



Functional morphology of the proboscis of the fly *Prosenia siberita* (Diptera, Tachinidae)

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Abstract: Functional morphology of the proboscis of the fly *Prosenia siberita* (Diptera, Tachinidae). The flower-visiting behaviour and mouthpart morphology were studied in the long-proboscid fly *Prosenia siberita* (FABRICIUS, 1775) (Tachinidae) for the first time using light microscopy and scanning electron microscopy. *Prosenia siberita* visits inflorescences to extract nectar. The proboscis consists of the basal rostrum and the long haustellum, which measures about half the body length. In resting position, the conspicuously thin haustellum points forward. The haustellum articulates with the extensible rostrum, where it can be folded downward for feeding. In the proximal haustellum, the u-shaped labrum-epipharynx unit forms the food canal along with the rod-shaped hypopharynx. Both components are surrounded from posterior by the laterally bent up prementum of the labium. In the distal haustellum, the prementum is tubular and forms the food canal; labrum and hypopharynx do not reach this section. At the tip, the short labella are directed forward. The labella form a gap leading to three longitudinal pseudotracheae which merge with the food canal in the prementum. Only a few other representatives of the Tachinidae have proboscises longer than the head. Some of these long-proboscid tachinid flies possess proboscises with long labella, which are foldable backwards. These findings suggest independent evolution of particularly long proboscises within Tachinidae.

Keywords: mouthparts, long-proboscid fly, nectar-feeding, feeding behaviour, flower-visiting insects, Diptera

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Introduction

Many adult flies are frequent and important flower visitors, but the flower-handling and morphology of the feeding organs are rarely studied in detail, even in Central European species (KEVAN & BAKER 1983, PROCTOR et al. 1996, GILBERT & JERVIS 1998, LARSEN et al. 2001). The mouthparts of adult Diptera allow the ingestion of fluids from a variety of food sources including nectar from flowers and blood from various hosts. For these tasks, most flies have a proboscis composed of the trough-like labium, which surrounds the other mouthpart components from posterior, i.e., the labrum-epipharynx and hypopharynx as well as the paired mandibles and maxillae in some taxa (CUMMING & WOOD 2017). Apart from females of blood-sucking nematoceros families and lower

Brachycera, the mandibles are missing and the maxillae are rudimentary, of the latter only the maxillary palpi remain in most flies.

In Brachycera, the labial prementum and the labella, which correspond to the labial palpi are the predominant parts of the proboscis (CUMMING & WOOD 2017). The proboscis operates according to a sponging mode of feeding with the labella at the tip as the primary organs for fluid uptake (KRENN et al. 2005).

In Cyclorrhapha, the mouthparts consist of a basal, moveable unit, called the rostrum, and the haustellum. The latter is composed of the elongated labrum plus the interior epipharynx and hypopharynx, which both form the food canal, as well as a pair of short laciniae in some taxa. All components are enclosed by the prementum bearing the apical labella, where the food canal opens into grooves called pseudotracheae (SCHUHMACHER & HOFFMANN 1982, CUMMING & WOOD 2017). The rostrum is composed of the modified and moveable clypeus, the cibarium unit inside the head and the proximal parts of the labium as well as maxillary rudiments. The rostrum forms the movable connection to the frontal head capsule and bears the maxillary palpi at the distal end. The proximal labium composes the posterior rostrum and is particularly mobile in brachyceran Schizophora and Calyptrata, which comprise Tachinidae among many other fly taxa. The rostrum is connected to the lower cranial margin by a thin cuticle that allows extension and retraction of the proboscis basis under the head (CUMMING & WOOD 2017).

Most flower-visiting Diptera extract nectar from flowers with easily accessible nectaries using a short proboscis (KRENN et al. 2005). However, among many brachyceran families there are species with strikingly long and thin proboscises that evolved independently as an adaptation to long floral tubes with concealed nectar inside (KRENN et al. 2005, BAUDER & KAROLYI 2019). Their morphology has only been studied in detail in a few representatives of Syrphidae (GILBERT 1981, SCHUHMACHER & HOFFMANN 1982), Bombyliidae (SZUCSICH & KRENN 2000, 2002), Nemestrinidae (KAROLYI et al. 2012, 2013), and Tabanidae (KAROLYI et al. 2014). Morphological descriptions of the mouthparts are missing in many other long-proboscid flies, like representatives of Acroceridae, Vermilionidae, Conopidae, Chloropidae and Tachinidae or their feeding organs are studied only superficially (ELZINGA & BROCE 1986, summarized in PROCTOR et al. 1996, KRENN et al. 2005, KRENN 2019). However, the short mouthparts of calyptrate muscoid flies are studied extensively (summarized in CUMMING & WOOD 2017). Detailed studies of the functional morphology and the mechanism of proboscis movements are available for some Calliphoridae (GRAHAM-SMITH 1930, THOMSEN 1977). Since this taxon is part of the same superfamily as Tachinidae (O'HARA et al. 2008, STIREMAN et al. 2018), the results on Calliphoridae can be used for comparisons with tachinid flies.

Although Tachinidae are represented by approximately 600 species in Central Europe (TSCHORSNIG & HERTING 1994) and about 10,000 species worldwide (O'HARA 2008), detailed functional morphological studies of the mouthparts are lacking. Morphological descriptions are limited to line drawings (BECHER 1882, TSCHORSNIG & HERTING 1994, PROCTOR et al. 1996) and the examination of the labellum (ELZINGA & BROCE 1986).

Drawings of the head in the identification key of TSCHORSNIG & HERTING (1994), images published in the phylogenetic study of STIREMAN et al. (2018) and the checklist of Tachinidae (O'HARA et al. 2020) indicate that most species possess a short, fully retractable proboscis. However, some representatives of the subfamilies Tachininae and Dexiinae have a proboscis that is significantly longer than the head and clearly visible in resting position (TSCHORSNIG & HERTING 1994, STIREMAN et al. 2018, O'HARA et al. 2020). In these species, the length of the haustellum and the shape of the labella obviously differs from most other Tachinidae.

In the present study, we use the widespread tachinid fly *Prosenia siberita* (FABRICIUS, 1775) to examine a long-proboscid representative of Dexiini. The flower handling behavior, the proboscis movements and the composition of the proboscis are studied for the first time in a long-proboscid tachinid fly using micromorphological techniques. We deduce the mechanisms of proboscis movements from our findings on mouthpart morphology and feeding behaviour. Finally, we discuss the evolution of a long proboscis within the Tachinidae and compare the functional morphology of the proboscis in *P. siberita* with other anthophilous Diptera.

Material and methods

Sampling sites

The examined specimens of *P. siberita* were collected from meadows in Kaunertal (47.0266° N; 10.740° E) and Fendels (47.0536° N, 10.670° E), Tyrol (Austria), as well as from the vegetation along the banks of the Ill river in Montafon (46.9638° N, 10.075° E), Vorarlberg (Austria). A total of 14 individuals of both sexes were captured and fixed in 80% ethanol.

Observations on the flower-visiting behaviour were conducted mainly on *Knautia arvensis* (Caprifoliaceae) in August 2023 in Kaunertal (Tyrol). Photographic documentation of proboscis movements was done using a Nikon D7200 SLR camera with AF Micro Nikkor 105 mm, 1:2.8 with image series of 6 frames/sec. To get an overview of the nectar host plants in Central Europe, photos were searched on the internet (search term *Prosenia siberita*) showing *P. siberita* on flowers and, if possible, the plant was determined on genus level, see appendix.

Mouthpart anatomy

Anatomical studies on the long fly proboscis were carried out using microphotography, serial semi-thin sections, and scanning electron microscopy. A stereomicroscope (Wild Heerbrugg AG, Heerbrugg) equipped with a drawing mirror was used at 12× and at 50× magnification for light microscopical studies and measurements of ethanol-fixed fly proboscises. The measurements are presented as minimum and maximum values of studied individuals. Images of the fly head and proboscis were taken with a Nikon SMZ 25 microscope using the NIS Elements software (Nikon, Tokyo, Japan). We used the image stacking function to produce depth-focused photomicrographs with magnifications up to 100× magnification.

Scanning electron microscopy (SEM)

The heads of three ethanol-fixed flies were separated from the body and dehydrated in an ascending ethanol series. Subsequently, heads were transferred to hexamethyldisilazane for 15 minutes and air-dried overnight (BOCK 1987). The samples were mounted on SEM specimen holders with double adhesive graphite strips. Conductive silver was used to ensure charge dissipation prior to sputter coating with gold. Samples were imaged with a Philips XL 30 ESEM (Philips, Amsterdam, NL) at 15 kV. Images were stored using the Scandium software (Olympus, Tokyo, Japan).

Serial semi-thin sections

The heads of four fixed flies were dehydrated in an ascending ethanol series, transferred to acetone and finally immersed in Agar Low Viscosity Resin (Agar Scientific, Stansted, United Kingdom). Fly heads were put into silicone molds and covered with resin. To ensure complete infiltration of the resin, samples were put into a vacuum chamber at 40 °C. Afterwards, the specimens were hardened at 60 °C. Serial sections of the proboscis were cut with a Leica UC6 ultramicrotome (Leica, Wetzlar, Germany) and a Histo Jumbo diamond knife (Diatome, Switzerland) at a thickness of 1 µm. Sections were stained with toluidine blue and sealed with Agar Low Viscosity Resin and coverslips. The section preparations were examined with a Nikon Labophot 2 microscope and imaged with a Nikon Eclipse Ni light microscope (Nikon, Tokyo, Japan) and processed with NIS Elements software. Muscle terminology is based on origin and insertion as it is used in the anatomical studies of Bombyliidae (SZUCSICH & KRENN 2000, 2002).

Adobe Photoshop 23.5.5 (Adobe Systems, San Jose, USA) was used to process the brightness and contrast of the micrographs as well as to compile and label the image plates.

Results

Proboscis movements and flower-visiting behaviour

Prosenia siberita is an approximately 10 mm ($n = 14$) long, long-legged, slender fly that consumes nectar from flowers using a long, conspicuously thin proboscis (Fig. 1). The flies were observed to probe the florets of the multi-flowered inflorescences of *Centaurea* sp. (Asteraceae) and *K. arvensis* (Caprifoliaceae) (Fig. 1). Pollen uptake was not observed.

The proboscis is composed of the basal unit (called rostrum) and the long haustellum bearing the short, forward directed labella at the tip (Figs. 1, 2). In resting position, the rostrum lies hidden in a ventro-frontal depression of the head capsule (the oral cavity), while the haustellum points forward and greatly protrudes the head (Fig. 1). The slender haustellum is slightly bent down as it progresses (Fig. 1A); thus, the labella are lowered and point downwards obliquely. To take up nectar, the proboscis tip is inserted into a floret from above (Fig. 1B–E). To achieve this feeding position, the rostrum rotates out of the oral cavity and the haustellum turns into a vertical position, so that the haustellum tip projects downwards. The rostrum elongates the proboscis base during flower probing, depending on the length of the flower tube (Fig. 1D–F). Full elongation of the rostrum increases the total length of the proboscis by approximately the height of the

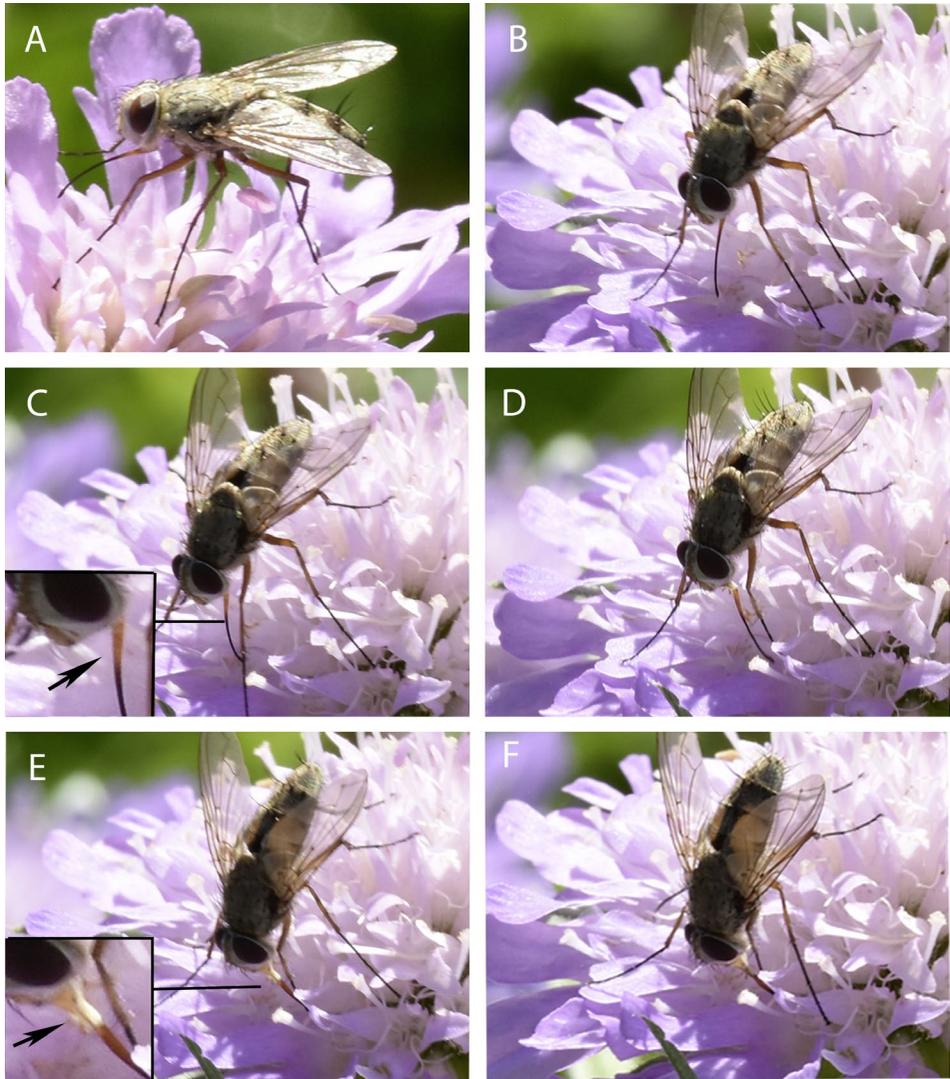


Fig. 1. Flower handling of *Prosema siberita* (Tachinidae) on the inflorescence of *Knautia arvensis* (Caprifoliaceae). A. Proboscis in resting position; haustellum in front of the head; B–F. Movements of the proboscis (image series of 6 images/sec); B–C. Haustellum extends in a downwards position (arrow in inserted magnification); D–E. Elongation of rostrum (arrow in inserted magnification); F. Fly delving into a floret for nectar uptake.

head. In addition, the fly may tilt the body so that the head also dips deeper into the flower (Fig. 1F). This body bend is accomplished by the flexion of the front legs as well as by the extension of the hind legs. The middle legs act as a pivot point, while the head remains immovably attached to the thorax. The sequence of movements is documented in Fig. 1. After extracting nectar, the fly removes the haustellum out of the corolla, turns, and moves to the next floret of the inflorescence.

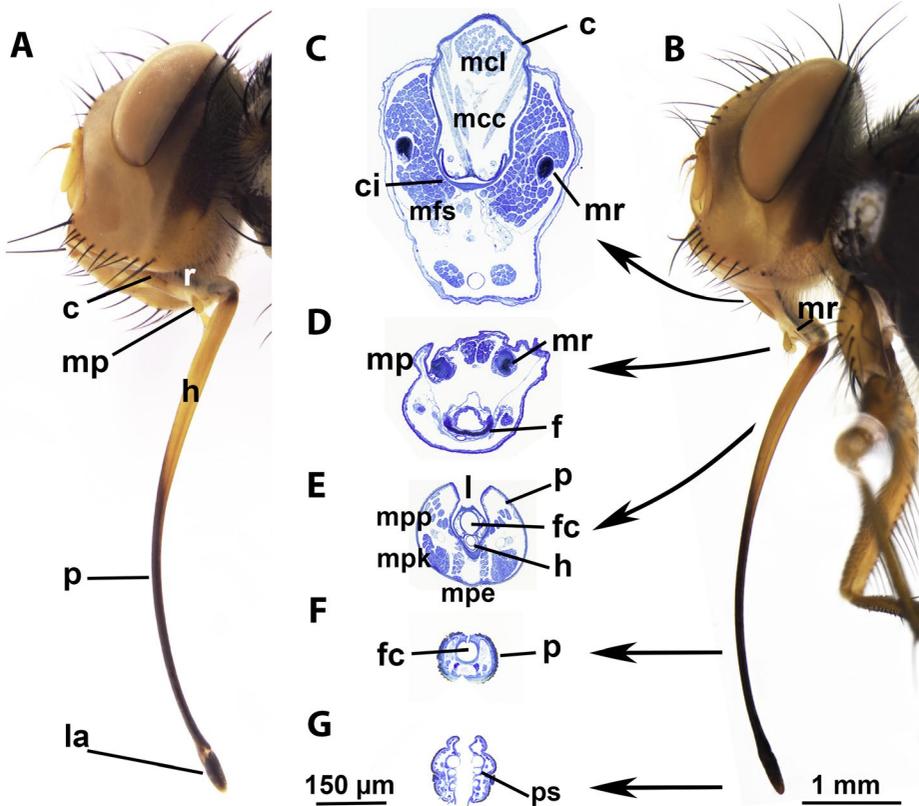


Fig. 2. Proboscis of *Prosema siberita* (Tachinidae). A. Rostrum in resting position, haustellum partly extended; B. Rostrum partly elongated, haustellum further folded down; C-G. Anatomy of rostrum and haustellum, semithin cross sections; C. Proximal rostrum; D. Distal rostrum; E. Proximal haustellum; F. Distal haustellum; G. Labella; c clypeus, ci cibarium, f fulcrum, fc food canal formed by labrum-epipharynx unit, h haustellum, hy hypopharynx, l labrum-epipharynx, la labella, mp maxillary palpus, mr maxillary rod, mcs musculus clypeo-cibarialis, mcl musculus clypeo-labralis, mfs musculus fulcro-stipitalis, mpe musculus praemento-epifurcalis, mpk musculus praemento-kappalis, mpp musculus praemento-paraphysalis, p prementum, ps pseudotracheae

Proboscis length and morphology

The length of the haustellum measures about half of the body length (Fig. 1). The median length of the haustellum is 4.57 mm (N = 9). If the proboscis is extended to its maximal length, another 1–2 mm are added by the elongated rostrum (Fig. 1E, and in some photos from the internet, see appendix). This results in an estimated maximum operational length of the proboscis of at least 6–7 mm.

Articulation of the proboscis to the head

The basal unit of the feeding apparatus is the rostrum, which forms a moveable and extensible connection with the head capsule. The clypeus at the frontal side is the only sclerotized external part, thin cuticle forms the lateral and posterior sides. The proximal end of the clypeus attaches to the frons forming a hinge joint (Fig. 3A). The distal end

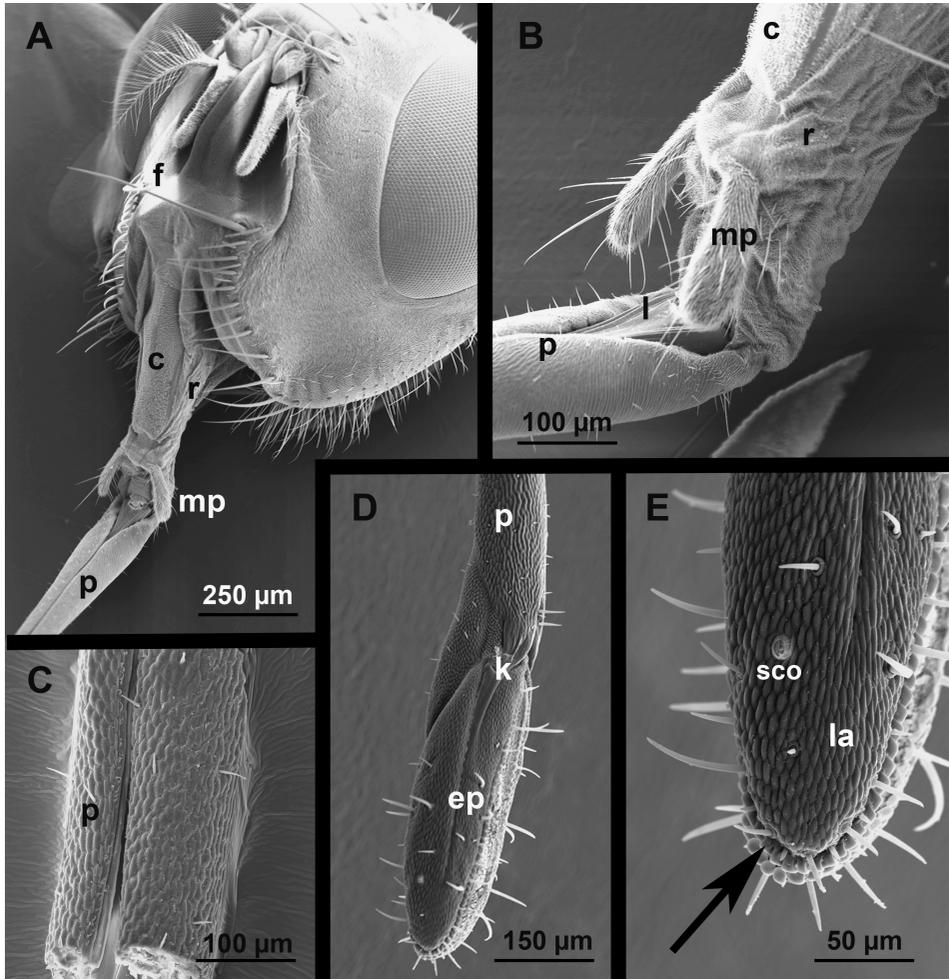


Fig. 3. Head and mouthparts of *Prosenia siberita* (Tachinidae) (SEM); A. Rostrum rotated out of the head cavity, haustellum partly extended (oblique frontal view); B. Articulation of rostrum and haustellum (oblique lateral view); C. Prementum forms haustellum in distal proboscis (oblique dorsal view); D. Labella in direction of the prementum (lateral view); E. Tip of labella (lateral view), arrow indicates the gap between labella; c clypeus, ep epifurca, f frons, k kappa, l labrum, la labellum, mp maxillary palpus, p prementum, r rostrum, sco sensillum coeloconicum

of the clypeus is articulated with the proximal end of the labrum. Laterally and posteriorly, the cuticle of the rostrum has a light color and forms an extensible connection to the head capsule (Fig. 2A, B, 3A, B). The thin cuticle is wrinkled and densely covered with spine-like microtrichia (Fig. 3B). Inside the rostrum, there is a pair of dark, rod-shaped cuticular parts that presumably correspond to rudiments of maxillary basis (Fig. 2B). The cibarium lies under the clypeus and consists of a trough-shaped sclerite, called the fulcrum. Both structures form the expandible preoral cavity of the food pathway (Fig. 2C).

The two maxillary palpi originate from the distal half of the rostrum (Figs. 2A, 3A, B). The one-segmented maxillary palpus is about 180–215 μm long ($N = 10$), club-shaped and weakly bristled. At its tip there are a few conspicuously long bristles that are about half as long as the palpus (Fig. 3B). In the resting position of the proboscis, the maxillary palps are hardly visible in lateral view because they lie hidden under head structures. However, when the haustellum is in the feeding position, the rostrum is elongated and the maxillary palpi are clearly visible under the head. In this position, the light-coloured cuticle of the rostrum stretches and the maxillary rudiments are advanced (Fig. 2B).

To achieve the feeding position, the fronto-clypeal joint rotates the rostrum out of the oral cavity and the articulation of the clypeus and labrum is extended whereby the haustellum tip is lowered. The musculature of the rostrum consists of a pair of short muscles between the genae and the cibarium (musculi geno-cibarialis) and muscles extending from the frons to the proximal end of the fulcrum (musculi fronto-cibarialis). The paired clypeo-labralis muscles lie under the clypeus. They originate from the middle of the clypeus and inserts on the proximal end of the labrum (Fig. 2C). Distal from the musculus clypeo-labralis, a pair of fulcro-stipitalis muscles extend longitudinally from the base of the maxillary rod to the distal half of the cibarium (Fig. 2C). The posterior side of the rostrum contains longitudinal muscles, the paired musculus tergo-labialis that originate from the posterior head capsule and insert at the posterior end of the prementum. In addition, several small clypeal muscles (musculi clypeo-cibarialis) extend to the anterior roof of the cibarium forming a fluid pump in the underlying preoral cavity (Fig. 2C).

Haustellum, the conspicuous part of the proboscis

The haustellum protrudes under the head in all functional positions. The entire haustellum is rigid and cannot be angulated, shortened or lengthened. The haustellum tapers from proximal to distal: Near the head it measures about 280 μm in diameter, while at its distal end the diameter is about 140 μm ($N = 8$). The cuticle of the prementum is wrinkled proximally and is patterned distally with knob-like elevations. The prementum bears numerous sensilla chaetica that vary in length (10–50 μm , $N = 2$) and are sometimes slightly curved (Fig. 3C). In the distal half of the prementum, a longitudinal furrow is visible on each side below the laterally upcurved margins, which presumably interlock and form a tongue-and-groove connection (Fig. 3C).

The haustellum arises from a hinge-like junction at the distal end of the rostrum (Fig. 3A, B). The anterior-dorsal side of the joint forms the labral sclerite, which is hinged to the clypeus. The proximal haustellum is light-coloured and composed of the (1) labrum-epipharynx unit which forms the food canal, (2) the hypopharynx with the salivary duct and (3) the u-shaped prementum which covers both components from posterior (Fig. 2E). In the proximal region, the labrum-epipharynx unit is almost closed and forms the food canal, which is covered ventrally by the hypopharynx (Fig. 2E). Small oblique muscles (musculi labro-epipharyngeales) run within the labrum-epipharynx unit in the proximal portion of the haustellum. The cross sections of the proximal prementum show a nerve, tracheae, and musculature running in a longitudinal direction (Fig. 2E). Three pairs of muscles can

be distinguished: the musculus praemento-paraphysalis, musculus praemento-kappalis, musculus praemento-epifurcalis. Remarkably, the labrum and hypopharynx are only about one-quarter as long as the prementum.

Somewhat proximally of the middle of the haustellum, the cuticle becomes darker and the diameter distinctly thinner (Fig. 2). The distal proboscis consists only of the greatly elongated, very thin prementum, which is circular in cross-section (Fig. 2F). The upturned margins of the prementum form the circular food tube along three-quarters of the haustellum. The food tube measures 35 - 37 μm in diameter. In the distal half, no muscles appear in the cross sections of the prementum. However, parallel to the nerve there are dark colored components that could represent tendons. Presumably, these tendons connect the proximal end of the labella with the muscles in the proximal prementum and allow minor movements of the labella.

Labella at the proboscis tip

The proboscis terminates in the paired labella, which are short and oval. They are 0.5–0.6 mm ($N = 10$) long and arise obliquely at the distal end of the prementum. They are slightly thicker than the distal end of the prementum and appear as a swelling at the apex (Fig. 2). The greatest height is 140–150 μm ($N = 8$). The diameter of both labella is 145–150 μm , whereas the distal end of the prementum measures only 98–125 μm in cross section. The labella are connected to the prementum by an oblique joint, which appears as a narrow, lightly sclerotized articulate membrane (Fig. 2A). A small sclerite, the kappa, is evident on the proximal end of each labellum; the lateral sides show an elongated sclerite called epifurca (Fig. 3D).

The median sides of the labella lie close together. A gap between the two apical parts measures 10–30 μm (Fig. 3E). There are numerous, bristle-shaped sensilla on the labellum, which are 36–54 μm ($N = 9$) long (Fig. 3D, E). Some of the bristles are longitudinally grooved. Just behind the apex, there is a single, conspicuous sensillum coeloconicum on the lateral labellum (Fig. 3F). The inner surface of the labellum is lined with a rough, but thin cuticle. The surface is densely furnished with multi-pointed microtrichia, which also cover the pseudotracheae. Transverse sections show three pseudotracheae on the median side of each labellum (Fig. 2G). These small half-tubes open at the apical end of the labellum. They measure about 7–10 μm in cross-section apically and become thicker proximally measuring 20–25 μm ($N = 2$). They unite at the base of the labella to form four circularly arranged pseudotracheae, which merge into the feeding tube formed by the prementum.

Discussion

Proboscis length and flower choice

Adult flies out of 50 families have been observed to feed nectar from flowers (KEVAN & BAKER 1983, PROCTOR et al. 1996, GILBERT & JERVIS 1998, LARSEN et al. 2001). At the same time, only 9 dipteran families are known to comprise species with remarkably long mouthparts specialized for nectar extraction (GILBERT & JERVIS 1998,

KRENN et al. 2005). Detailed morphological examinations of long proboscises exist only for some Syrphidae (GILBERT 1981, 1985, SCHUHMACHER & HOFFMANN 1982, SCHIEMENZ 1957), Bombyliidae (SZUCSICH & KRENN 2000, 2002), and single species of Nemestrinidae (KAROLYI et al. 2012, 2013) and Tabanidae (DIERL 1968, KAROLYI et al. 2014).

Most Central European flies can be observed on flowers where the nectaries are easily accessible and can be exploited by dabbling movements of a short proboscis and spread labella (PROCTOR et al. 1996). Such sponging-feeding mouthparts are equipped with broad cushion-shaped labella and represent the ancestral feeding organs in adult Diptera, while elongated proboscises are derived. Evolution of long proboscises that significantly exceed the height of the head occurs in some piercing blood-feeding flies and nectar-feeding taxa of Diptera (KRENN ET AL. 2005, KRENN & ASPÖCK 2012, KRENN 2019, BAUDER & KAROLYI 2019, BARTON et al. 2023). In nectarivorous flies, long and siphoning proboscises represent adaptations to long nectar spurs or deep flower tubes with nectaries at the base. In most taxa, such nectar-extracting organs are characterized by an elongated haustellum and slender labella with a small number of pseudotracheae (ELZINGA & BROCE 1986, GILBERT & JERVIS 1998, KRENN et al. 2005).

Within Tachinidae, illustrations of various flies show that most species possess a short proboscis that is completely retractable under the head (PROCTOR et al. 1996, STIREMAN et al. 2018, O'HARA et al. 2020). Short-proboscid representatives tend to take up honeydew and easily accessible nectar from open flowers (ALLEN 1929, MENZEL & ZIEGLER 2001), whereas tachinid flies with a proboscis that exceeds the height of the head suck nectar mainly from deep flowers with concealed nectar (GILBERT & JERVIS 1998). The present observations in *P. siberita* show that this fly mainly visits multi-floral inflorescences with rather small florets, although the proboscis is at least half as long as the body. A comparison with images of flower-visiting flies of this taxon likewise indicates that they forage mostly on inflorescences with small, funnel-shaped individual flowers like those of Asteraceae, Caprifoliaceae, and Lamiaceae as well as open florets of Apiaceae (for a list of plant species visited by *P. siberita*, see appendix). By contrast, long-proboscid representatives of Bombyliidae, Tabanidae or Nemestrinidae are specialized on long-tubed single flowers (PROCTOR et al. 1996, SZUCSICH & KRENN 2002, KAROLYI et al. 2013, 2014, KRENN et al. 2021). Representatives of the latter taxa may act as specialized and important pollinators of their nectar host plants in the Cape Floristic Region of Southern Africa (GOLDBLATT & MANNING 2000, PAUW 2022).

Prosenia siberita flies are generalist flower visitors that exploit various flowers with its extended proboscis from above. The flower-handling behavior is characterized by vertical movements of the proboscis into the small florets of the inflorescence, like the behaviour of short-proboscid Brachycera (PROCTOR et al. 1996, KRENN et al. 2005). Although *P. siberita* usually moves over the inflorescences to extract nectar from the single florets there is no indication for transfer of pollen grains onto the body. Therefore, we conclude that *P. siberita* does not pollinate its nectar host plants. Furthermore, there is no indication for pollen-feeding as it is known for many flower-visiting Syrphidae, which regularly take up and ingest pollen with the labella (GILBERT 1981, 1985).

Morphology and functional mechanism of the proboscis

The proboscis of *P. siberita* is characterized by the following traits: (1) the proboscis is very thin, exceeds half of the body length and bends downwards to the tip; (2) labrum and hypopharynx are short and restricted to the proximal quarter of the haustellum; (3) the prementum is tubular in the distal three quarters of the haustellum and solely forms the circular food canal; (4) the short, barely mobile labella point forward and possess only few pseudotracheae.

The morphology and movements of the mouthparts of *P. siberita* allow conclusions on the mechanics of the proboscis movements. We found that the anatomy of the rostrum and the basis of the haustellum is similar to other Calyptrata. Therefore, we conclude that the rotation movement of the rostrum and the extension motion of the haustellum can be explained by the same principle as in Bombyliidae (SZUCSICH & KRENN 2000, 2002) and Calliphoridae (GRAHAM-SMITH 1930, THOMSON 1977, VAN DER STARRE & RUIGROK 1980). The rostrum rotates at the hinge joint of the clypeus and frons, thus stretching this basal unit of the mouthparts. At the same time, the haustellum is extended at the joint between labrum and clypeus. The movements of the clypeus-cibarium unit of the rostrum are presumably achieved by the antagonistic head muscles that insert on the proximal region of the cibarial fulcrum, as in the Bombyliidae and Calliphoridae (KRENN & SZUCSICH 2000, 2002, GRAHAM-SMITH 1930). Down-folding of the haustellum at the hinge joint to the clypeus is probably achieved by the movements of the maxillary rods, which are probably homologous to the stipes. Despite the absence of the laciniae in Tachinidae, muscles of the maxillae seem to be responsible for the haustellum extension and bending, as it was found in Bombyliidae and Nemestrinidae (SZUCSICH & KRENN 2000, 2002, KAROLYI et al. 2012). The elongation of the rostrum is substantial and increases the reach of the proboscis tip. In this position of the proboscis, the maxillary palpi are clearly visible under the head. Their downward movements indicate that the maxillary rudiments are involved in the extension of the haustellum, as it has been concluded from the functional anatomy of the proboscis of Bombyliidae (SZUCSICH & KRENN 2000, 2002). Despite the restriction of the maxillary rudiments to the rostrum in *Prosenia* in contrast to Bombyliidae, it is likely that the contraction of the muscles running from the labrum basis to the maxillary structures move the haustellum in feeding position.

In contrast to *Cyclorrhapha* with a short proboscis, the labella of *Prosenia* cannot be fully spread for sponging up fluid. However, some mobility of the labella is likely retained, since we found tendons, which extend to the labella sclerites. Muscles inside the prementum probably open the gap between the labella. This movement probably improves active nectar uptake by expanding the space between the labella in a similar fashion as it was shown for the glossal hairs of honeybees (WU et al. 2015). In addition, we assume that the median sides of the labella, the pseudotracheae, and the food canal are hydrophilous promoting fluid ingestion like in houseflies (LEHNERT et al. 2017).

Tachinidae with a long proboscis

In most Tachinidae, the proboscis is shorter than the head and can be folded in z-shape under the head in resting position, where the broad labella lie close to the head capsule

(PROCTOR et al. 1996, TSCHORSNIG & HERTING 1994, STIREMAN et al. 2018, O'HARA et al. 2020). However, in *P. siberita* the proboscis is directed forward and protrudes the head remarkably, and the short labella point forward in all positions. A similar proboscis morphology can be found in some long-proboscid representatives of the subfamily Dexiinae, like in species of *Prosenoides*, *Chaetogyne*, *Imitomyia*, *Jurino-dexia*, *Mochlosoma*, and *Nimioglossa*. Photos show that all these flies have a long, thin, forward pointing haustellum and small labella (O'HARA et al. 2020). However, within the Tachininae two morphologies of long proboscises can be seen: The species of the genera *Adejeania*, *Deopalpus*, *Jurinella*, *Pararchydas*, *Peleteria*, and *Protodejeania* show similar proboscis morphology as *P. siberita*, but in contrast to the Dexiinae all have long maxillary palpi (O'HARA et al. 2020), whereas the fly species from the genus *Siphona* have a proboscis characterized by long labella which are folded backwards under the head in resting position (TSCHORSNIG & HERTING 1994, ELZINGER & BROCE 1986). Based on these morphological differences and since long-proboscid Tachinidae occur in different subfamilies (CERRETTI et al. 2014, STIREMAN et al. 2019), we conclude that their long proboscis evolved independently multiple times within the Tachinidae. However, comparative studies of the functional morphology of these tachinid species are missing.

Long proboscises in other flower-feeding insects

The proboscis of *Prosenia* is characterized by a changing composition of mouthpart components in the proximal and the distal haustellum regions. Such a complex proboscis has also been studied in *Prosoeca* (Nemestrinidae), *Philoliche* (Tabanidae) (KAROLYI et al. 2012, 2013, 2014) but also occur in some Conopidae (KRENN et al. 2005, KRENN 2019). In all these cases, the food canal in the distal region of the proboscis is composed only of the prementum. This striking change of the haustellum composition was found within the Calyptrata for the first time in this study. It represents a remarkable case of convergent evolution that led to a particularly thin fly proboscis, which is adapted to extract concealed nectar out of very thin floral tubes. Such a simple one-part composition of the food canal was found elsewhere only in the Masarinae (Vespidae). These rather small flower-visiting Hymenoptera possess a particularly thin, siphoning proboscis that is composed of the tubular glossa only. It is retractable in a loop into the head for its resting position (KRENN et al. 2002, KRENN 2019). All other long-proboscid flower-visiting insects possess a proboscis which is composed of either two elongated components, as in glossatan Lepidoptera (e.g. KRENN 2010) and meloid beetles (WILHERMI & KRENN 2012); or it includes five single mouthpart components, like in Apoidea and some other Apocrita (KRENN et al. 2005). In long-tongued bees, the hairy glossa functions like a tongue, which can be further protruded to load nectar between glossa hairs, while the other proboscis components form the food canal around it. The food canal enables fluid transport into the mouth (SNODGRASS 1956, KRENN et al. 2005, WU et al. 2015, DÜSTER et al. 2018, SHI et al. 2020). In other long-proboscid flies, for example from the Acroceridae or Vermileonidae five or six structures form the haustellum and seem elongated (KRENN et al. 2005). However, detailed morphological studies of species of these families are missing yet.

Zusammenfassung

Bau und Funktion der Mundwerkzeuge der langrüsseligen Raupenfliege *Prosenia siberita* (FABRICIUS, 1775) (Tachinidae) wurden mit Licht- und Elektronenmikroskopie erstmals untersucht. *Prosenia siberita* besucht Blütenstände, aus deren Einzelblüten Nektar gesaugt wird. Der Rüssel besteht aus der Rüsselbasis (Rostrum) und dem auffällig langen Haustellum, welches an der Spitze die Labellen trägt. Das Haustellum ist etwa halb so lang wie der Körper und gelenkig mit dem Rostrum verbunden. In Ruhestellung ist das Haustellum nach vorne gestreckt. Zur Nahrungsaufnahme wird dieses nach unten geklappt und das Rostrum verlängert. Im proximalen Viertel besteht das Haustellum aus Labrum-Epipharynx, das zusammen mit dem stabförmigen Hypopharynx das Nahrungsrohr bildet, sowie dem Prämentum, welches alle Teile von posterior umfasst. Im distalen Abschnitt besteht das Haustellum nur aus dem röhrenförmigen Prämentum, welches das dünne Nahrungsrohr bildet; Labrum und Hypopharynx sind nicht so stark verlängert und fehlen im distalen Rüssel. Die Rüsselspitze besteht aus den kurzen, nach vorne gerichteten Labellen. Diese bilden einen schmalen apikalen Spalt, der zu drei längs verlaufenden Pseudotracheen führt, welche ins Nahrungsrohr münden. Nur wenige andere Vertreter der Tachinidae besitzen einen Rüssel, der länger als der Kopf ist. Bei einigen dieser langrüsseligen Arten sind jedoch die Labellen lang und können zurückgeklappt werden, was darauf hinweist, dass auffällige Verlängerungen des Saugrüssels bei Tachinidae mehrfach unabhängig entstanden sind.

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Appendix

List of 10 nectar host plants of *Prosenia siberita*, extracted from internet sources, January 25, 2024

1. *Cirsium arvense* (Asteraceae)
Wikipedia: Tachinidae - Prosenia siberita-001.jpg|thumb|Tachinidae - Prosenia siberita-001
<https://swiatmakro.com/2015/07/23/szarnica-lakowa-prosenia-siberita-warszawianka>
<https://www.insekten-sachsen.de/pages/TaxonomyBrowser.aspx?ID=144450>
<https://inaturalist.lu/taxa/522328-Prosenia-siberita>
2. *Cirsium* sp. (Asteraceae)
<https://www.insecte.org/forum/viewtopic.php?t=172859;joyce30;23.5.13;Bouillargues30230>
3. *Erigeron annuus* (Asteraceae)
<https://inaturalist.lu/taxa/522328-Prosenia-siberita>
4. *Senecio* sp. (Asteraceae)
<https://inaturalist.lu/taxa/522328-Prosenia-siberita>
https://www.preboggion.it/Insecta_SP_Prosenia_siberita.htm
5. *Centaurea* sp. (Asteraceae)
<https://www.izeltlabuak.hu/talalat/13175>
<https://www.sydhavnstippen.dk/2021/10/fluor-i-sommerfugleengen/>
<https://inaturalist.lu/taxa/522328-Prosenia-siberita>
6. *Solidago virgaurea* (Asteraceae)
<https://www.gbif.org/species/1472219>
7. *Knautia* sp. (Dipsacaceae)
<https://tachinidae.myspecies.info/taxonomy/term/24>
<https://inaturalist.lu/taxa/522328-Prosenia-siberita>
<https://insektarium.net/diptera-2/tachinidae-raczycowate/prosenia-siberita-szarnica-lakowa/>
<https://www.naturbasen.dk/observation/2355672/prosenia-siberita>
http://danskessvampe.dk/?page_id=9583

8. *Scabiosa* sp. (Dipsacaceae)
<https://www.naturbasen.dk/observation/2355672/prosena-siberita>
9. *Mentha* sp. (Lamiaceae)
https://www.preboggion.it/Insecta_SP_Prosema_siberita.htm
10. Apiaceae
<https://www.gbif.org/species/1472219>

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