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Evolutionary functional morphology of the proboscis and feeding apparatus of hawk moths (Sphingidae: Lepidoptera)

Caroline Reinwald | Julia A.-S. Bauder | Florian Karolyi | Michael Neulinger | Sarah Jaros | Brian Metscher | Harald W. Krenn

Department of Evolutionary Biology, University of Vienna, Vienna, Austria

Correspondence
Harald W. Krenn, Department of Evolutionary Biology, University of Vienna, Djerassiplatz 1, Vienna 1030, Austria.
Email: harald.krenn@univie.ac.at

Abstract

The morphology of the proboscis and associated feeding organs was studied in several nectar-feeding hawk moths, as well as a specialized honey-feeder and two supposedly nonfeeding species. The proboscis lengths ranged from a few millimeters to more than 200 mm. Despite the variation in proboscis length and feeding strategy, the principle external and internal composition of the galeae, the stipes pump, and the suction pump were similar across all species. The morphology of the smooth and slender proboscis is highly conserved among all lineages of nectar-feeding Sphingidae. Remarkably, they share a typical arrangement of the sensilla at the tip. The number and length of sensilla styloconica are independent from proboscis length. A unique proboscis morphology was found in the honey-feeding species *Acherontia atropos*. Here, the distinctly pointed apex displays a large subterminal opening of the food canal, and thus characterizes a novel type of piercing proboscis in Lepidoptera. In the probably nonfeeding species, the rudimentary galeae are not interlocked and the apex lacks sensilla styloconica; galeal muscles, however, are present. All studied species demonstrate an identical anatomy of the stipes, and suction pump, regardless of proboscis length and diet. Even supposedly nonfeeding Sphingidae possess all organs of the feeding apparatus, suggesting that their proboscis rudiments might still be functional. The morphometric analyses indicate significant positive correlations between galea lumen volume and stipes muscle volume as well as the volume of the food canal and the muscular volume of the suction pump. Size correlations of these functionally connected organs reflect morphological fine-tuning in the evolution of proboscis length and function.

KEYWORDS

insects, mouthparts, sensilla, suction pump

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1 | INTRODUCTION

Sphingidae are among the most fascinating insects as many of them are impressively large, exhibit powerful flight maneuvers, including hovering during nectar feeding, and are involved in the pollination of numerous remarkable plants. Therefore, it is no surprise that this group of Lepidoptera has received ongoing attention in insect science and has been mentioned in the earliest stages of evolutionary biology (Darwin, 1862; Müller, 1873). Today, Sphingidae are famous models for morphological co-adaptations of insects and flowers (e.g., Arditti et al., 2012; Miller, 1997; Nilsson, 1988, 1998; Wasserthal, 1998), pollination biology (e.g., V. Grant & Grant, 1983; Houlihan et al., 2019; Johnson & Raguso, 2016; Nilsson et al., 1985), mutualistic insect–plant networks (Johnson et al., 2017; Sazatornil et al., 2016), sensory physiology of flower-visiting behavior (e.g., review Stöckl & Kelber, 2019), and biophysics of fluid-feeding (Kornev et al., 2017). Many Sphingidae are critical for the pollination of specialized sphingophilous flowers, where some plant species with particularly deep flowers depend on pollination by sphingid moths with a matching proboscis (e.g., V. Grant & Grant, 1983; Houlihan et al., 2019; Johnson et al., 2017; Nilsson, 1988; Wasserthal, 1997).

Hawk moths include over 1700 species worldwide and are most species-rich in the tropics (Kitching, 2022). The most recent phylogenetic analysis, including more than 130 hawk moth species and sequences from five protein-coding nuclear genes, corroborated morphological evidence for the monophyly of the Sphingidae and supported the basal divergence between Smerinthinae (s.s.) + Sphinginae and Macroglossinae (Kawahara et al., 2009). Based on this phylogeny, the taxon sampling of the present comparative study covers all high-rank taxa and includes European as well as American species.

The Lepidopteran proboscis is a prime example for a long, tube-shaped, siphoning organ that evolved for the uptake of concealed floral nectar. Regardless of variations in length, the proboscis possesses the same principle composition in most glossatan Lepidoptera (e.g., Krenn, 2010, 2019; Krenn & Kristensen, 2000, 2004; Scoble, 1995). The mouthparts of adult Lepidoptera entail a small labrum covering the base of the proboscis. The labrum possesses lateral lobes, the piliferes, which bear bristles on their lateral edges that contact the galeal base on the dorsal side (Krenn & Kristensen, 2000). The mandibles are reduced in all glossatan Lepidoptera. Two greatly elongated galeae of the maxillae form the proboscis and enclose the central food canal via ventral and dorsal coupling. Cuticular structures, the ventral legulae, form double hooks and act as a stable junction between the two galeae along the total length of the proboscis. Dorsal plate-shaped legulae overlap each other, thereby closing the food canal dorsally, except in the most distal region (Krenn & Kristensen, 2000). Close to the apex, the dorsal legulae form slits into the food canal referred to as drinking slits. The cuticle of the drinking region has hydrophilic properties that promote the ingestion of fluid into the food canal (Lehnert et al., 2013, 2017; reviewed in Lehnert & Wei, 2019). Each galea contains sensilla, a trachea, a nerve, and a basal galeal muscle as well as numerous intrinsic muscles inside the lumen (e.g., Eastham & Eassa, 1955; Eaton, 1971; Krenn, 1990; Krenn & Kristensen, 2004). The basal parts of the maxillae form a hemolymph pump, the stipes pump, which is responsible for uncoiling the proboscis from its coiled resting position via a hydraulic mechanism (Bänziger, 1971; Krenn, 1990, 2010; Wannenmacher & Wasserthal, 2003). Coiling of the proboscis is achieved by the contraction of the intrinsic intragaleal musculature, as has been verified by electrophysiological studies with sphingid moths (Wannenmacher & Wasserthal, 2003). The labium forms a triangular plate on the ventral side of the head and bears bristle-covered, three-segmented labial palpi. They are equipped with an apical sensory pit-organ that is involved in CO_2-perception in some hawk moths (Guerenstein et al., 2004). In two high-ranked taxa, the piliferes and the labial palps are functionally coupled to form ultrasound-sensitive hearing organs (Göpfert & Wasserthal, 1999; Göpfert et al., 2002; Roeder, 1972; Roeder et al., 1968, 1970).

The sensilla equipment of the lepidopteran proboscis has previously been studied in multiple taxa. A review by M. J. Faucheux (2013) provides an overview of various sensilla types and discusses their functions. The surface of the galeae bears several morphological types of sensilla: bristle-shaped sensilla, that is, sensilla chaetica (termed as sensilla trichodea by Krenn, 1998); sensilla basiconica equipped with a peg-shaped sensory cone (with various subtypes according to pore numbers); sensilla styloconica composed of a variously sculptures stylus and a short sensory cone; sensilla coeloconica, which are positioned in deep cavities of the cuticle; rarely found sensilla filiformia with long thin bristles and flat sensilla campaniformia. At least four sensilla types have been described in some hawk moths (Faucheux, 2013; Krenn & Kristensen, 2000). Furthermore, a generalized pattern of the arrangement of different sensilla has been identified on the proboscis of Glossata (Krenn & Kristensen, 2000). Bristle-shaped sensilla chaetica occur throughout the entire length of the external galeae and decrease in length toward the distal end. Sensilla basiconica are arranged in rows on the outside of the galeal surface and in one row inside the food canal. Short sensilla coeloconica in cuticle pits have been described in some Sphingidae and Papilionoidea (Faucheux, 2013; Ma et al., 2019). Sensilla styloconica occur only in the distal half of the galeae in Myoglossata (Faucheux, 2007, 2008, 2013; Krenn & Kristensen, 2000). They may display a great morphological diversity in some Lepidopteran taxa (Krenn et al., 2001; Ma et al., 2019; Petr & Stewart, 2004). In addition to their function as combined mechano-chemosensilla, they have been proposed to form brush-like nano-sponges in the drinking region of certain species which do not feed on floral nectar (Krenn et al., 2001; Lehnert et al., 2016; Lehnert & Wei, 2019; Molleman et al., 2005).

Although mouthparts are indispensable for feeding, the morphology of the proboscis and the feeding apparatus as a whole has not yet been studied comparatively in Sphingidae. Proboscis length varies greatly within the Sphingidae and ranges from only 1 mm in nonfeeding imagines to 280 mm; the recorded maximum for a flower-visiting insect (Amsel, 1938; Kitching & Cadiou, 2000). Unique honey-feeding behavior is confirmed for *Acherontia* species (Dvořák, 2017; Kitching, 2003; Moritz et al., 1991). These cleptoparasitic hawk moths are known to invade beehives of *Apis mellifera* and are capable of penetrating sealed honeycombs (Ebert et al., 1994; Kitching, 2003). Many species of the Smerinthinae on the other hand, are reported to have a reduced proboscis (Ebert et al., 1994). Since there have been no observations of flower-visiting behavior to date, they are suspected not to feed on nectar.

Like the proboscis, the cibarial suction pump is essential for fluid uptake. This large muscular organ has been particularly well studied in terms of morphology and neuroanatomy based on the sphingid *Manduca sexta* (Davis & Hildebrand, 2006). In all Lepidoptera, its lumen is connected continuously with the food canal. Fluid is sucked into the cibarium and pumped further into the esophagus through the consecutive contractions of dilator and contractor muscles, respectively (Socha et al., 2007). In *Acherontia atropos*, the suction pump is also involved in sound production. In this functional context, it generates pressure differences, resulting in the suction and release of air through the proboscis (Brehm et al., 2015).

The proboscis, the stipes pump at the maxillary base and the suction pump inside the head capsule compose the lepidopteran feeding apparatus. This set of organs is functionally integrated, as it is crucial for proboscis movements, food uptake, and fluid transport into the alimentary tract. Previous morphometric comparisons of these organs have suggested morphological fine-tuning of proboscis lengths in various butterfly taxa (Bauder et al., 2013, 2015; Krenn & Bauder, 2017), but has not yet been studied in hawk moths. Sphingidae are especially diverse as this taxon encompasses species with an extremely long proboscis as well as species with a rudimentary proboscis, which are believed to not take up food as adults. Hence, this taxon provides the opportunity to study mouthpart evolution under functional aspects as well as the reduction of organs that have lost their primary function. The aim of this study is to compare the morphology of the feeding apparatus in representatives of all high-rank taxa of Sphingidae. The external and internal proboscis morphology, as well as the stipes and the suction pump, are examined in different nectar-feeding species displaying various proboscis lengths, one honey-feeding species as well as hawk moths with short mouthparts and reduced feeding abilities.

2 | MATERIAL AND METHODS

2.1 | Studied species

The taxon sampling followed the high-rank systematics of Sphingidae including 14 species (Table 1) with various proboscis lengths from all tribes except for the paraphyletic Spingulini (Kawahara et al., 2009). The studied neotropical species were collected by F. Bodmer from August 2011 to November 2012 in Ecuador (Research permits: N°. 016-IC-FAU-DPL-MA, N°. 023-2011-IC-FLO-DPL-MA; export permits: N° 001-2012-IC-FAU-DPL-MA). Samples were collected in the Reserva Biológica San Francisco, adjacent to Podocarpus National Park in the province Zamora-Chinchipe, located on the eastern slope of the Andes. This area is mostly covered by nearly pristine mountain rain forest and by various types of succession vegetation after anthropogenic interventions (Beck et al., 2008). Hawk moths were collected from the lit windows of the Estación Científica San Francisco and by light trapping in the adjacent forest between 1820 and 1900 m asl (3°58.3′S, 79°4.7′W). The European species were sampled in the surroundings of Vienna and Carinthia (Austria) by J. B. or obtained from breeders in Austria and from London Pupae Supply Ltd. The individuals of *A. atropos* and *Smerinthus ocellata* are a donation of M. Staggl who reared them in captivity in Vienna during 2020 and 2021.

The species were all assigned to one of three feeding categories: (1) nectar-feeding species with a long or very long proboscis, (2) honey-feeding, and (3) probably nonfeeding hawk moths with a greatly reduced proboscis length (references are given in Table 1).

Body lengths were measured using a caliper before fixing the specimens in Formalin-Aceto-Alcohol (FAA) and storing them in 80% ethanol (EtOH). The FAA was prepared by mixing concentrated formalin (=35%), 80% EtOH, and concentrated acetic acid (3:10:1). One or two individuals of each species were selected for scanning electron microscopy (SEM). Different specimens were used for the light microscopical analyses. One individual per species was studied using microCT to assess the galea- and stipes anatomy as well as the dimensions of the suction pump, with *Neococtytius cluentius* as an exception. Due to the time-consuming methodology, *Pachylia ficus*, *Sphinx pinastri*, *S. ocellata*, and *Macroglossum stellatarum* were not included in the analysis of the galea anatomy; only 10 species were analyzed in this regard (Table 1).

2.2 | Light microscopy and biometry of external features

Proboscis lengths were determined based on microscopic slides of the galeae or in microCT images. Measurements were taken on the longitudinal axis of the proboscis. The number of coils was measured on preserved specimens before further preparation for SEM or using microCT images. Photos of the heads were made using a Nikon SMZ25 stereomicroscope and NIS imaging software (Nikon). The length of the drinking region was measured using microscopic slides. The numbers of sensilla styloconica and sensilla basiconica of the food canal were counted on microscopic slides of the galeae. Sensilla types were identified based on their external morphology according to the review of M. J. Faucheux (2013). EtOH preserved specimens as well as one pinned specimen from the insect collection (Faculty of Life Sciences, University of Vienna) were used for the preparation of the proboscis slides. Proboscises were removed with small scissors, kept in 30% lactic acid for several days, and separated into two galeae before glycerin embedment on glass slides. The galeae were positioned in such a way that the inner side displaying the food canal and the outer side could be inspected in the same specimen. Individual sensilla lengths and numbers were determined using a Nikon Laborphot 2 equipped with a camera lucida and a Nikon

TABLE 1 Studied Sphingidae, numbers, origin, applied methods (LM, SEM, µCT, and histology using semithin sections), and feeding preferences

Species	*N* studied individuals	Origin	Study method	Feeding strategy (references)
Smerinthinae				
Protambulyx strigilis (Linnaeus, 1771)	3	Ecuador, RBSF (F. Bodmer)	LM, SEM, µCT, semithin sections	Nectar-feeding (Danaher et al., 2020)
Mimas tiliae (Linnaeus, 1758)	3	Klopeinersee (Carinthia, Austria)	LM, SEM, µCT, semithin sections	Probably nonfeeding (Ebert et al., 1994)
Smerinthus ocellata (Linnaeus, 1758)	3	Breeder M. Staggl (Vienna)	LM, SEM, µCT	Probably nonfeeding (Ebert et al., 1994)
Sphinginae				
Neococytius cluentius (Cramer, 1775)	3	Ecuador, RBSF (F. Bodmer)	LM, µCT, semithin sections	Nectar-feeding (Haber & Frankie, 1989)
Sphinx pinastri (Linnaeus, 1758)	3	Insect collection Faculty of Life Science, Vienna	LM, SEM, µCT,	Nectar-feeding (Ebert et al., 1994)
Euryglottis paper (Boisduval, 1875)	2	Ecuador, RBSF (F. Bodmer)	LM, SEM, µCT, semithin sections	Nectar-feeding (Nattero et al., 2003)
Manduca scutata (Rothschild & Jordan, 1903)	3	Ecuador, RBSF (F. Bodmer)	LM, SEM, µCT, semithin sections	Nectar-feeding (genus *Manduca*: V. Grant & Grant, 1983; Haber & Frankie, 1989)
Agrius convolvuli (Linnaeus, 1758)	3	Lower Austria, insect collection Faculty of Life Science, Vienna	LM, SEM, µCT, semithin sections	Nectar-feeding (Ebert et al., 1994)
Acherotia atropos (Linnaeus, 1758)	4	Breeder M. Staggl (Vienna)	LM, SEM, µCT, semithin sections	Honey-feeding (Ebert et al., 1994)
Macroglossinae				
Pachylia ficus (Linnaeus, 1758)	3	Ecuador, RBSF (F. Bodmer)	LM, SEM	Nectar-feeding (Danaher et al., 2020)
Eumorpha triangulum (Rothschild & Jordan, 1903)	2	Ecuador, RBSF (F. Bodmer)	LM, SEM, µCT, semithin sections	Nectar-feeding (Danaher et al., 2020)
Macroglossum stellatarum (Linnaeus, 1758)	2	Lower Austria, insect collection Faculty of Life Science, Vienna	LM, SEM	Nectar-feeding (Ebert et al., 1994)
Deilephila elpenor (Linnaeus, 1758)	3	London Pupae Supply Ldt.	LM, SEM, µCT, semithin sections	Nectar-feeding (Ebert et al., 1994)
Xylophanes pyrrhus (Rothschild & Jordan, 1906)	3	Ecuador, RBSF (F. Bodmer)	LM, SEM, µCT, semithin sections	Nectar-feeding (genus *Xylophanes*: Haber & Frankie, 1989; Sazima et al., 1994)

Note: The insect collection of the Faculty of Life Sciences (University of Vienna) includes ethanol-fixed specimens sampled in Europe.

Abbreviations: LM, light microscopy; SEM, scanning electron microscopy; µCT, x-ray microtomography.

Eclipse E800 light microscope (Nikon). All length measurements were taken from the base to the apex of the sensillum.

2.3 | SEM

For SEM imaging, the proboscises were removed from individuals that had previously been fixed in FAA for several days and then stored in 80% EtOH. All samples were dehydrated with an ascending EtOH series (1 ×9 6%, 3 × 100%, 30 min/step), submerged in hexamethyldisilazane and air dried overnight (Bock, 1987). They were then mounted on stubs fitted with graphite plates and coated with gold. Scanning electron micrographs were taken on a Philips XL30 ESEM (Philips) at 15 kV. Images were taken using the Scandium software (Olympus). One or two specimens per species were studied.

2.4 | Microcomputed tomography

Specimens were fixed in FAA and stained with 1% iodine in EtOH. Only *S. ocellata* and *M. stellatarum* were fixed in 70% EtOH. MicroCT scans of 12 species (Table 1) were either performed with an Xradia MicroXCT imaging system (Carl Zeiss X-Ray Microscopy; Pleasanton) at the Vetcore Facility Imaging Unit (Veterinary Medical University of Vienna) or a Bruker SkyScan 1272 (Bruker Micro-CT; Kontich) at the Theoretical Biology Imaging Lab of the Department of Evolutionary Biology (University of Vienna). Scans were conducted at an optical magnification ranging from 0.4 to 4. The specimens were scanned at 80 kV peak source and 100 μA intensity. Projections were acquired with a 10–30 s exposure time (camera binning = 1). Only *S. pinastri* was scanned with an exposure time of 1.32 s. Depending on specimen size, isotropic voxel resolution varied between 2.45 and 7.28 μm. Tomographic reconstructions were made with the XMReconstructor or Bruker NRecon software supplied with the system and volume renderings of the scans were performed in Amira software 6.4.0 (ThermoFisher Scientific).

2.5 | Histology of proboscis anatomy

The proboscises were removed from the head, fixed in FAA for 2 days, and then stored in 70% EtOH before dehydration with an ascending EtOH series. This was followed by a 10 min acetone wash that was repeated twice. The proboscises were kept in a 50:50 resin-acetone mixture overnight, transferred to an 80:20 mixture for 6 h, and then kept in pure resin overnight before being fitted into a mold. Polymerization was induced at 60°C. Semithin sections (thickness 1 μm) were cut using a Leica UC6 ultracut microtome (Leica). At least 200 μm of serial cross sections were acquired for the proximal, distal, and tip regions of the proboscises. The series was transferred onto microscope slides and stained with toluidine blue for 20 s. Finally, the slides were sealed with acrylic low viscosity resin and covered with coverslips. Semithin serial sections were produced and analyzed for 10 species (Table 1).

2.6 | Digitalization and image processing

For each of the 10 histologically processed species, 20 sections (equivalent to 20 μm) of three proboscis regions (proximal, distal, and tip) were photographed, which yielded three image stacks per species. The proximal region was apportioned to 20%–30%, the distal region to 50%–60%, and the tip region to 80%–90% of the entire proboscis length. The 20 sections of each region were chosen at random from 200 μm using a random number generator (App developed by Bennett Bock, 2012). All sections of the proboscises were photographed with a Nikon eclipse E800 by applying magnifications ranging from 4× to 10×. To calculate voxel size, photos of a micrometer scale were taken while operating under the same magnifications as the stacks of the different regions. Using the scale as a reference in Photoshop, a length of 1000 or 500 μm was defined with the rectangle tool. For every region, this determined the amount of pixels equivalent to this length. The results were then downscaled to one pixel to determine voxel size. The image stacks were all converted from RGB to greyscale and image size was reduced by 50% in Photoshop CC 2018 (Adobe Systems).

2.7 | Segmentation and 3D reconstruction

For each of the 20 μm stacks, a volume rendering (volren tool) and a scale bar (scale bar tool) were generated using Amira software 6.4.0 to check for any distortions or wrong size ratios resulting from a potentially incorrect voxel size. Lateral intrinsic muscles (lim), median intrinsic muscles (mim), the galea lumen (excluding muscles, the trachea, the cuticle, and the nerve), and the food canal were segmented in the segmentation window using the threshold, the brush tool, and the interpolation tool. Only the left or the right galea was segmented as previous work has shown that values for the separate galeae are nearly identical (Reinwald, 2021). For stacks containing only one galea, only half of the food canal was segmented. For the stipes pump, the left external stipes muscle (esm) and the left internal stipes muscle (ism) were segmented. For the suction pump, the dilator muscles (dil), compressor muscles (com), and the lumen of the suction pump (lum) were segmented. Purely for imaging purposes, both sides of the stipes pump and the basal galea musculature in *A. atropos*, *Agrius convolvuli*, and *Mimas tiliae* were segmented in Amira. For visualization of the stipes pump and suction pump, a volume rendering of the head capsule was generated with the volren tool and a gray colourmap. The stipes- and suction pump musculature as well as the basal galea muscles were displayed by generating an iso-surface of the created materials, reducing the number of faces, smoothing the surface, and enabling the surface view. Snapshots were saved as TIFF files, and the values for render tiles and antialias were increased to 3 to improve image quality.

2.8 | Biometric measurements and analyses

For each of the 20 µm stacks, the volume of the lateral intrinsic musculature, the median intrinsic musculature, the galea lumen, and the food canal was computed based on the materials created in Amira. This yielded the volumes specific to one galea over a length of 20 µm. The individual volumes were computed and displayed by applying the material statistics tool and selecting the materials as source. The same principles were applied for volume computation of the stipes, the stipes pump muscles, the suction pump and suction pump musculature as well as the head capsule based on the microCT scans (Table 1). All values were transformed to mm^3. In cases where stacks contained only one galea, the computed food canal volume was doubled as only half of the food canal could be segmented. All volumetric values for the intragaleal muscles (lim and mim), the galea lumen, the cuticle, and the food canal were extrapolated onto 200 µm by multiplying the values by 10. As has been shown by preliminary work for this study (Reinwald, 2021), the differences between this method and manually segmenting all 200 slices, are less than 0.00001 mm^3. These biometric samples from three proboscis regions (at 20%–30%, 50%–60%, 80%–90% of the proboscis length) were used to calculate a proxy for the total volume of the intragaleal muscles, as well as the volumes of the lumina of the galeae and the food canal.

Data analyses were performed with R version 4.1.1 (R Core Team, 2021). Wilcoxon rank sum tests were used to compare the number and length of sensilla styloconica as well as the number of sensilla basiconica between five short-proboscid sphingid species (proboscis length divided by body length <1) and five long-proboscid sphingid species (proboscis length divided by body length >1). Spearman's rank correlations were calculated to determine the relation between the volume of the galeal lumen and the volume of the stipes musculature, as well as between the volume of the food canal and the volume of suction pump musculature.

3 | RESULTS

3.1 | External proboscis morphology

All studied sphingid species share the same mouthpart morphology, although the proboscis length differs remarkably (Figure 1). The length of the proboscis may vary from a few millimeters (2.9 mm) in certain studied species to more than 200 mm in others (Table 2). Likewise, the number of coils during resting position varies remarkably (Figure 1), ranging from about 1 up to 10 (Table 2). The highest number of coils were found in *N. cluentius*. This species displays the longest proboscis, which measures nearly three times the body length (Table 2).

The proboscis continuously tapers from the head toward the tip, becoming especially slender near the tip in nectar-feeding species. In these hawk moths, a specialized drinking region occurs distally, where the dorsal legulae are longer and loosely arranged to form slits that lead into the median food canal (Figures 2a and 3d). The length of this drinking region makes up 6.2% to 20.9% of the total proboscis length (Table 3). The ventral legulae firmly close the proboscis along the entire length up to the apex. The proboscis cuticle is smooth except at the base, where the ventral wall of the galeae is sparsely covered by short microtrichia. In most species, a patch of longer microtrichia exists on the dorsal side close to the head. The galeal wall is composed of externally visible cuticular ribs which are curved and extend from the dorsal galea side to the ventral legulae. At the lateral side of the galea, the cuticular ribs display a ventro-lateral bend toward the proboscis base (Figure 2a). In contrast, the wall of the

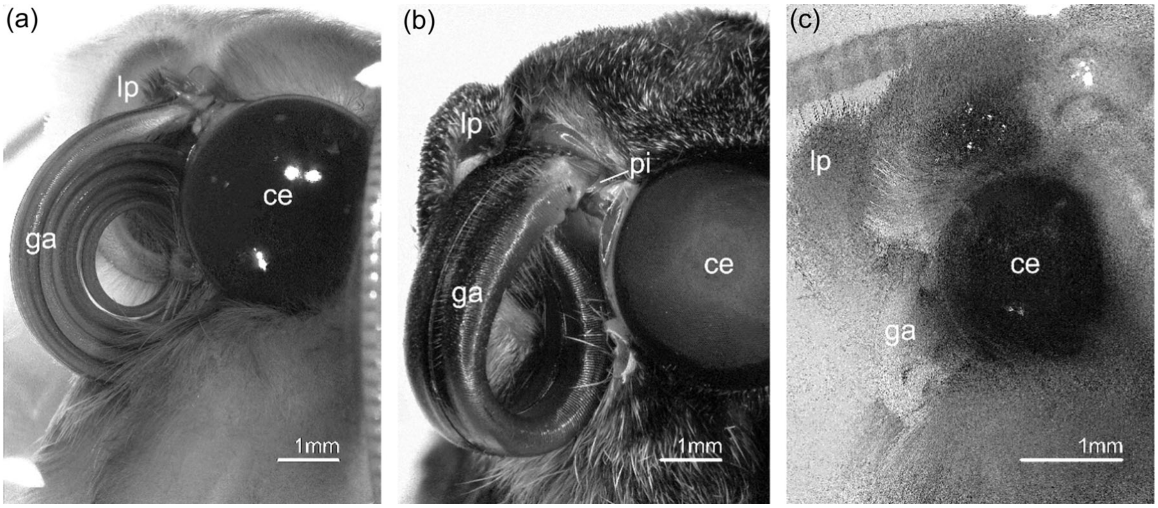

FIGURE 1 Head and coiled proboscis of three Sphingidae, left labial palpus removed (LM photos). (a) Nectar-feeding *Xylophanes pyrrhus*; proboscis (45.2 mm long) coiled in about 5 coils; (b) Honey-feeding *Acherontia atropos*, stout proboscis (12.4 mm long) coiled in approximately 1 coil; (c) Probably nonfeeding *Mimas tiliae*; galeae not interlocked throughout the entire length; rudimentary proboscis (4 mm long), coiled in 1.5 coils. ce, complex eye; ga, galea; lp, labial palpus; pi, piliferes.

TABLE 2 Length of body and proboscis of studied individuals of Sphingidae, relations of proboscis length to body length, and numbers of coils in resting position

Species	Body length (mm)	Proboscis length (mm)	Relativ proboscis length (% body length)	Number of coils resting position
Protambulyx strigilis	45.7	31.1	68	7
	52.7	28.3	54	
Mimas tiliae	27.9	5.8	20	1.5
	33.1	4.0	12	
Smerinthus ocellata	31.0	2.9	9	0.75
Neococytius cluentius	71.2	198.8	279	10
	71.8	210.9	294	
Sphinx pinastri	35.4	29.6	84	5–6
	32.9	31.0	94	
Euryglottis aper	49.9	69.2	139	7–8
	56.8	98.0	173	
Manduca scutata	51.8	96.2	188	8–9
	55.1	90.3	164	
Agrius convolvuli	44.4	93.7	211	8
	48.7	85.9	177	
Acherontia atropos	53.1	10.8	20	1
	55.4	12.4	22	
Pachylia ficus	58.8	49.1	84	6–7
	65.4	47.7	73	
Eumorpha triangulum	54.6	50.9	93	7
	62.0	47.8	77	
Macroglossum stellatarum	22.4	23.3	104	7
	19.2	20.3	106	
Deilephila elpenor	29.6	23.4	79	5–6
	34.7	23.5	68	
Xylophanes pyrrhus	37.8	45.2	120	5–6
	42.6	45.3	106	

food canal is composed of narrow, straight, vertical cuticular ribs which are interspaced by short ridges. The ribs extend from the ventral to the dorsal legulae (Figure 3d). In the honey-feeding *A. atropos*, the stout proboscis tapers abruptly toward the distal end and forms a sharply pointed drinking region (Figure 2c,d). The galeae form a large, elliptical opening of the food canal, located dorsally and subterminally of the apex (Figure 2c). Throughout the rest of the short and robust-looking proboscis, the food canal is enclosed by the interconnection of dorsal and ventral legulae. Ventral legulae extend from the proboscis base to the apex while dorsal legulae are absent in the tip region (Figure 2c). The external wall of the galeae is composed of regular, vertical cuticular ribs that curve slightly (Figure 2d). They extend into the ventral and dorsal linking structures and exert an additional bend toward the apex within the dorsal region. The ribs do not fully extend into the tip region and fade into the ventral side of the tip. Therefore, the cuticula surrounding the food canal opening is smooth (Figure 2c,d). The food canal is lined with smooth, uniform cuticular ribs, arranged vertically throughout the entire proboscis.

The reduced galeae of the nonfeeding species exhibit light hues as well as indistinct cuticular ribs that are covered with microtrichia in *M. tiliae*. The proboscis is coiled in one to two irregular coils (Figure 1c). The galeae of these hawk moths are only loosely connected at their base. Dorsal and ventral legulae are present and rather elongated. However, the ventral legulae are not hook-shaped and do not interlock the galeae. A particular drinking region is not recognizable (Figure 2e).

3.2 | Proboscis sensilla

All studied nectar-feeding species and the honey-feeding hawk moth display the same morphological sensilla types on the dorsal, lateral, and ventral sides of the galeae, that is, aporous bristle-shaped sensilla chaetica of various lengths, sensilla basiconica in more or less deep cuticular depressions and club-shaped sensilla styloconica located in cuticle cavities (Figures 2 and 3). Sensilla coeloconica could be identified in *A. atropos* at the proboscis apex where they are located in pits (Figure 3g). The median galea side, which composes the food canal, exhibits only sensilla basiconica.

3.2.1 | Bristle shaped sensilla

Sensilla chaetica are arranged in several irregular rows placed along the ventral, lateral, and dorsal sides of the galeae (Figure 2a). They increase in length and number from the tip to the proximal region. Particularly short sensory bristles occur in the drinking region. In the outermost coil, long bristle-shaped sensilla chaetica occur in higher densities in some species; the longest are located on the ventral side of the galeae. The bristles extend from a minor cuticular depression and are tilted slightly toward the apex of the proboscis. In the nectar-feeding species *A. convolvuli*, for instance, about 35 long sensilla chaetica are found on one galea. Within the tip region, sensilla chaetica length ranges from 11.5 to 33.3 μm, while sensilla from the proximal galea region are 31.2–43 μm long.

In addition to sensilla chaetica, each galea of the species *A. atropos* encompasses more than 150 long, thin, aporous bristle-shaped sensilla, which are termed as sensilla filiformia by M. J. Faucheux (2013). Here, the bristles vary in length from 105 to 646 μm (Figure 2b).

3.2.2 | Sensilla basiconica

Sensilla basiconica occur in longitudinal rows alongside the dorsal legulae and the dorsolateral side of the external surface. They bear

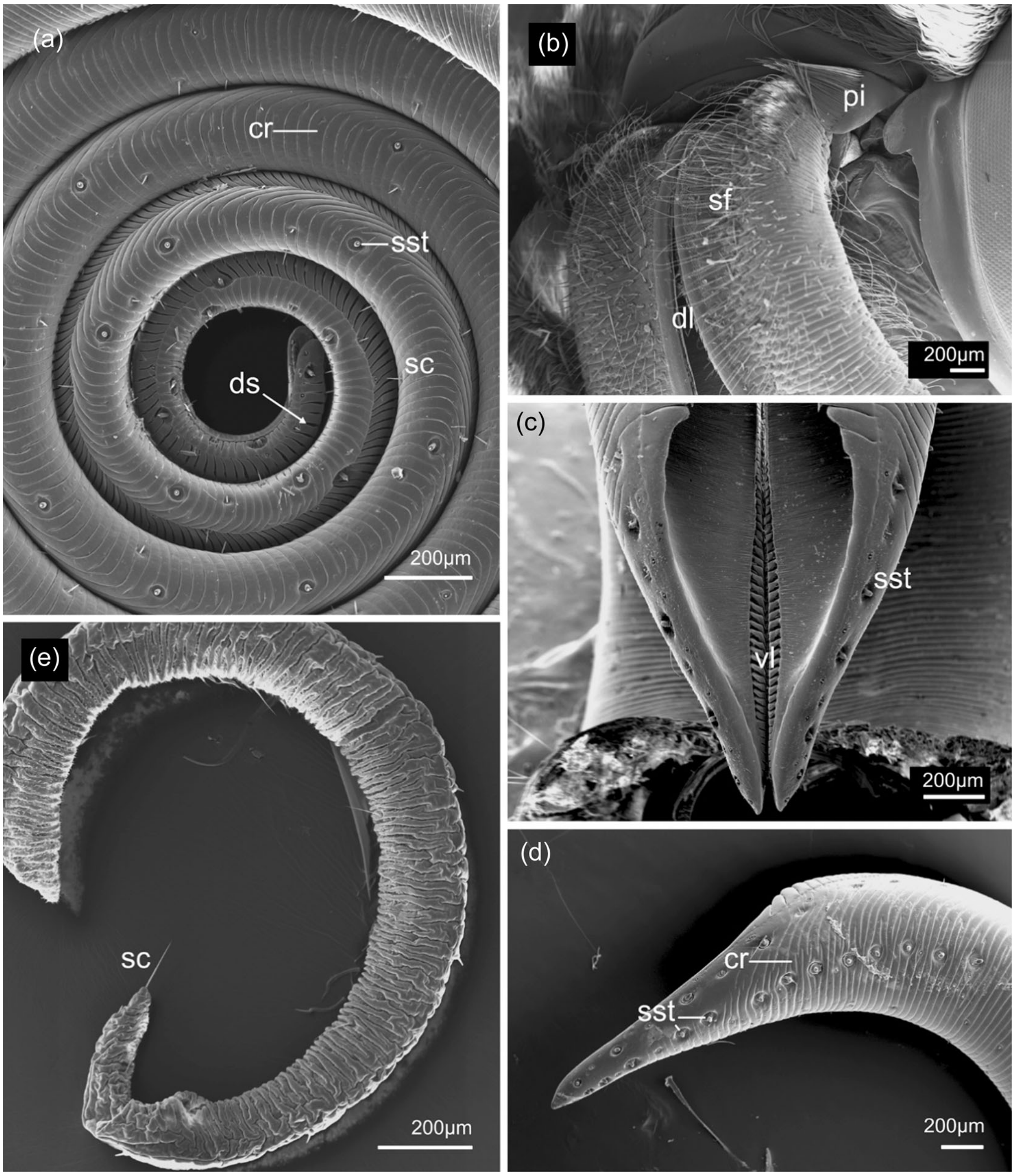

FIGURE 2 Proboscis of various Sphingidae (SEM). (a) Nectar-feeding *Deilephila elpenor*; the galeal surface of the long slender proboscis displays cuticle ribs. Sensilla of various types in great distance to each other, drinking region with drinking slits; (b–d) Robust proboscis of the honey-feeding *Acherontia atropos*; (b) proximal region of the galea covered with long bristle-shaped sensilla filiformia; sensilla of the piliferes are in contact with the galeal base; (c) wide opening of the food canal on the dorsal side of the tip; (d) distal half of the proboscis (lateral view); distinct decrease of thickness forms pointed tip; (e) rudimentary galea of *Smerinthus ocellata* (lateral view). cr, cuticle rib; dl, dorsal legulae; ds, drinking slits; pi, pilifer; sc, sensillum chaeticum; SEM, scanning electron microscopy; sf, sensilla filiformia; sst, sensillum styoconicum; vl, ventral legulae.

sockets and blunt-tipped sensory cones that stick out from cuticle depressions, which are particularly deep in the drinking region (Figure 3a,b). Only few sensilla basiconica are present in the proximal galea region where the cuticle depression are relatively shallow (Figure 3e).

In all species, sensilla basiconica occur in the food canal, at least in low numbers (Table 3). They are arranged in one row from the base to the apical drinking region while being positioned closely to the dorsal legulae. The sensilla are spaced more loosely in the distal proboscis region and exhibit higher densities at the base and tip. Sensilla basiconica of the food canal are characterized by an indistinct socket and an elongated, peg-shaped sensory cone (Figure 3f). Their numbers range from 2 in *S. ocellata* to 72 in *N. cluentius*. Species with a proboscis exceeding the body length possess significantly more food canal sensilla than species with a proboscis that is shorter than the body ($W = 8.0$, $p = 0.04$, $N = 14$).

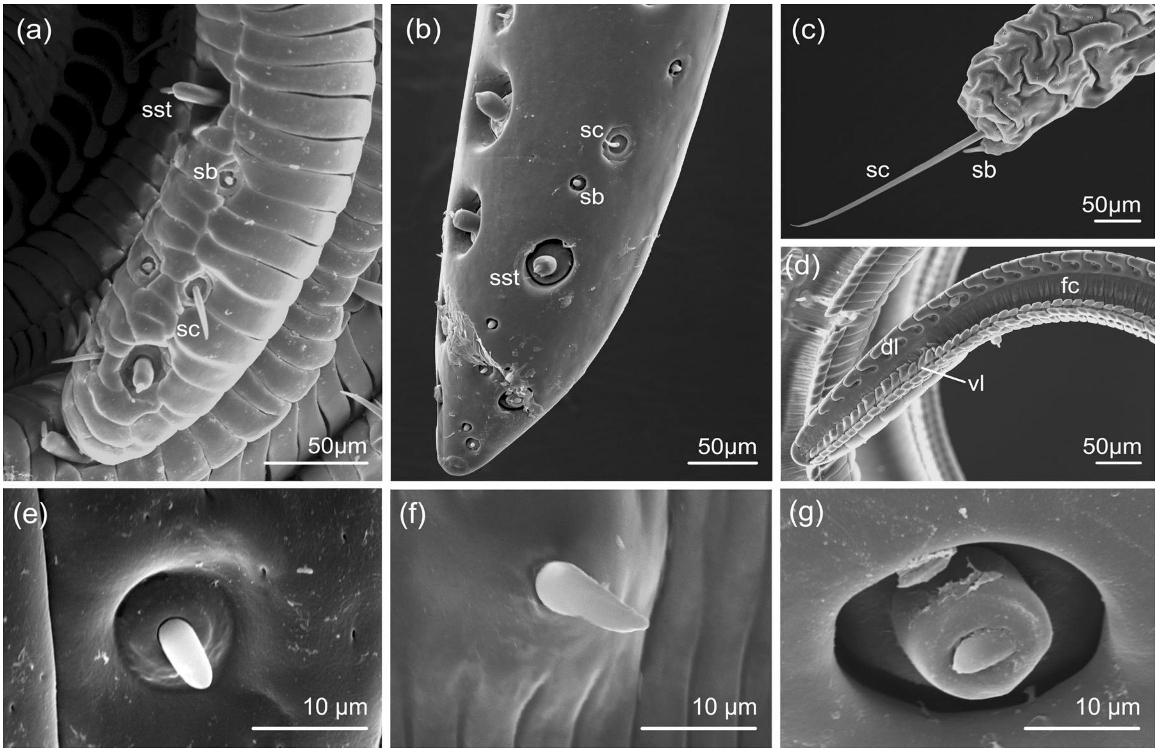

FIGURE 3 Proboscis sensilla in various Sphingidae (SEM). (a) Galea tip of *Sphinx pinastri*; nectar-feeding species; (b) galea tip of *Acherontia atropos*; honey-feeding species (c) *Smerinthus ocellata*; apex with a sensillum chaeticum and a sensillum basiconicum (d) *Protambulyx strigilis*; food canal in drinking region; (e) *Deilephila elpenor*; sensillum basiconica located in depression of the cuticle (f) *P. strigilis*; sensillum basiconicum in the food canal; (g) *A. atropos*; sensillum coeloconicum close to the apex. dl, dorsal legulae; fc, food canal; sb, sensillum basiconicum; sc, sensillum chaeticum; SEM, scanning electron microscopy; sst, sensillum styloconicum; vl, ventral legulae.

TABLE 3 Relative proboscis length (%) indicates species with a proboscis shorter or longer than the body length; drinking region (% of proboscis length) equivalent to area with dinking slits; number and length of sensilla styloconica on the distal proboscis, and sensilla basiconica of the food canal

			Sensilla styloconica			
Species	Relative proboscis length (% body length)	Drinking region (% prob. length)	Number per proboscis	Length (µm)	Area (% proboscis length)	Sensilla basiconica food canal (*n* per proboscis)
Protambulyx strigilis	68	13.8	24	28.8	25	32
Mimas tiliae	20	NA	0	NA	NA	20
Smerinthus ocellata	9	NA	0	NA	NA	2
Neococytius cluentius	279[a]	6.4	16	33.3	<10	72
Sphinx pinastri	84	15.9	16	25.4	20	42
Euryglottis aper	139[a]	13.9	26	28.3	25	68
Manduca scutata	188[a]	6.2	34	31	20	64
Agrius convolvuli	211[a]	9.1	24	29.8	20	60
Acherontia atropos	20	15.7	54	39.8	33	26
Pachylia ficus	84	12.4	24	37.1	12.5	54
Eumorpha triangulum	93	8.4	24	36.2	16.6	40
Macroglossum stellatarum	104[a]	10.7	24	23.8	16.6	48
Deilephila elpenor	79	20.9	40	26.7	40	64
Xylophanes pyrrhus	120[a]	7.1	32	28.3	25	32

[a] Proboscis longer than the body.

3.2.3 | Sensilla styloconica

In all studied species, sensilla styloconica are composed of a stylus and a rounded sensory cone; pores could not be detected. The stylus is smooth and cylindriform aspinate in shape (see terminology of Petr & Stewart, 2004). These sensilla always extend from a depression of the cuticle (Figures 2a,c,d and 3a,b). In some species, for example, in *A. convolvuli*, the club-shaped shaft tapers toward the base. They are arranged in two longitudinal rows in the most distal 10-40% of the distal proboscis in nectar- and honey-feeding species (Table 3). The dorsal row is short and extends closely to the dorsal legulae, while the ventral/ventrolateral row extends further into proximal direction. As it progresses, this row of sensilla also extends toward the lateral side of the galea. The distance between individual sensilla styloconica increases toward the head and sensilla styloconica are successively replaced by sensilla basiconica. The drinking region displays only 6–12 sensilla styloconica per galea. The apex of the galeae exhibits a characteristic pattern of sensilla in nectar-feeding species. This sphingid-type comprises a sensillum styloconicum on the dorso-lateral side, which is accompanied by short sensilla chaetica and one or two sensilla basiconica on the lateral and/or ventral side. Here, a second sensillum styloconicum can be found in most species, like in *S. pinastri* (Figure 3a). Sensilla styloconica never stick-out beyond the apex of the galeae (Figures 2a,c,d and 3a,b). In the ventral tip region, each sensillum styloconicum is followed by a short sensillum chaeticum. Sensilla styloconica measure 23.8–39.8 µm in total length and count approximately 8–20 per galea in nectar-feeding species (Table 3). The number and length of sensilla styloconica do not significantly differ between species with a proboscis that is longer than the body and species with a proboscis that is shorter than the body (sensilla number: $W = 18.5$, $p = 0.47$, $N = 14$; sensilla length: $W = 23$, $p = 0.42$, $N = 14$).

Compared to other Sphingidae, sensilla styloconica are relatively long and numerous in *A. atropos*, despite the short proboscis (Table 3). As in other species, they are arranged in two rows on each galea (Figure 2d). The dorsal row surrounding the opening of the food canal consists of about 5 sensilla styloconica, while the ventro-lateral row consists of 22 sensilla on one galea. All sensilla styloconica extend from cavities; their sensory cones only slightly protrude above the cuticular depression (Figures 2c,d and 3b). Close to the apex, there are particularly short sensilla in deep pits which are identified as sensilla coeloconica (Figure 3g).

Sensilla styloconica are missing on the proboscis of both nonfeeding species *S. ocellata* and *M. tiliae*. Only bristle-shaped sensilla chaetica and a few sensilla basiconica occur on the lateral side of the rudimentary galeae. In *M. tiliae*, the median side additionally exhibits a few sensilla basiconica. One prominent sensillum chaeticum along with a sensillum basiconicum can be found at the apex of the galea in *S. ocellata* (Figure 3c).

3.3 | Proboscis anatomy

The internal composition of the galea is similar in all studied Sphingidae, regardless of proboscis length. Even in species with greatly reduced galeae, a trachea, a nerve, and the intrinsic galeal muscles extend throughout the entire proboscis (Figure 4). The basal galeal musculature (musculus stipito-galealis) extends from the proximal end of the galea at the stipes to the dorsal galeal wall (Figure 5). In *A. atropos*, the basal galeal musculature is especially distinct with longer muscle fibers in the median portion (Figure 5b).

In all species, the volume of the galeae decreases from the proximal region to the drinking region (Supporting Information: Table S1). The lateral and ventral lumen of the galeae is occupied by the lateral and median intrinsic galeal muscles (musculi intragaleales) which display slightly different arrangements in the three proboscis regions (Figure 4a–c). Lateral intrinsic muscles progress obliquely from the lateral to the ventral galea wall while median muscles extend longitudinally along the ventral wall. In the drinking region, all intrinsic galeal muscles extend in longitudinal direction and the two types of intrinsic muscles cannot be distinguished in all species. The volume of the intrinsic galeal muscles decreases from the proximal to the drinking region (supplementary online material, Table S1). Extrapolated onto the entire galea, lateral intrinsic muscles have approximately 3 times the volume of median intrinsic muscles. The total volume of the intrinsic galeal musculature ranges from 0.01 mm^3 in *M. tiliae* to 1.29 mm^3 in *N. cluentius* (Table 4).

The galeae of the honey-feeding species *A. atropos* display a sickle-shaped cross section in the drinking region (Figure 4d–f). The volume of the intrinsic galeal musculature decreases from the proximal proboscis to the tip. Distally, the intrinsic galeal muscles still occupy large areas of the galeal volume (Figure 4e,f).

The rudimentary proboscis of nonfeeding hawk moths is characterized by small diameter and rounded galeae in cross-section. Furthermore, the galeae are not connected. The galeal wall is composed of a thin cuticle. The intrinsic galeal musculature has small volume (Table 4) and cannot be assigned to lateral or median intrinsic musles (Figure 4g–i).

3.4 | Stipes pump

The composition of the stipes as well as the arrangement of the internal and the external stipes musculature is similar in all studied hawk moths (Figure 5). The external stipes musculature consists of two distinct portions that differ in origin and attachment points. The external stipes muscle 1 (musculus clypeo-stipitalis) originates on the clypeus, progresses laterally past the anterior tentorium and attaches at the lateral side of the stipes sclerite via a tendon. The external stipes muscle 2 (musculus tentorio-stipitalis externalis) originates on the ventral side of the tentorium and attaches to the flat part of the stipes sclerite. The internal stipes muscle (musculus tentorio-stipitalis internalis) has its origin at the posterior tentorium and extends almost longitudinally through the head to the median part of the stipes.

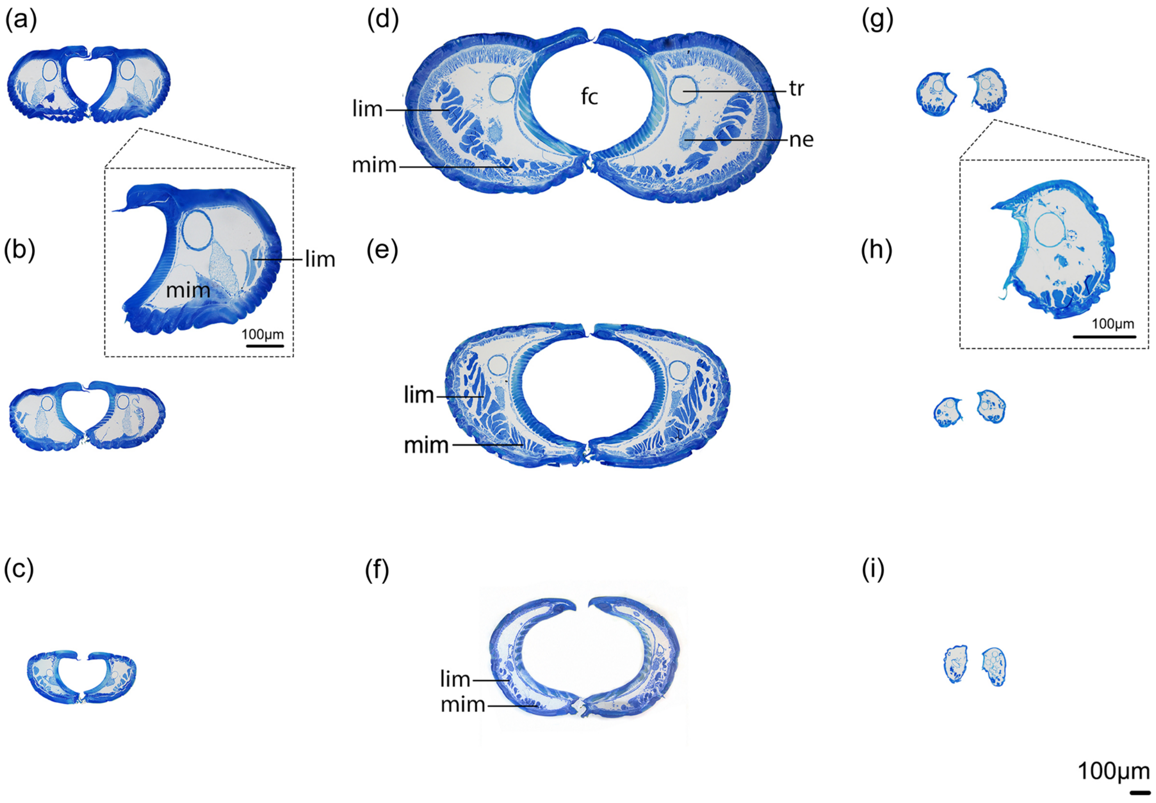

FIGURE 4 Cross sections of proboscises of three Sphingidae (semithin sections; all sections at the same scale); despite various sizes, the same principle composition can be found; proximal proboscis region in the first row, distal proboscis region in the second row, drinking region in the third row, (a–c) Nectar-feeding *Agrius convolvuli*; one galea magnified, (d–f) honey-feeding *Acherontia atropos*; (g–i) nonfeeding *Mimas tiliae*; galeae not connected; one galea magnified; lim and mim could not be separated. fc, food canal; lim, lateral intrinsic muscles; mim, median intrinsic muscles; ne, nerve; tr, trachea.

The external stipes muscles have three to five times the volume of the internal stipes muscles in the studied species (Table 4). The added volume of the external and internal stipes musculature ranges from 0.36% of the head capsule volume in *M. tiliae* to 5.06% in the long-proboscid *A. convolvuli*. The volume of the stipes lumen itself varies distinctly among the studied species: The largest stipes volume is found in *A. atropos*, although this species has a short, voluminous proboscis. The stipes lumen is nearly 30 times smaller in *M. tiliae* (Table 4).

Sphingidae with a proboscis exceeding the body possesses larger stipes muscles compared to species with a proboscis shorter than the body. The volume of the galeal lumen correlates significantly with both, the volumes of the external ($\rho(8) = 0.96$, $p = 2.2 \times 10^{-16}$) and the internal stipes musculature ($\rho(8) = 0.88$, $p = 9.1 \times 10^{-4}$; Figure 6a,b).

3.5 | Suction pump

A suction pump with similar anatomy is present in all Sphingidae, regardless of proboscis length and feeding strategy. The lumen is smallest in *M. tiliae*, with a rudimentary proboscis, and largest in *N. cluentis*, the species with the longest proboscis (Table 4). Dilator muscles extend from the dorsal side of the suction pump to the clypeus. Compressor muscles encompass the lumen of the pump (Figure 7). The dilator muscles have approximately 3 times the volume of the compressor muscles, except in *M. tiliae*, where the compressor muscles are larger than the suction pump dilators (Table 4). The volume of the food canal correlates significantly with the volume of the suction pump dilator musculature ($\rho(8) = 0.69$, $p = 0.04$) and the volume of the compressor musculature ($\rho(8) = 0.65$, $p = 0.05$; Figure 6c,d). Sphingid species ($N = 10$) with a proboscis length greater than the body have significantly larger suction pump muscles than species with a proboscis that is shorter than the body.

4 | DISCUSSION

4.1 | Proboscis in nectar-feeders, honey-feeders, and nonfeeding species

All nectar-feeding Sphingidae display a similar proboscis morphology, despite greatly differing proboscis lengths. A reduction in proboscis length was found in the honey-feeding hawk moth *A. atropos* and in presumably nonfeeding species. While the slender proboscis of flower-visiting, nectar-feeding Sphingidae may measure up to more than twice their body length, *A. atropos* displays a much shorter and

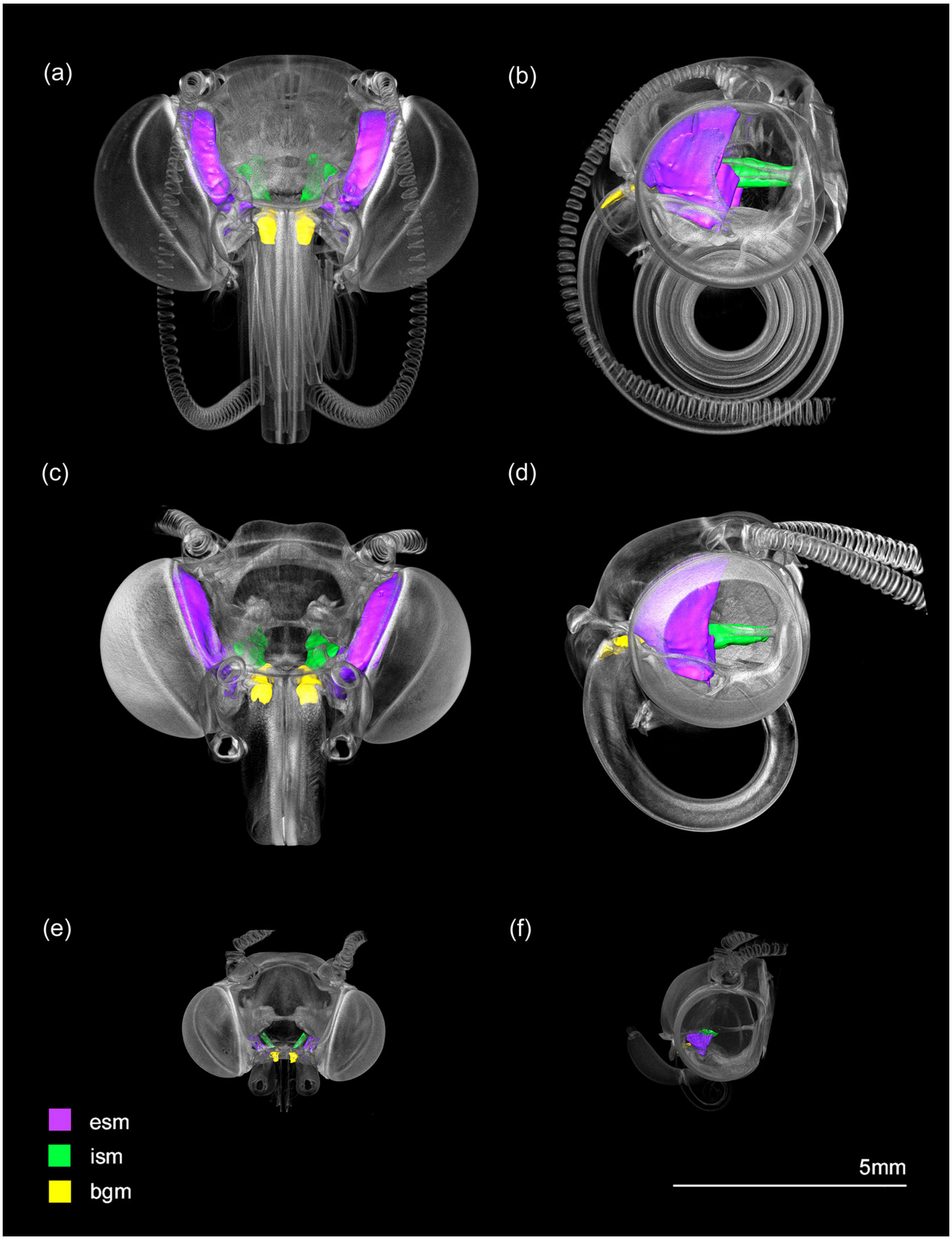

FIGURE 5 Head, muscles of the proboscis base, and the stipes pump of three Sphingidae; reconstruction from microCT images (all species at same scale); (a) nectar-feeding *Agrius convolvuli*, frontal view; (b) *A. convolvuli*, lateral view; (c) honey-feeding *Acherontia atropos*, frontal view; (d) *A. atropos*, lateral view; (e) *Mimas tiliae*, proboscis rudimentary, frontal view; (f) *M. tiliae* lateral view. External stipes muscles (esm) in purple; internal stipes muscles (ism) in green; basal galeal muscles (bgm) in yellow.

robust proboscis with a unique morphology. Like most sphingid moths, adult heteroneuran Lepidoptera primarily feeds on floral nectar and use a coilable siphoning proboscis, formed by the maxillary galeae, for fluid uptake (Krenn & Kristensen, 2000; Krenn, 2010; Krenn, 2019; Mitter et al., 2017; Scoble, 1995). Based on the phylogeny of Sphingidae (Kawahara, 2009), it can be concluded that floral nectar feeding is the plesiomorphic feeding preference in hawk moths. Therefore, honey-feeding or the reduction of feeding practices in adults is derived. Many Sphingidae are well-known for their long proboscises, which enable them to reach the nectar inside deep-tubed flowers, mostly during hovering flight in front of flowers (e.g., Johnson et al., 2017; Nilsson, 1998; Stöckl & Kelber, 2019). Extremely long proboscises (i.e., longer than 1.5 times the body length) have been recorded in Sphingini. Examples are the

TABLE 4 Biometry of the proboscis (% indicate species with a proboscis longer or shorter than body length), galea (volumes of lumen and intragaleal musculature), the stipes pump (volumes of esm and ism), food canal volume, and suction pump (volumes of lumen, dilator muscles, and compressor musculature)

Species	Relative proboscis length (% of body length)	Intragaleal muscles (mm^3)	Galea lumen (mm^3)	Stipes volume (mm^3)			Food canal (mm^3)	Sucking pump volume (mm^3)		
				Lumen	esm	ism		Lumen	Dilator	Compressor
Protambulyx strigilis	54	0.12	0.49	0.02	1.02	0.35	1.93	2.97	6.20	1.86
Mimas tiliae	12	0.01	0.03	0.01	0.02	0.01	na	0.22	0.15	0.20
Neococytius cluentius	294[a]	1.29	10.65	0.15	3.56	1.18	15.11	4.25	11.40	2.15
Sphinx pinastri	94	na	na	0.05	0.32	0.07	0.33	0.27	0.64	1.37
Euryglottis aper	173[a]	0.46	3.89	0.26	2.07	0.42	5.47	5.20	5.14	1.90
Manduca scutata	164[a]	0.42	2.69	0.22	2.13	0.42	4.18	0.76	6.15	2.14
Agrius convolvuli	177[a]	0.37	2.09	0.18	1.91	0.47	3.29	2.11	3.73	1.37
Acherontia atropos	22	0.41	1.28	0.29	1.64	0.40	3.18	1.10	4.46	2.38
Eumorpha triangulum	77	0.34	1.97	0.12	1.97	0.74	2.38	1.13	6.57	2.46
Deilephila elpenor	68	0.06	0.32	0.03	0.38	0.12	0.48	0.22	0.73	0.37
Xylophanes pyrrhus	106[a]	0.09	0.60	0.01	0.82	0.21	1.14	1.56	2.67	0.74

Abbreviations: esm, external stipes muscle; ism, internal stipes muscle.

[a] Proboscis longer than the body.

record holder in proboscis length *Amphimoea walkeri*, the famous Malagasy *Xanthopan morganii praedicta* or several *Cocytius* and *Neococytius* species (Amsel, 1938; Bauder & Karolyi, 2019; Netz & Renner, 2017; Wasserthal, 1997). In the present data set, the mouthpart morphology of sphingid moths with an extremely long proboscis has been compared to species with moderate proboscis lengths for the first time. Apart from extraordinarily long galeae, they do not exhibit any unique features. In contrast, the short but specialized proboscis of *A. atropos* is most likely an adaptation to the moth's way of obtaining honey inside beehives. The length of the proboscis matches the depth of the honeycombs, which are typically 12.5–17.7 mm deep (Hüsing & Nitschmann, 1987). A short and robustly built proboscis may be crucial when piercing through the wax seals of the honeycombs. It is also intuitively obvious that a long proboscis is not useful inside the beehives, as there is only limited space and a long proboscis may impede the generation of the force adequate for penetrating the seals of the honeycombs. Interestingly, the noctuid moth *Calyptra eustrigata*, which has been found to pierce fruit as well as the skin of humans or even ungulates and elephants (Bänziger, 1968, 1980), displays a similarly short proboscis, compared to related taxa (Bänziger, 1970). Therefore, piercing behavior in different Lepidoptera seems to be related to a reduction of proboscis length relative to body size.

The principle galeal anatomy and even the particular arrangement of intragaleal muscles are nearly identical in all studied species, regardless of proboscis length. Similar internal proboscis anatomy occurs in all glossatan Lepidoptera, regardless of systematic affiliation and feeding preference (Eastham & Eassa, 1955; Krenn & Kristensen, 2004). Our results corroborate the anatomical findings for the only sphinigid studied thus far, *M. sexta* (Eaton, 1971; Krenn & Kristensen, 2004). It can be concluded that *M. sexta* possesses the typical proboscis morphology of a sphingid moth, despite its extraordinary length. Likewise, the piercing proboscis of *A. atropos* shares an identical anatomy, including the galeal muscles, the trachea, and a nerve. The conservation of galeal muscles among all studied species can most likely be traced back to the functional purpose of the intrinsic galea musculature, as it coils the proboscis in both, long- and short-proboscid species, alike (Wannenmacher & Wasserthal, 2003). Even the reduced proboscis of *M. tiliae* contains muscles and appears to be moveable, as has been suggested by previous observations (Kernbach, 1962). Our anatomical results corroborate early findings that even the short galeae of probably nonfeeding hawk moths possess all features of a lepidopteran proboscis, including galeal muscles, a nerve, a trachea as well as sensilla (Hättich, 1907).

4.2 | Adaptations of the drinking region

The proboscis of the nectivorous Sphingidae is characterized by a slender and smooth distal region, equipped with drinking slits between the dorsal legulae and comparatively few, short sensilla which extend from cuticle depressions. Such a tip morphology facilitates proboscis entry into floral tubes, which has been proven to be an adaptation for optimized nectar uptake in nymphalid butterflies (Lehnert et al., 2021). A similar morphology has been described in Hesperiidae as well as long-proboscid Riodinidae (Bauder et al., 2013; Krenn & Bauder, 2017) and seems to be a feature displayed by all nectar-consuming Lepidoptera that are adapted to particularly long nectar spurs (Bauder & Karolyi, 2019). Some other nectar-feeding insects, i.e., nemestrinid or tabanid flies, display similar morphological adaptations comprising a long, slender

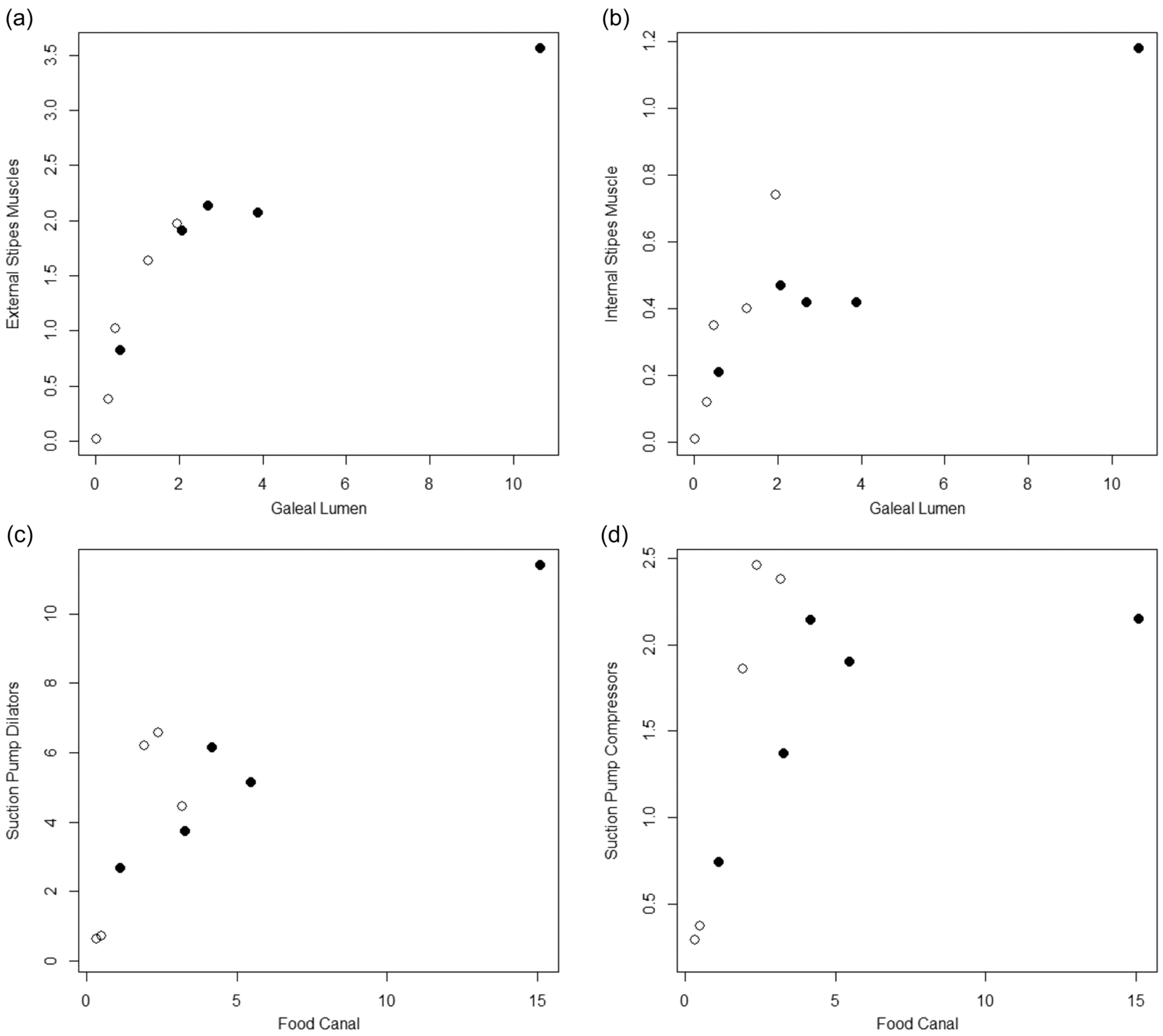

FIGURE 6 Volumes of functionally connected organs of the feeding apparatus correlate significantly. Spearman's rank correlation of morphometrical variables was measured in 10 sphingids with varying proboscis length. Each data point represents a measurement obtained from one individual of a species. Black-filled dots indicate species with a proboscis longer than the body, and white-filled dots indicate species with a proboscis shorter than the body (see Table 4). Values are given in mm^3. (a) Galeal lumen and external stipes musculature of the hemolymph pump ($\rho(8) = 0.96$, $p = 2.2 \times 10^{-16}$). (b) Galeal lumen and internal stipes musculature of the hemolymph pump ($\rho(8) = 0.88$, $p = 9.1 \times 10^{-4}$). (c) Suction pump dilator musculature and food canal ($\rho(8) = 0.69$, $p = 0.04$). (d) Suction pump compressor musculature and food canal ($\rho(8) = 0.65$, $p = 0.05$).

proboscis and a smooth apical region with remarkably few and short sensilla (Karolyi et al., 2012, 2014; Krenn et al., 2021).

The apical drinking region in the honey-feeding *A. atropos* however, displays noteworthy morphological differences. The distinct proboscis tip morphology in *A. atropos* is unique within the Sphingidae and even the entire lepidopteran order. The tip is smooth and sharply pointed while displaying one large opening of the food canal. Although pointed proboscis tips can also be found in certain fruit-piercing and/or blood-feeding moths, their morphology is noticeably different from the tip of *A. atropos*. In blood-sucking species such as *C. eustrigata*, the proboscis displays a conoid, stiff point that is heavily sclerotized (Bänziger, 1980). In fruit-piercers such as *C. thalictri* or *Scoliopteryx libatrix*, a sudden, almost triangular pointing of the proboscis tip is noticeable (Bänziger, 1970). Furthermore, the tips of these piercing moths display prominent tearing hooks, erectile barbs and/or rasping structures (Bänziger, 1970, 1980; Büttiker et al., 1996; Zaspel et al., 2011), which are not present in *A. atropos*. The single, subterminal opening of the food canal recorded in *A. atropos* has not yet been discovered in other Lepidoptera. It is likely that this type of apical drinking region is a functional adaptation

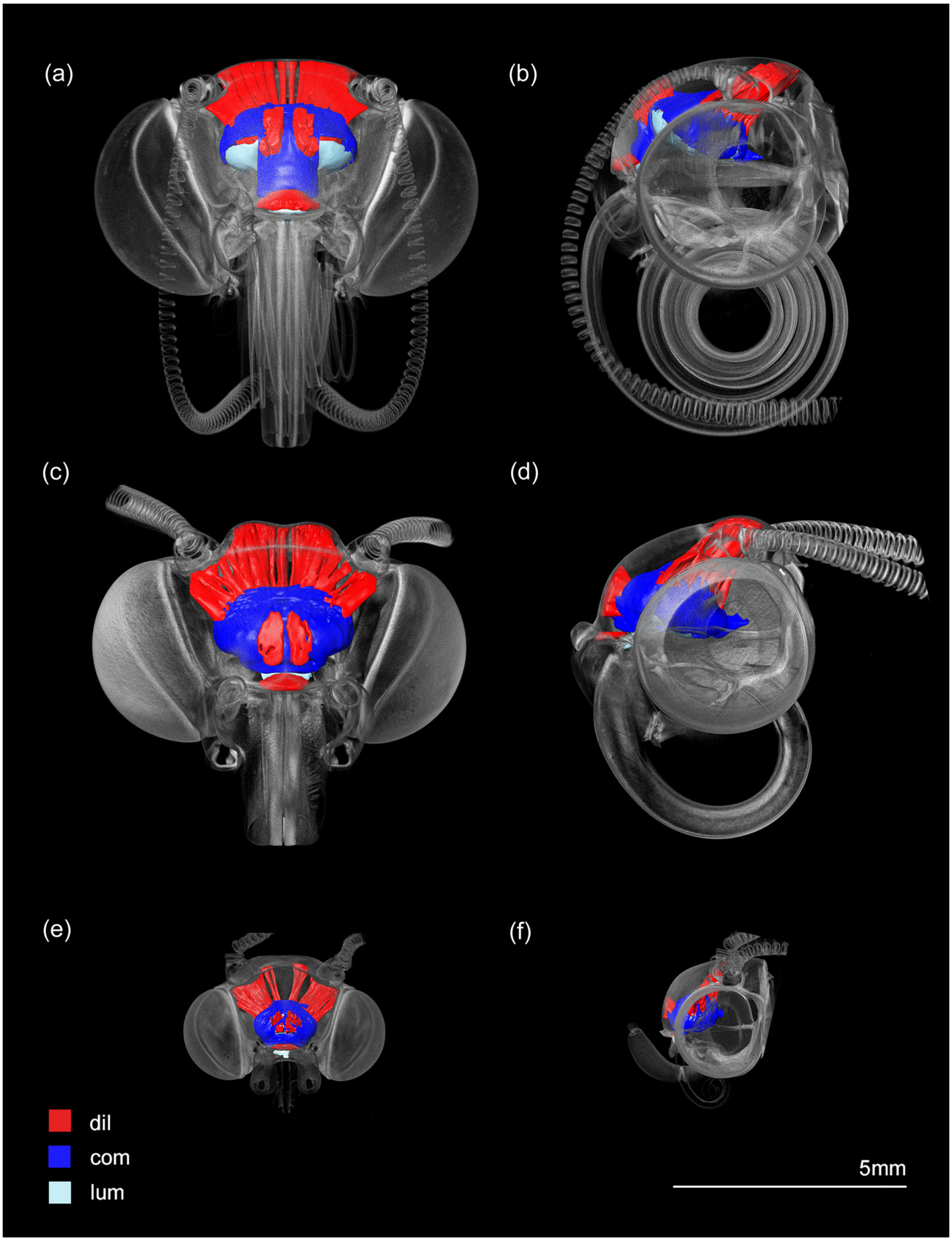

FIGURE 7 Suction pumps of three Sphingidae; reconstructions from microCT images (all species at same scale); (a) nectar-feeding *Agrius convolvuli*, frontal view; (b) *A. convolvuli*, lateral view; (c) honey-feeding *Acherontia atropos*, frontal view; (d) *A. atropos*, lateral view (e) nonfeeding *Mimas tiliae*, frontal view; (f) *M. tiliae*, lateral view. Dilator muscles (dil), which expand the suction pump lumen (lum), in red; compressor muscles (com), which decrease the suction pump lumen, in the dark blue; lumen in light blue.

to the moth's feeding mode, but it might also be related to sound production in which air is blown out of the proboscis (Brehm et al., 2015). In context with piercing, however, an opening like this is postulated to provide stability while preventing blockages of the canal (Foelix, 2011). Subterminal openings have further been found in various substance-piercing structures across multiple animal taxa. Presumably, as the outcome of natural selection, the chelicerae of spiders, the stingers of scorpions, and the fangs of venomous snakes have all convergently developed identically structured tips and openings (Du Plessis et al., 2018; Foelix, 2011; Zhao, et al., 2016). Likewise, hypodermic needles, which are applied in human medicine, are fabricated with an elliptical, subterminal opening and a tapered tip (Huber, 1946). From a mechanical point of view, such tapered, slightly bent structures attain maximum stress in the near-tip region upon penetration and consequently provide exceptional load-bearing capabilities. Therefore, this structural design, along with a sub-terminal, elliptical opening of the canal, proves to be a common solution in liquid-injecting or extracting structures (Bar-On, 2019), but is unique within the Lepidoptera.

The probably nonfeeding Sphingidae display disconnected galeae at the tip and reduced sensory equipment; they do not have a drinking region like other feeding Lepidoptera (Krenn, 2010). There are no observations of the studied species visiting flowers (Ebert et al., 1994). However, feeding experiments in the laboratory have indicated that *M. tiliae* and *S. ocellata* are capable of feeding from droplets of water (Kernbach, 1962) as the food canal is open in the distal half of the proboscis rudiment. A reduced proboscis typically suggests a loss of feeding abilities; however, there are other examples of Lepidoptera with reduced, nevertheless functional proboscises (Smedley & Eisner, 1995). Further experiments with colored fluids have indicated that rudimentary proboscises can take up liquid and might rely on capillarity for fluids to enter between the loosely connected galeae (Grant et al., 2012). In addition, it cannot be ruled out that rudimentary proboscises have other biological functions, as has been shown in the nonfeeding gelechid *Tuta absoluta* (Abd El-Ghany & Faucheux, 2022).

4.3 | Sensilla at the proboscis tip

The detection of flowers, flower-visiting behavior, and nectar uptake are controlled by a multitude of sensory organs including the eyes, antennae, and mouthpart sensilla (reviewed in Stöckl & Kelber, 2019). Various proboscis sensilla taste or sense the odor of nectar (Haverkamp et al., 2016; Kelber, 2003) while mechano sensilla localize the floral entrance and give information on surface properties during flower probing (Campos et al., 2015; Deora et al., 2019; Goyret, 2010; Goyret & Kelber, 2011). The sensilla of the proboscis tip control the proboscis insertion into flowers, the nectar uptake and provide feedback on flight muscle control (reviewed in Deora et al., 2019; Stöckl & Kelber, 2019). Our results show that the proboscis apex has a unique and characteristic arrangement of sensilla in all nectar-feeding Sphingidae, which we have termed the sphingid type. All tip sensilla are short and barely protrude from cuticular depressions. This has been believed to protect the sensilla from mechanical stress during the probing of food sources (Faucheux, 2013). Such sensory equipment was previously described in the sphingid *M. sexta*, where the ultrastructural features, neurophysiological results as well as experimental approaches have revealed the olfactory sensibility of the proboscis tip (Haverkamp et al., 2016). Since we found the same arrangement of sensilla in the present study, we conclude that all nectar-feeding sphingid moths are likely capable of detecting the scent of flowers with the tip of their proboscis.

The sensilla equipment of all regularly feeding Sphingidae, including *A. atropos*, shows a slight variation in types and distribution. Structurally, sensilla chaetica, sensilla basiconica, and sensilla styloconica exhibit only minor differences in size. As the mechanoreceptive function of aporous bristle-shaped sensilla has been widely accepted (Faucheux, 2013; Gnatzy & Tautz, 1980), sensilla chaetica probably perceives tactile stimuli throughout the entire length of the proboscis. In nectar feeders, apical bristle-shaped sensilla are responsible for detecting the opening of corolla tubes, which is also assumed for Sphingidae (Bauder et al., 2011; Deora et al., 2019; Krenn, 1998). Sensilla spread throughout the length of the proboscis are supposed to provide information on corolla diameter as well as proboscis insertion depth (Faucheux, 2013; Krenn, 1998). In most Sphingidae, the sensilla chaetica are short, rather inconspicuous and only barely protrude above the galea surface at the distal region of the proboscis. Due to their ultrastructure, sensilla basiconica are regarded as contact chemosensilla (Faucheux, 2013; Krenn, 1998; Walters et al., 1998). Depending on their positioning on the proboscis, they are stimulated at first contact with the food source or after deeper insertion of the galeae into the liquid (Faucheux, 2013). Internal sensilla basiconica, which functions as taste receptors inside the food canal (Inoue et al., 2009), presumably gives information about liquid flow (Krenn, 1998). Like in other studied lepidopteran proboscises, sensilla basiconica are positioned in one row along the inner wall of the food canal. Their numbers were found to increase with proboscis length in Hesperiidae (Krenn & Bauder, 2017) as well as in the presently studied Sphingidae. The number and micromorphology of sensilla styloconica often varies greatly in the feeding guilds of various Glossata, particularly in butterflies (e.g.; Bauder et al., 2013; Faucheux, 2013; Krenn et al., 2001; Lehnert et al., 2016; Ma et al., 2019; Zenker et al., 2011). In contrast to the high diversity in length, shape, and arrangement of sensilla styloconica in Noctuoidea, Nymphalidae, and Riodinidae (Bauder et al., 2013; Büttiker et al., 1996; Krenn et al., 2001; Lehnert et al., 2021; Molleman et al., 2005; Pinterich & Krenn, 2020; Zaspel et al., 2011; Zenker et al., 2011), the sensilla styloconica of Sphingidae are small, uniform, and simple in micromorphology. Within the Sphingidae, they extend from cuticle cavities and their sensory cones extend out of the depression. While sensilla styloconica is restricted to the drinking region in butterflies, one row of greatly interspaced sensilla styloconica extends far into the proximal direction in sphingid moths. Similar sensory equipment of the proboscis tip that comprises only few and small uniform sensilla styloconica has previously been described in nectivorous, long-proboscid Riodinidae and Hesperiidae (Bauder et al., 2011, 2013; Krenn & Bauder, 2017; Lehnert et al., 2016). In Hesperiidae, the number of sensilla styloconica was found to be independent of proboscis length (Krenn & Bauder, 2017) as it is in the Sphingidae. We conclude that a proboscis drinking region with only a few and short sensilla styloconica is characteristic for Lepidoptera with a particularly long proboscis. This is most likely an adaptation for feeding from long and narrow floral tubes, as has been proven for Nymphalidae (Lehnert et al., 2016, 2021). Since the same arrangement of different sensilla types and equally small numbers of sensilla were found in all studied Sphingidae, it can be concluded that similar neuronal mechanisms guide all flower-visiting

Sphingidae while finding entrances to nectar spurs. Yet, it remains surprising that so few sensilla are responsible for the exploration of the flower surface. The sensilla at the apex of the piercing proboscis of *A. atropos* extend from deep depressions of the cuticle. This is reminiscent of the proboscises of fruit-piercing Charaxinae, which display, smooth sensilla styloconica typically arranged in a groove (Krenn et al., 2001; Molleman et al., 2005; Pinterich & Krenn, 2020). All sensilla of the proboscis tip in Sphingidae extend from more or less deep cuticle depressions. In the sphingid *M. stellatarum*, some particularly small spherical sensilla have been termed as sensilla coeloconica (Faucheux, 1978). Similar sensilla coeloconica, which are located in annular cuticle cavities close to the proboscis tip were found to replace the sensilla styloconica in some butterflies (Ma et al., 2019). In *A. atropos*, the sensilla termed as coeloconica have been considered as sensilla styloconica with small spherical sensory cones that are completely sunken in a cuticle pit at the pointed apex of the proboscis (Faucheux 2013). Comparative ultrastructural investigations of Lepidoptera, which could help to decide whether these sensilla share similar internal features or not, are missing. These results could be used to better discuss whether these sensilla belong to the same type or should be classified as separate types.

As has been described for the apex of other Lepidoptera with a rudimentary proboscis, the supposedly nonfeeding Sphingidae also bear a reduced set of sensilla. As mentioned, the presence of sensilla in various nonfeeding species indicates possible other functions of the rudimentary mouthparts such as checking egg-laying sites (Abd El-Ghany & Faucheux, 2022; Faucheux, 1978; Faucheux, 2013).

4.4 | Food source and morphological fine-tuning of the feeding apparatus

The proboscis features and head organs reflect morphological fine-tuning in context with length adaptations of the feeding apparatus to nectar spur size in nectivorous Sphingidae. The galea lumen is connected to the stipes pump and the cibarial suction pump is continuous with the central food canal of the proboscis. The biometric correlations of the galeal lumen and the volume of the stipes musculature can be interpreted as match in size of organs that are functionally depended. The significant correlation of stipes muscle volumes in short- and long-proboscid species reflect their function in proboscis movements: They are responsible for producing the hemolymph pressure necessary to uncoil the proboscis by increasing the pressure in the galeal lumen (Bänziger, 1971; Krenn, 1990; Wannenmacher & Wasserthal, 2003).

Similar results exist when comparing the volumes of the food canal and the suction pump. The volumes of the muscles of the suction pump showed significant size correlations in species with a proboscis shorter versus longer than the body. Such morphological fine-tuning occurs in skipper butterflies where interconnected organs of the feeding apparatus inside the head and the proboscis have been found (Krenn & Bauder, 2017). It has been suggested that the pressure drop produced by the suction pump correlates positively with the flow rate of the liquid passing through the food canal (Kornev & Adler, 2019). In this context, the remarkably large food canal in *A. atropos* could additionally help to overcome the flow resistance of the highly viscose honey (Garcia et al., 2005). Kornev et al. (2017), however, have suggested that *A. atropos* only invests little muscular energy in combating viscous drag when moving fluids through the proboscis. Viscosity alone seems not to be the sole determinant of the muscle strength required to move liquid through the proboscis. Apparently, the suction pump dimensions in the short-proboscid *A. atropos* and the long-proboscid *M. sexta* are similar. This has led to the conclusion that the pump chambers of the two species are theoretically interchangeable, without compromising the ability of *A. atropos* to take up honey (Kornev et al., 2017). In the probably only water up-taking species *M. tiliae*, the suction pump has the smallest dimensions compared to the other Sphingidae. In contrast to all other hawk moths, the compressor muscles, which are responsible for pushing liquid into the esophagus, are larger than the dilator muscles. This is in line with the biophysical considerations of Kornev and Adler (2019) that insects with very short proboscises expend more energy for swallowing than for the up-take of liquids through the food canal. This might be due to the hydrophilic properties of the cuticle that play an important role in fluid ingestion through the tiny openings at the tip and the transport of liquid through the rather slim food canal in Lepidoptera (Lehnert et al., 2013; Lehnert & Wei, 2019). This phenomenon is probably especially important in sphingids with a rudimentary and split proboscis. In these species, water feeding could be experimentally initiated and observations recorded the wettability of the mouthparts (Kernbach, 1962).

5 | CONCLUSIONS

All major established subfamilies of the Sphingidae (Kawahara et al., 2009) include nectar-feeding species and are characterized by the same proboscis morphology. Especially their hovering capabilities can be regarded as a precondition for the evolution of superlong proboscises, as uncoiling a very long proboscis only works in mid-air and not after landing on the flower. Co-evolutionary interactions of long-tubed flowers with Sphingidae must be seen as an evolutionary process that involves the hover-flight of big insects with correlatingly long proboscises (Haber & Frankie, 1989). This feeding organ enables access to large quantities of nectar in big flowers, which in turn are needed to compensate the energy-consuming flight behavior. However, it was shown that long-proboscid hawk moths are generalistic flower visitors (Haber & Frankie, 1989; Johnson et al., 2017; Miller, 1997) and the evolution of particularly long proboscises expands access to matching long nectar spurs in flowers of which some are specialized to use these insects as pollinators (Houlihan et al., 2019; Wasserthal, 1997).

The morphology of the proboscis apex is nearly identical in all flower-visiting hawk moths. Since all major lineages of the Sphingidae have been studied in this data set, it can be concluded that the characteristic sensilla equipment at the proboscis tip is plesiomorphic in this family and the sphingid-type belongs to the ground pattern of this moth family. The piercing proboscis is derived in the honey-feeding *A. atropos*, which has evolved in the Acherontiini, providing access to honeycombs as a high-energy food source. This adaptation is exceptional regarding the tip morphology and represents a new type of piercing mouthparts in Lepidoptera. Another evolutionary pathway has led to reduced proboscises in the Smerinthini. In these hawk moths, supposedly only water-feeding species have retained the basic set of organs of the feeding apparatus.

Proboscis length has previously been found to correlate with body size in Sphingidae (Haber & Frankie, 1989; Miller, 1997). The evolution of a greatly elongated proboscis is coupled with matching sizes of functionally connected pump organs that are necessary for proboscis movement and fluid uptake. Morphological costs of a proboscis length evolution include a larger volume of the muscles inside the galea, the enforcement of the associated stipes, and the cibarial suction pump. Since the dimensions of the feeding apparatus are dependent and related to body size, it can be concluded that functionally coupled organs increase or decrease according to the evolution of body size. An exception has been found in the sensory equipment of the proboscis, as the number and length of sensilla styloconica did not correlate with proboscis length in the studied hawk moths.

AUTHOR CONTRIBUTIONS

Caroline Reinwald: Investigation; methodology; data curation; visualization; writing—original draft; writing—review & editing; software. **Julia A.-S. Bauder**: Data curation; methodology; investigation; writing—review & editing; resources. **Florian Karolyi**: Visualization; writing—review & editing. **Michael Neulinger**: Writing—review & editing; investigation; data curation; software. **Sarah Jaros**: Investigation; writing—review & editing; data curation. **Brian Metscher**: Methodology; writing—review & editing. **Harald W. Krenn**: Conceptualization; writing—original draft; writing—review & editing; project administration; supervision.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request. Studied animals will be stored in the insect collection of the Faculty of Life Science (Department of Evolutionary Biology, University of Vienna, Austria).

ORCID

Harald W. Krenn http://orcid.org/0000-0003-4323-5099

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