

Stefan H. Eberhard · Harald W. Krenn

## Salivary glands and salivary pumps in adult Nymphalidae (Lepidoptera)

Received: 19 December 2002 / Accepted: 12 May 2003 / Published online: 24 June 2003  
© Springer-Verlag 2003

**Abstract** The salivary glands and salivary pumps were investigated by means of dissection and serial semithin sections in order to expose the anatomy and histology of Nymphalidae in relation to feeding ecology. The paired salivary glands are tubular, they begin in the head, and extend through the thorax into the abdomen. The epithelium is a unicellular layer consisting of a single cell type. Despite the uniform composition, each salivary gland can be divided into five anatomically and histologically distinct regions. The bulbous end region of the gland lies within the abdomen and is composed of highly prismatic glandular cells with large vacuoles in their cell bodies. The tubular secretion region extends into the thorax where it forms large loops running backward and forward. It is composed of glandular cells that lack large vacuoles. The salivary duct lies in the thorax and also shows a looped formation but is composed of flat epithelial cells. The salivary reservoir begins in the prothorax and reaches the head. Its cells are hemispherical and bulge out into the large lumen of the tube. In the head the outlet tube connects the left and right halves of the salivary gland, and its epithelial cells are flat. The salivary pump lies in the head ventral to the sucking pump and leads directly into the food canal of the proboscis. It is not part of the salivary gland but is derived from the salivarium. Both the thin cuticle of the roof of the salivary pump and the thick bottom are ventrally arched. Paired muscles extend from the hypopharyngeal ridges and obviously serve as dilators for the pump. A functional interpretation of the salivary pump suggests that when not in use, the dilators are not contracted and the pump is tightly closed due to its own elasticity. When the dilator muscles repeatedly contract, the saliva is forced forward into the food canal of the proboscis. The salivary gland anatomy was found to be similar to other Lepidoptera.

Furthermore, the histology of the salivary glands is identical in all examined butterflies, even in the species which exhibit specialized pollen-feeding behavior.

**Keywords** Salivary gland · Salivary pump · Salivarium · Functional anatomy · Lepidoptera · Nymphalidae

### Introduction

Lepidoptera are among the best investigated insects in terms of ecology and systematics. Despite this, some aspects of the anatomy and histology of adult Lepidoptera await detailed study, for example, the salivary glands.

In the head of insects a series of paired glands may occur in connection with the mouthparts; most frequently, as in adult Lepidoptera, these are the labial glands (Chapman 1998). In the caterpillars of Lepidoptera the labial glands produce silk (Chapman 1998), whereas the mandibular glands produce saliva (Weber 1933). The salivary glands are bean-shaped in the pollen-feeding basal taxa of Micropterigidae (Hannemann 1956), while in the Glossata they are tubular (Chapman 1998) and extend to the abdomen. Dauberschmidt (1933) described the anatomy of the intestine and the salivary glands of both the larvae and the adults of a number of Lepidoptera by dissection and touched on aspects of the histology. He distinguished several parts within the salivary glands, such as an unpaired duct, the paired ducts, and the secretion parts which were found to be enlarged in some species. Hakim and Kafatos (1974) described the anatomy, histology, ultrastructure, and physiology of the salivary glands of *Manduca sexta* (Linnaeus, 1764) (Sphingidae). Their study represents the only comprehensive investigation of a salivary gland in Lepidoptera. A detailed examination of the salivary glands in Papilionoidea is lacking up to now.

The salivarium lies below the hypopharynx and receives the duct of the salivary glands. It is composed of salivary muscles and suspensorial sclerites of the hypopharynx and is known as the salivary pump or, as

S. H. Eberhard (✉) · H. W. Krenn  
Institut für Zoologie,  
Universität Wien,  
Althanstrasse 14, 1090 Vienna, Austria  
e-mail: stefan.eberhard@univie.ac.at  
Fax: +43-1-42779544

termed by Snodgrass (1935), the salivary syringe. It can be reasonably expected that Lepidoptera possess a salivary pump like all other fluid feeding insects, however a detailed description of the functional anatomy is lacking and its biological role has never been discussed from a feeding ecological point of view.

There is no doubt that the saliva facilitates the intake of food. Saliva is not only admixed with the liquid food, but in the Glossata can also be discharged from the tip of the proboscis (Kirbach 1884; Weber 1933; Wigglesworth 1972). With regard to the elaborated pollen-feeding behavior of butterflies of the genus *Heliconius* (Nymphalidae), it has been proposed that saliva is essential for the extraction of amino acids from pollen grains which have been previously collected with the proboscis during flower-probing (Gilbert 1972; Boggs 1987; Penz and Krenn 2000).

This study concentrates on the anatomy and histology of the salivary glands and the functional anatomy of the salivary pump of three species of Nymphalidae (Papilionoidea). The salivary pump deserves special attention since it is the motor which drives the saliva out to the tip of the proboscis. The comparison with other Lepidoptera allows us to identify special features in Nymphalidae. Since saliva obviously plays a crucial role in the derived feeding behavior of nectar- plus pollen-feeding species of *Heliconius*, a derived anatomy of the salivary gland and the salivary pump can be anticipated in comparison to related but purely nectarivorous Nymphalidae.

## Material and methods

### Animals

Three nymphalid species were investigated: *Heliconius melpomene* (Linnaeus, 1758), *Dryas julia* (Fabricius, 1775), and *Vanessa cardui* (Linnaeus, 1758). The first two species belong to the Heliconiinae (Brown 1981), whereas *V. cardui* belongs to the Nymphalinae (Tolman and Lewington 1998).

Pupae of *H. melpomene* were purchased from Worldwide Butterflies and Lullingstone Silk Farm (Dorset, UK) and pupae of *D. julia* from London Pupae Supplies, The Granary Manor Farm (Oxford, UK). Eggs of *V. cardui* were obtained from Carolina Biological Supply Company (Burlington, N.C., USA) and raised in our laboratory on an artificial diet and thistle (*Cirsium arvense*, Asteraceae). The adult butterflies were kept in a greenhouse of the Institute of Ecology and Conservation Biology of the University of Vienna and in a climate chamber of the Institute of Zoology of the University of Vienna. They were maintained at a temperature of about 25°C, with 40% to 90% relative humidity and 12 h of light. The butterflies were fed with an artificial nectar (Alm et al. 1990), which mimics the nectar of *Lantana camara* (Verbenaceae), and with Butterfly Nectar (The Birding Company, Yarmouth, Me., USA). Pollen was provided to *H. melpomene* (Carlisan Blütenpollen, Pronatura, Ebreichsdorf, Austria).

### Light microscopic techniques

The salivary glands and pumps were investigated by studying serial semithin sections with a light microscope. The animals were fixed in Duboscq-Brazil and their bodies were divided up into head, pro-, meso-, metathorax, and abdomen. Animals were dehydrated with ethanol or with 2,2-dimethoxypropane. Single specimens were

embedded in ERL-4206 resin by vacuum impregnation and thereafter polymerized at 70°C for 20 h. Semithin sections (1 µm) were cut on a Reichert OmU3 microtome with glass knives and diamond knives. The sections were stained on a hot-plate at 90°C for about 30 s with a 1:20 diluted mixture of 1% azure II and 1% methylene blue in 1% borax solution. Selected sections were embedded in Araldite and photographed using a Nikon Microphot-FXA light microscope and a Nikon Coolpix 950 digital camera. A comprehensive description of the entire procedure is given in Pernstich et al. (2003).

Additionally, dissections of the salivary glands were conducted. Microscopic preparations of the complete salivary glands were embedded without dehydration in polyvinylalcohol on microscopic slides. In this way the entirety of the salivary glands could be examined under the microscope.

## Results

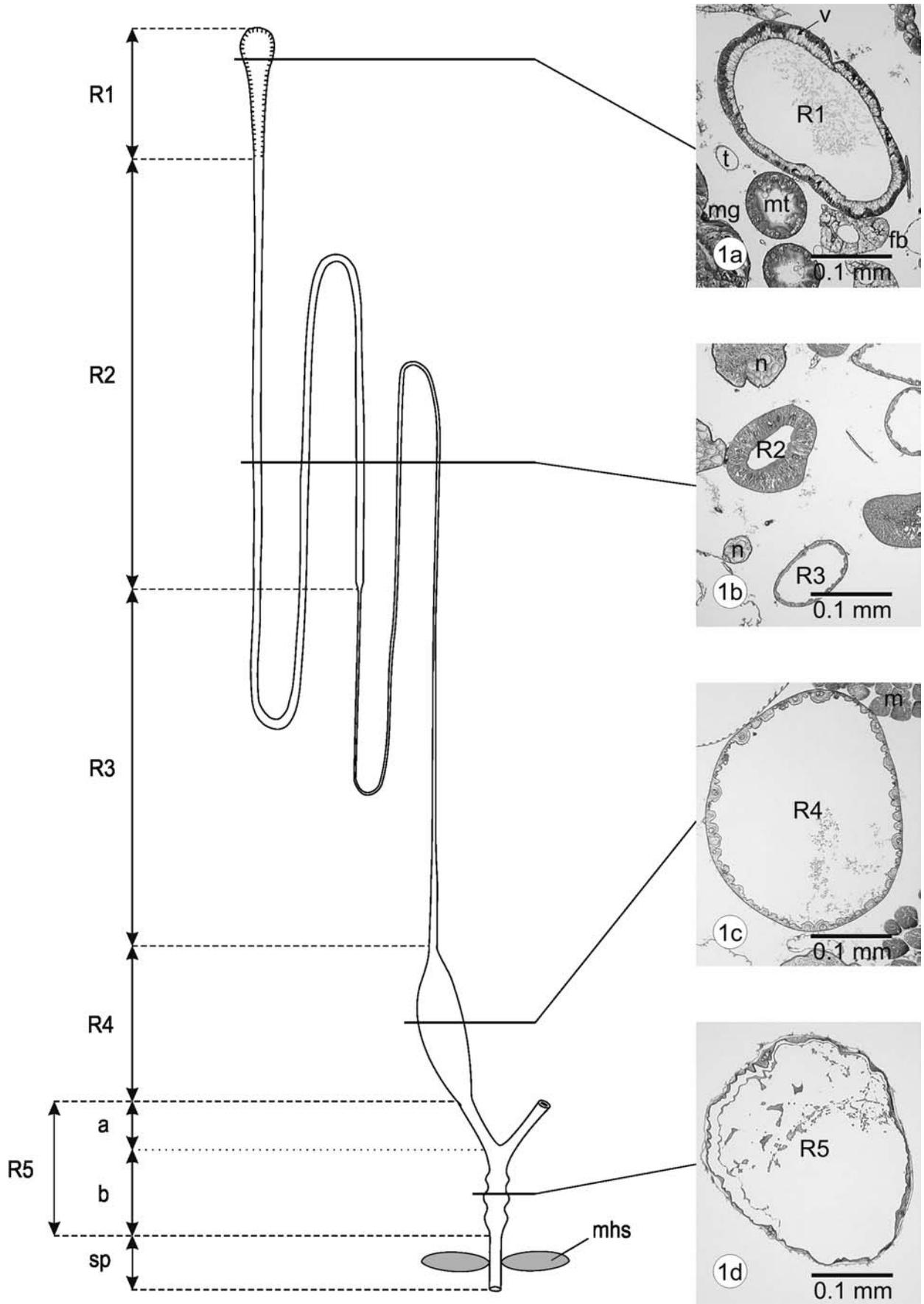
### Anatomy and histology of the salivary gland

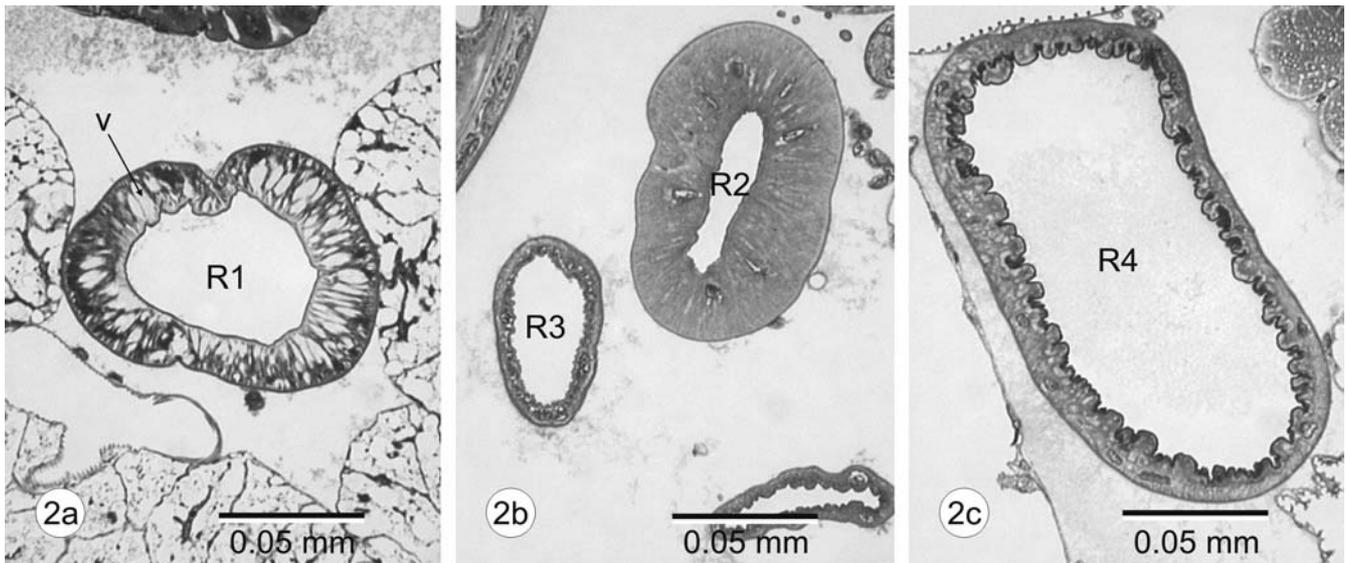
The gross morphology of the salivary gland is identical in all three investigated Nymphalidae. The salivary glands are paired and extend in the form of two, more or less convoluted tubes through the thorax into the abdomen. In the head the left and right salivary gland unite to form the unpaired salivary outlet tube, which leads to the salivary pump.

The five distinct regions of the salivary gland are recognized according to their histology and position within the body: bulbous secretion region (R1), tubular secretion region (R2), salivary duct (R3), salivary reservoir (R4), and salivary outlet tube (R5) (Fig. 1). These five regions are histologically different, each consisting of one particular cell type. The epithelium is always a unicellular layer. On the outer surface, i.e., toward the hemolymph, this layer is confined by a basement membrane. On the inner surface, i.e., toward the lumen of the gland, a cuticular intima is detectable in R4 and R5.

The salivary secretion regions, the bulbous end region (R1) and the tubular secretion region (R2), are composed of glandular cells. R1 has an epithelium about four times thicker than R2 (Figs. 1, 2), but it comprises only approximately 10% of the total length of the secretion part of the salivary gland (Fig. 1). This bulb-like end of the gland is located in the foremost

**Fig. 1a–d** Schematic illustration of the salivary gland and its division into distinct regions including the salivary pump (*left side*). Only one of the two salivary glands is shown in entirety. The different regions are not shown at the same scale, for example, the salivary pump (*sp*) is outlined as comparatively longer than the rest of the gland only for purpose of illustration. On the *right (a–d)* are semithin sections through the five regions of the salivary gland of *Heliconius melpomene*. **a** Cross-section of the bulbous secretion region (*R1*). **b** Cross-sections of both the tubular secretion region (*R2*) and the salivary duct (*R3*). These regions are side by side, due to forward and backward extending loops which overlap partially. **c** Cross-section through the salivary reservoir (*R4*). **d** Cross-section through the main part of the salivary outlet tube (*R5b*). *fb* Fat body, *m* muscle, *mg* midgut, *mhs* *musculus hypopharyngo-salivarialis*, *mt* Malpighian tubule, *n* nerve, *t* trachea, *v* vacuole





**Fig. 2a–c** Semithin sections through the first four regions (R1–R4) of the salivary gland of *Dryas julia*. **a** Cross-section of the bulbous secretion region (R1). This section does not exhibit the largest diameter of this region. **b** Cross-sections of both the tubular

secretion region (R2) and the salivary duct (R3). **c** Cross-section through the salivary reservoir (R4). This section also does not exhibit the largest diameter of this region. *v* Vacuole

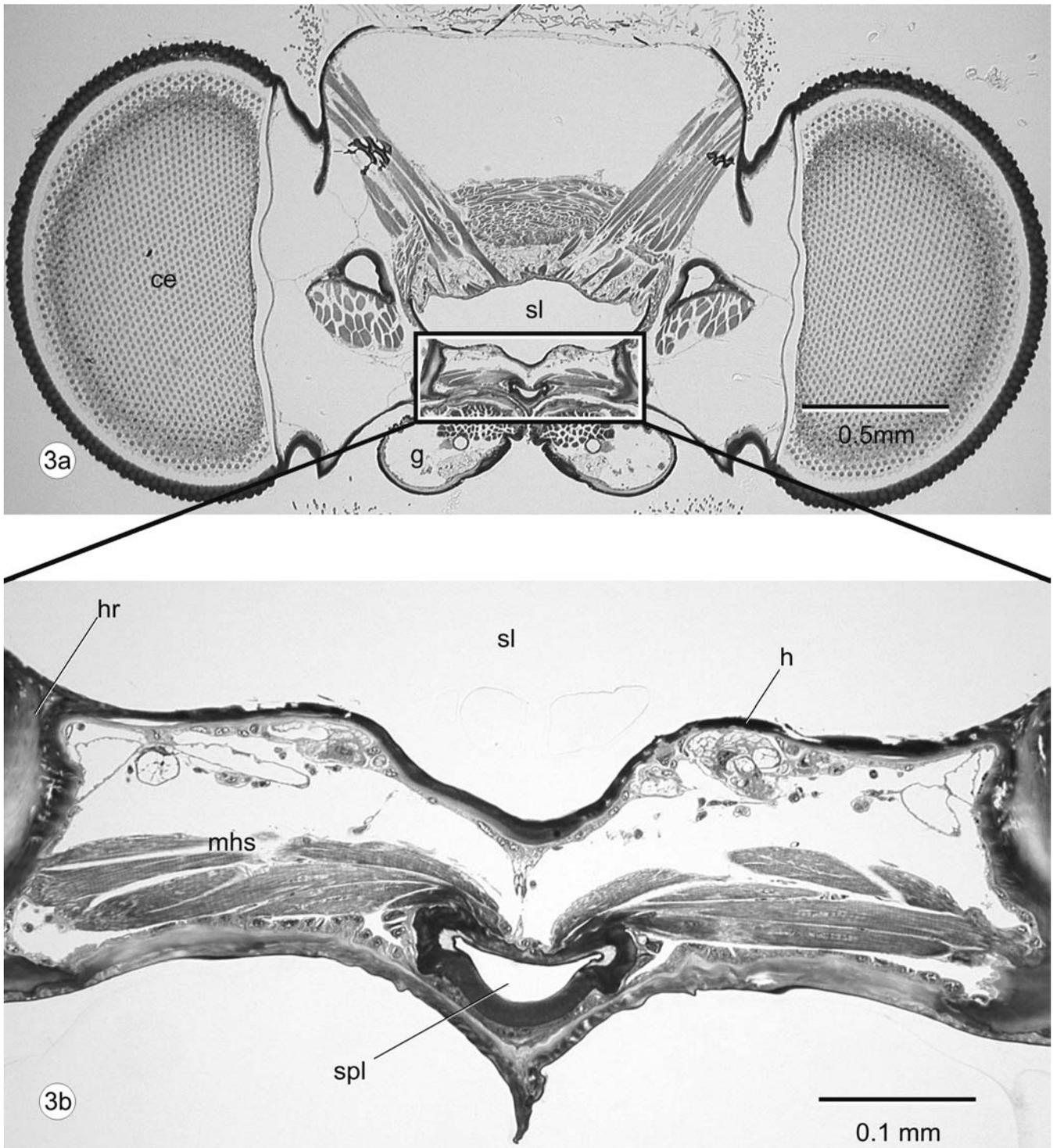
part of the abdomen next to the beginning of the midgut. It is composed of highly prismatic glandular cells with large vacuoles in their apical (lumen-orientated) parts. These vacuoles take up 80% of the volume of the cell bodies. Their position is identifiable by its translucent appearance (Figs. 1a, 2a). The nuclei of the cells lie close to the basement membrane. There is a gradual transition between R1 and R2 (Fig. 1). R2 is positioned within the thorax, lateral to the esophagus. It runs forward and backward in tight U-shaped loops and is strongly convoluted. This region is furthermore very well supplied with tracheae. In contrast to R1, the region is composed of highly prismatic glandular cells without large vacuoles (Figs. 1b, 2b). The nuclei are located in the middle of these cells. The salivary duct (R3) has a distinct boundary. The duct is located in the thorax lateral to the esophagus just as R2. It also is convoluted, but not as strongly as R2. Both regions overlap within the pro- and mesothorax. Therefore cross-sections of both R2 and R3 lie side by side (Figs. 1b, 2b). Within the metathorax only the salivary secretion region is detectable, i.e., the salivary duct maximally reaches to the beginning of the metathorax. The diameter of the salivary duct and that of the salivary secretion region may slightly vary in their courses. Histologically, R3 is composed of flat epithelial cells (Figs. 1b, 2b).

The major part of the salivary reservoir (R4) is situated within the prothorax but also reaches through the neck into the hindmost part of the head. There is no clear boundary between R3 and R4. However, the diameter of R4 increases rapidly and reaches its greatest extension within the prothorax. In the neck region and head the diameter of R4 decreases again. The largest diameter of

the lumen of R4 is approximately three times larger than the lumen of R3 (Figs. 1b, c, 2b, c). The lumen of R4 is spacious and the cells of the region are histologically conspicuous. They are not flat as the cells in R3, rather they are hemispherical and bulge out into the lumen of the tube (Figs. 1c, 2c). The conjunction to the salivary outlet tube is gradual and lies in the head.

The salivary outlet tube (R5) is also located in the head and consists of a paired and an unpaired part (Fig. 1). The paired part (R5a) connects the salivary reservoir with the unpaired outlet tube (R5b) so that the left and right salivary glands join together within R5. The major part of R5 is formed by the unpaired outlet tube, which extends forward ventral to the brain and connects the salivary gland with the salivary pump. Its lumen is conspicuously wide, and it approaches the width of the salivary reservoir. Both parts are histologically similar. The cell layer is a flat epithelium similar to that of R3, but a cuticular intima is detectable and the wall as a whole is slightly creased (Fig. 1d).

The histological comparison of the salivary glands in its various regions reveals a striking similarity between the three investigated species, the pollen-feeding *H. melpomene* (Fig. 1a–d), the non-pollen-feeding *D. julia* (Fig. 2), both Heliconiinae, and *V. cardui* which belongs to the Nymphalinae. Because of the great resemblance of the salivary glands of *D. julia* and *V. cardui*, only cross-sections of the former are illustrated. The cells of R1 of *D. julia*, *V. cardui*, and *H. melpomene* show the same typical large vacuoles. The vacuoles are easily detectable as a clear area in the cross-section shown in Fig. 2a. The cross-section was, however, not made at the largest diameter of R1, therefore the lumen seems to be small in comparison to that of R1 of *H. melpomene* (Fig. 1a). The salivary



**Fig. 3a, b** Cross-section through the head of *H. melpomene*. **a** Overview, showing the lumen of the sucking pump (*sl*) and the salivary pump below. **b** Detail of *boxed area* showing the salivary

pump (*spl*) and its dilator muscles (*mhs*) that extend between the hypopharyngeal ridges (*hr*) which connect the hypopharynx (*h*) to the ventral head capsule. *ce* Compound eye, *g* galea

secretion region (R2) and the salivary duct (R3) of *D. julia* (Fig. 2b) and *V. cardui* are developed as well as those in *H. melpomene* (Fig. 1b). R2 is composed of highly prismatic cells which contain no vacuoles, whereas R3 consists of flat cells. The salivary reservoir (R4) of *D. julia* (Fig. 2c)

and *V. cardui* has the same type of cells as the reservoir in *H. melpomene* (Fig. 1c). The region of the reservoir visible in Fig. 2c represents a marginal area and not the area with the largest diameter.

## Salivary pump

The salivary pump as a whole represents a fusion of the hypopharynx with the labium which encloses the salivarium. It is situated ventral to the well-developed sucking pump (Fig. 3). The salivary pump is connected with the labium on the ventral side of the head. Due to the dark coloration of the cuticle the salivary pump is easily distinguishable. The ventral side of the pump is very thick and consists of strongly sclerotized cuticula which is ventrally arched. On each side the cuticle is thick and strongly sclerotized, and it turns downward in a median direction. There, the cuticle is thinner and slightly sclerotized. This part composes the roof of the salivary pump. It is ventrally arched so that the lumen of the salivary pump resembles a downwardly arched crescent (Fig. 3b). It corresponds with the ventral part of the hypopharynx and the floor is derived from the dorsal side of the labium. Musculature extends from the hypopharyngeal ridges near the connection to the underside of the head capsule median to the roof of the salivary pump. Because of its association with the salivarium, this paired muscle is termed *musculus hypopharyngeo-salivarialis* (according to Kristensen 1968) and obviously functions as a dilator (Fig. 3b). The salivary pump has its orifice at the base of the proboscis below the sucking pump and it leads directly into the food canal of the proboscis. No significant anatomical differences were detected among the salivary pumps of *H. melpomene*, *D. julia*, and *V. cardui*.

## Discussion

### Salivary glands and salivary pumps

The present study is the first to focus on the anatomy and histology of the salivary glands and the functional anatomy of the salivary pump in adult Nymphalidae. Both organs form a functional unit for production and emission of saliva. Throughout the tubular glands various regions could be recognized based on anatomical and histological characters. In principle, a similar arrangement and composition has been described for *M. sexta* (Sphingidae) (see Hakim and Kafatos 1974) and a number of lepidopteran species (Dauberschmidt 1933). In the examined Nymphalidae and in *M. sexta* the glandular parts are characterized by highly prismatic cells and are presumably responsible for the production of the major quantities of the salivary fluid. In *M. sexta*, the production of the enzyme invertase and active  $K^+$  ion transport into the saliva was found in this region (Hakim and Kafatos 1974). The salivary duct is composed of flat epithelial cells in Nymphalidae and in *M. sexta*. It conveys saliva from the secretion region to the following enlarged region which is composed of specially shaped cells. In the examined Nymphalidae it may serve as a reservoir since this part was evidently filled with saliva during dissections. The physiological investigation in *M. sexta* indi-

cated that the saliva is modified in this region (Hakim and Kafatos 1974). Dauberschmidt (1933) presumed that this region is an expandable reservoir equipped with a muscular syringe. The first presumption could be demonstrated by our results, however the second could not be confirmed since no muscles are found in this region.

All three presently studied butterfly species reveal an identical composition in their salivary glands. The comparison of the gland histology of the pollen-feeding *H. melpomene* with that of the non-pollen-feeding *D. julia* (both Heliconiinae) and with *V. cardui* (Nymphalinae) indicated no differences.

The salivary duct opens into the salivarium which is formed by the hypopharynx and labium and is equipped with muscles (Snodgrass 1935). In the most basal taxon of Glossata, the Eriocraniidae, the musculature of the salivarium consists of a dilator muscle which originates on the hypopharynx and attaches to the salivarium and a second dilator muscle whose short fibers connect the salivarium to the hypopharyngeal floor (Kristensen 1968). As in other Ditrysia (Schmitt 1938), the Nymphalidae have only one pair of dilator muscles. Its attachment sites to the hypopharynx suggests that it is homologous with the dilator muscle (*m. hypopharyngeo-salivarialis anterior*) of Eriocraniidae.

A salivary pump has not been previously identified in *M. sexta* (Hakim and Kafatos 1974; Eaton 1988) and *Pieris brassicae* (Linnaeus, 1758) (Pieridae) (Eastham and Eassa 1955). It was believed that saliva flows passively out of the outlet tube which is closeable by the dilator muscles. This condition differs distinctly from that in the examined Nymphalidae where the saliva first enters the salivary pump and then is forced into the food canal of the proboscis. We clearly demonstrate that the salivarium, at least in the Papilionoidea, is not merely an opening of the salivary glands as indicated by Eastham and Eassa (1955) who did not attribute a pumping function to any structure.

### Discharge of saliva

It is unlikely to suppose that the flow of saliva through the entire length of the proboscis, which can reach considerable lengths, would rely entirely on passive means. Instead, saliva is actively discharged prior to the uptake of any food. The reconstruction of the serial sections of the salivary pump allows the following interpretation of the functional mechanism. At rest, the paired salivary pump dilators (*m. hypopharyngeo-salivarialis*) are not contracted yet the salivary pump is closed tightly because of the elasticity of the thin roof. Contraction of the dilators raises the roof and the saliva can flow into the free space. When the dilators relax, the roof of the salivary pump jerks back to the rest position and therefore the saliva is forced into the food canal. If this operation is rapidly repeated, a more or less continuous flow of saliva into the direction of the proboscis tip is produced.

The ability to discharge saliva is often neglected in studies on the feeding behavior of Lepidoptera, possibly because the proboscis operates like a drinking straw and the saliva cannot be easily observed. However, various butterflies have been observed to discharge a fluid from the proboscis to dissolve foods for uptake, for example *Morpho peleides* Kollar, 1850 (Nymphalidae) (Knopp and Krenn 2003) and *Vanessa atalanta* (Linnaeus, 1758) which was observed to leave small traces of moisture on a dry stone after touching it with the proboscis tip (Krenn, unpublished). In both cases it was evident that small quantities of fluid, probably saliva, are extruded as a solvent for sugar or minerals. The most remarkable example is found in the neotropical species of the genus *Heliconius*, which do not exclusively feed on nectar, but also actively collect pollen from the anthers of flowers with their proboscis (Gilbert 1972). The butterflies discharge a clear liquid from the tip of the proboscis which soaks into the load of pollen on the proboscis. These actions lead to the extraction of amino acids from the pollen grains which serve as an important source of nitrogen and are essential for reproduction (Gilbert 1972). The liquid which is used in pollen feeding is not regurgitated nectar (Boggs 1987) and therefore might be saliva. Although saliva obviously plays an important role in the feeding ecology, the histological composition of the salivary glands of *H. melpomene* does not differ from that of other pure nectar-feeding Nymphalidae.

**Acknowledgements** We are grateful to the Institute of Ecology and Conservation Biology (University of Vienna) for kindly granting us permission to use their greenhouse and to J. Plant for linguistic help.

## References

- Alm J, Ohnmeiss TE, Lanza J, Vriesenga L (1990) Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia* 84:53–57
- Boggs CL (1987) Ecology of nectar and pollen feeding in Lepidoptera. In: Slansky F, Rodriguez JG (eds) Nutritional ecology of insects, mites, spiders, and related invertebrates. Wiley, New York, pp 369–391
- Brown KS Jr (1981) The biology of *Heliconius* and related genera. *Annu Rev Entomol* 26:427–456
- Chapman RF (1998) The insects: structure and function, 4th edn. Cambridge University Press, Cambridge, UK
- Dauberschmidt K (1933) Vergleichende Morphologie des Lepidopteren Darmes und seiner Anhänge. *Z Angew Entomol* 20:204–267
- Eastham LES, Eassa YEE (1955) The feeding mechanism of the butterfly *Pieris brassicae* L. *Philos Trans R Soc Lond B Biol Sci* 239:1–43
- Eaton JL (1988) Lepidopteran anatomy. Wiley, New York
- Gilbert LE (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proc Natl Acad Sci USA* 69:1403–1407
- Hakim RS, Kafatos FC (1974) The structure and salivary function of the labial gland in adult *Manduca sexta*. *Tissue Cell* 6:729–750
- Hannemann HJ (1956) Die Kopfmuskulatur von *Micropteryx calthella* (L.) (Lep.): Morphologie und Funktion. *Zool Jahrb Anat* 75:177–206
- Kirbach P (1884) Über die Mundwerkzeuge der Schmetterlinge. *Arch Naturgesch* 50:78–119
- Knopp MCN, Krenn HW (2003) Efficiency of fruit juice feeding in *Morpho peleides* (Nymphalidae, Lepidoptera). *J Insect Behav* 16:67–77
- Kristensen NP (1968) The anatomy of the head and the alimentary canal of adult Eriocraniidae (Lep., Dacnonypha). *Entomol Medd* 36:239–292
- Penz CM, Krenn HW (2000) Behavioral adaptations to pollen-feeding in *Heliconius* butterflies (Nymphalidae, Heliconiinae): an experiment using *Lantana* flowers. *J Insect Behav* 13:865–880
- Pernstich A, Krenn HW, Pass G (2003) Method for preparation of serial sections of arthropods using 2,2-dimethoxypropane (DMP) dehydration and epoxy resin embedding under vacuum impregnation. *Biotech Histochem* 78:5–9
- Schmitt JB (1938) The feeding mechanism of adult Lepidoptera. *Smithson Misc Coll* 97:1–28
- Snodgrass RE (1935) Principles of insect morphology. McGraw-Hill, New York
- Tolman T, Lewington R (1998) Die Tagfalter Europas und Nordwestafrikas. Franckh-Kosmos, Stuttgart
- Weber H (1933) Lehrbuch der Entomologie. Fischer, Jena
- Wigglesworth VB (1972) The principles of insect physiology, 7th edn. Wiley, New York