGURE 7 | *Trichopria drosophilae*, siblicide of first-instar larvae and morphology of involved mouthparts (SEM): (A, G, I, J) General view of fighting larvae, (B, E, F, H) Head of the "winner" biting the caudal end of the "loser", (C) Head, (D) Labrum and labium (mouth opening).

inside the host puparium (Figure 10A). This instar is grub-like, about 1500 µm long, milky in color, poorly sclerotized, with traceable segmentation, and apneustic, similarly to the first instar. Morphologically, this instar is very different from the previous one.

Its cranium is larger than in the first instar (Figure 10B–D). It bears short but distinct antennae (Figure 10F), which were not discernible in the first instar. The labrum is short and evenly rounded (bearing two spines in the first instar), and the labial palpus is swollen and without a spine (the palpus in the first instar is a small swelling ending with a short tube; Figure). The mandibles are the most remarkable feature of this instar: they are wide, everted, and exodont, although poorly sclerotized (Figure 10B,C,E). The body consists of about 13 segments (Figure 10A,C), the sutures between which are poorly traceable.

3.7 | Final Instar Larva (Figure 11)

The larva is hymenopteriform (Figure 11A,B), with a developed tracheal system and strong sclerotized mandibles (Figure 11C,D). The freshly formed (young) final instar is about 1000 µm long and 700 µm wide (Figure 11B). The mandibles are large, stylet-like, about 100 µm long (Figure 11C,D,F). The larva is apparently 13-segmented, with three pairs of thoracic spiracles (Figure 11E).

3.8 | Pupa (Figure 12)

Pupation takes place inside the host puparium, under the host skin, after voiding the meconium (Figure 12A,B). The pupa is 1800–2000 µm long and enclosed within the host skin together

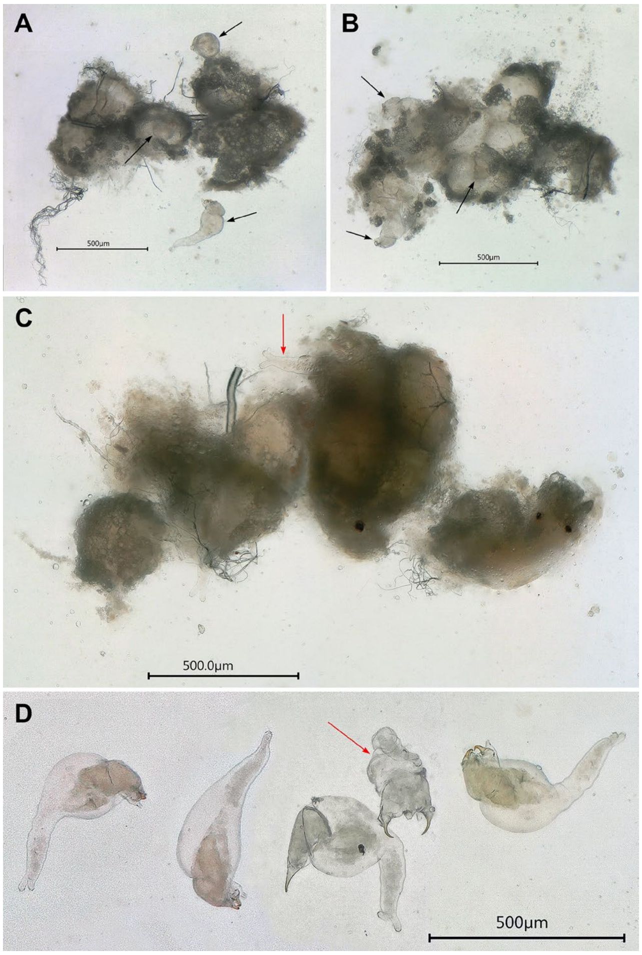

FIGURE 8 | *Trichopria drosophilae*, first-instar larvae encapsulated within the host as a result of superparasitism (light microscopy): (A–C) General view of serosa capsules, (D) Larvae isolated from serosa. Red arrow—“winner”, black arrows—larvae emerging from the capsules.

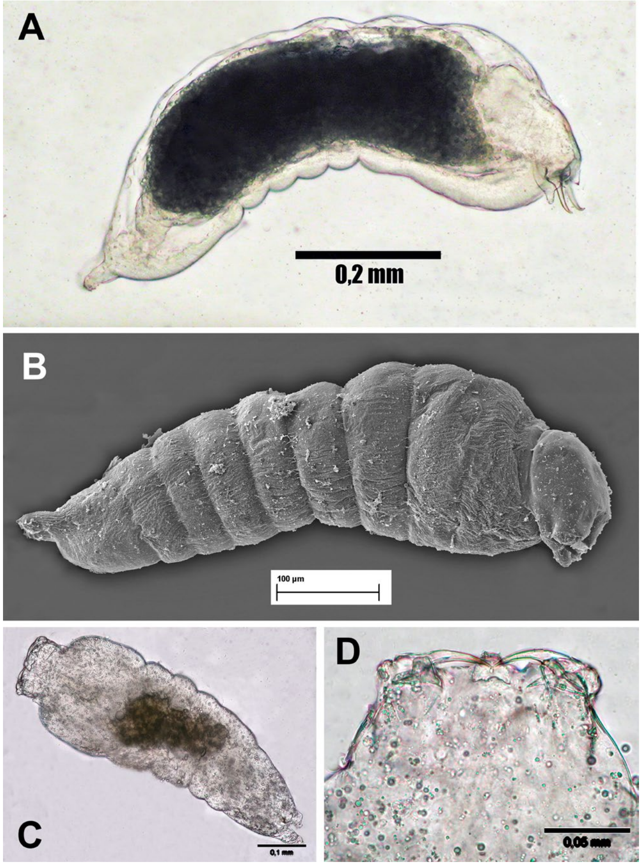

FIGURE 9 | *Trichopria drosophilae*, fully fed first instar (light microscopy and SEM): (A–C) Habitus, (D) Head.

with meconium pellets (Figure 12C,D). The freshly formed pupa is pale and darkens afterward.

4 | Discussion

Despite being in the focus of contemporary research, parasitoids of Drosophilidae still bring many novelties and unexpected parasitoid-host associations (e.g., parasitoids of adult flies: Moore et al. 2024). In addition, the diversity of diapriids as a whole and of the genus *Trichopria* is underestimated, with numerous species still undescribed (Masner and García 2002; Tymochko et al. 2021). New fossil records of the genus (Brazidec and Perrichot 2025) highlight that the limited knowledge of morphological variation among extant species hampers both the identification of fossil specimens and meaningful comparisons between past and present biotas. The proposed morphological and biological survey of *T. drosophilae* is expected to facilitate further discrimination of its adults among congeners and of its immature stages among other drosophilid parasitoids.

The obtained data demonstrate clear morphological differences among the immature stages of *T. drosophilae*. In general, these data correspond to the features of immature stages described by Coon et al. (2014) for *T. columbiana*, an aquatic parasitoid of *Hydrellia* flies (Diptera: Ephydridae). Similarly to *T. columbiana*, the egg of *T. drosophilae* is hymenopteriform with a small petiole, but it is shorter and thicker in *T. drosophilae*.

As in *T. columbiana*, three larval instars are recorded in *T. drosophilae*. The first instar of *T. drosophilae* is also similar to that of *T. columbiana*: they share the general habitus, the bifurcated and indentate terminal abdominal segment, and long sharp mandibles. Additional herein described characters that may or may not be present in *T. columbiana* include a bifurcate labrum and a pair of large labial palpi. All these structures are likely sensory and serve in locating rivals, followed by their physical elimination by the mandibles. One interesting feature of the first instar of *T. drosophilae* is its nearly twofold enlargement in size about 1 or 2 weeks after oviposition. This feature was not recorded for *T. columbiana* and is important, as the habitus of the larva changes and it may be mistaken for an additional instar if fine morphological details are overlooked.

The second and third larval stages in *T. columbiana* were mostly diagnosed by size differences (Coon et al. 2014). However, based on habitus and morphology, the larva shown

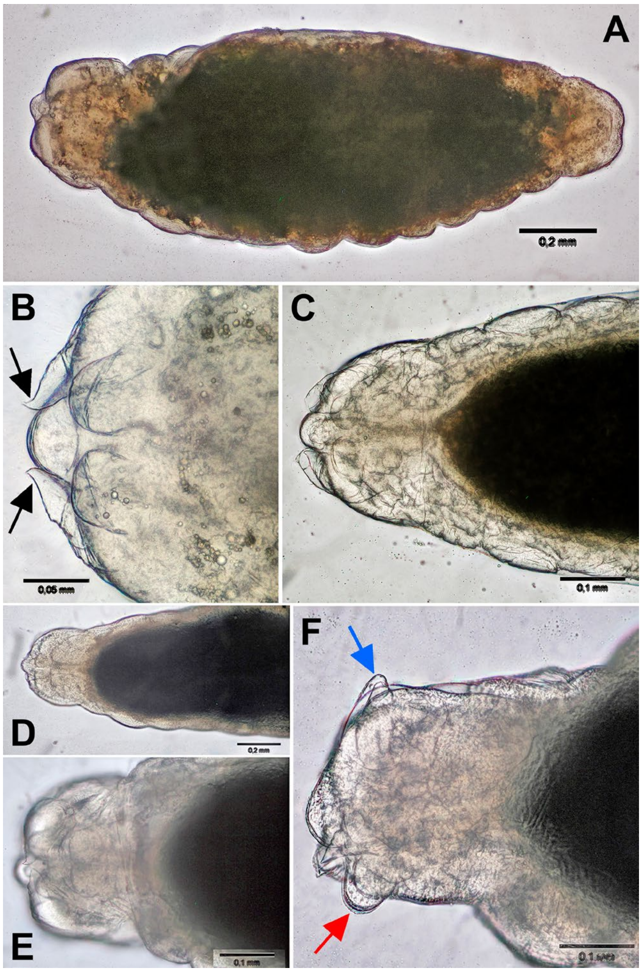

FIGURE 10 | *Trichopria drosophilae*, second instar (light microscopy): (A) Habitus, (B, C) Anterior part of body, ventral view, (D, E) Anterior part of body, dorsal view, (B) Head enlarged with everted mandibles (black arrows), ventral view, (F) Anterior part of body; antenna—blue arrow, labial palpus—red arrow.

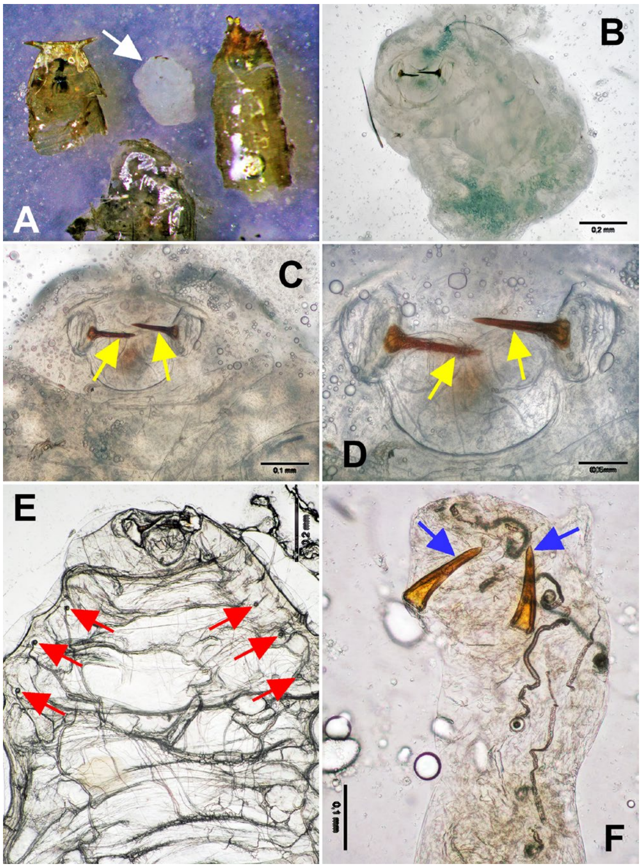

FIGURE 11 | *Trichopria drosophilae*, final instar (light microscopy): (A) Larva isolated from host puparium, (B) Habitus, (C, D) Head capsule, mandibles indicated by yellow arrows, (E, F) Molted skin: spiracles—red arrow, mandibles—blue arrow.

in Figure 2C of Coon et al. (2014) most likely represents a young final instar rather than a second instar. Indeed, the second instar of *T. drosophilae* has several diagnostic characters listed above, chiefly weaker sclerotization and smoother outlines. One of the most distinct characters is the mandibles, which are wide and exodont, unlike those of the first and third instars. The apparently less robust mandibles observed in the second larval instar are consistent with developmental patterns reported across parasitoid Hymenoptera. In many species, the second instar exhibits relatively weak sclerotization and reduced mandible strength compared with the first and especially the third (final) instar, which typically bears heavily sclerotized, functional mandibles adapted for intensive feeding. This trend has been documented in other parasitoids with three larval instars, including chalcidoids (Eulophidae: A. V. Gumovsky 2006, 2007, 2008) and ichneumonoids (Braconidae: Kawakami 1985; Xu et al. 2007), in which the mandibles of the second instar are much less sclerotized than those of the first and third instars. These observations emphasize that size and habitus alone are unreliable indicators for determining the number of larval instars in parasitoids and that distinct morphological characters should be used instead.

Our observations substantially extend the findings of Krüger et al. (2019), who provided valuable quantitative evidence on the influence of female density on oviposition activity and development duration, but inferred superparasitism and intraspecific competition in *T. anastrephae* primarily from numeric data of a laboratory population, without direct examination of immature stages. By documenting the morphology of immatures and parasitoid-host interactions, our studies demonstrate that superparasitism leads to intense larval competition in *T. drosophilae*, culminating in siblicide during the first instar. Importantly, survival was not strictly determined by larval age or size, indicating that competitive interactions are more complex than simple developmental asymmetry. Furthermore, our study provides the first direct evidence of encapsulation-like structures surrounding parasitoid larvae under extreme superparasitism pressure, a phenomenon not detected in previous studies on *Trichopria* parasitoids due to the absence of host dissections (e.g., Krüger et al. 2019).

Beyond general biological interest, the described immature morphology of *T. drosophilae* may be useful for tracing parasitoid biology in mineralized fossils. For example, van de Kamp et al. (2018) revealed fossil diapriid parasitoids within phosphatized fly pupae from the Paleogene of France using high-throughput synchrotron X-ray microtomography. These fossilized endoparasitoids were represented mostly by adults and rarely by pupae or late-instar larval skins. The data on the peculiar morphology of the first

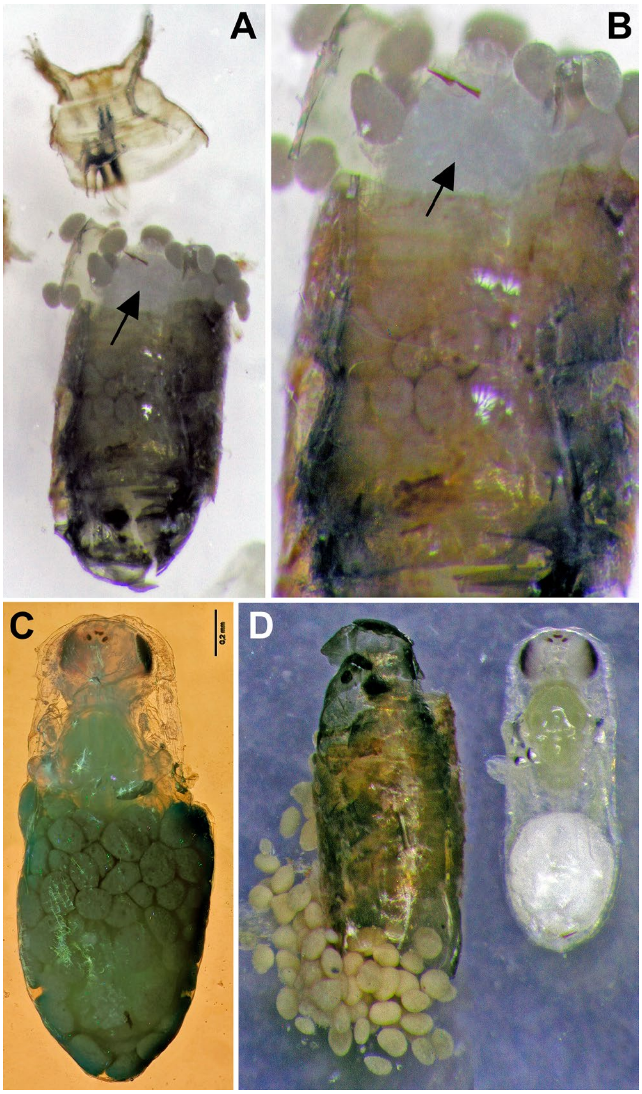

FIGURE 12 | *Trichopria drosophilae*, late immature stages (light microscopy): (A, B) Final instar (black arrowed) in host puparium, (C) Fresh pupa under membrane with meconium pellets, (D) Fresh pupa isolated from host puparium (meconium pellets fell out).

instar of *T. drosophilae* provide additional clues for identifying early instars within fossilized hosts.

Author Contributions

Jana Collatz: conceptualization, methodology, writing – review and editing, validation, resources. **Alex Gumovsky:** conceptualization, methodology, investigation, project administration, supervision, writing – original draft. **Lesia Tymochko:** writing – review and editing, data curation, investigation, methodology. **Lars Krogmann:** methodology, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All relevant data are included within the article.

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