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“Similar body size, variable proboscis length –
comparison of mouthpart morphology of two euglossine
bee species (Hymenoptera, Apidae, Euglossini)“

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CONTENTS

1	Introduction.....	1
1.1	Evolution of long-tongued insects.....	1
1.2	Mouthpart morphology of the honeybee.....	1
1.3	Food intake and capillary mechanisms.....	2
1.4	Euglossine bees.....	3
1.5	Aim of the present study.....	3
2	Material and Methods.....	5
2.1	Studied species.....	5
2.2	Microscopic observations and measurements.....	5
2.3	Experiments on nectar adhesion.....	6
2.4	Statistics.....	7
3	Results.....	8
3.1	Morphometry of the proboscis.....	8
3.2	Micromorphology of the proboscis.....	11
3.3	Nectar adhesion.....	17
4	Discussion.....	19
4.1	Morphometry and micromorphology.....	19
4.2	Nectar intake.....	20
4.3	Long-tongued flower visiting insects.....	21
5	Abstract.....	23
6	Zusammenfassung.....	24
7	References.....	25
8	List of figures.....	29
9	List of tables.....	29
	Acknowledgements.....	30
	Curriculum Vitae.....	31

1 INTRODUCTION

1.1 EVOLUTION OF LONG-TONGUED INSECTS

Flower visiting insects usually search for food on flowers and may act as pollinators while carrying pollen from one flower to another. Insects are adapted to this feeding behavior by the aid of sensory abilities (e.g. color vision), advanced flight abilities and mouthpart morphology. The flower's rewards for the insects are food (most common are pollen and nectar), places for protection or ovoposition sites. This giving and taking result in a mutually beneficial relationship (Kevan & Baker 1983, Proctor et al. 1996, Labandeira 1997, Grimaldi 1999, Krenn et al. 2005, Gullan & Cranston 2010). According to Darwin (1862) the association between long-tongued insects and deep corolla flowers has been supposed to be the result of a coevolution of plants and pollinators. This leads to reciprocal adaptations of plants and their pollinators and is supported by assumption of direct selection on corolla tube length used by one single pollinating species. The elongated mouthparts associated with nectar feeding evolved in glossatan Lepidoptera, some Diptera and multiple times in Hymenoptera. The hymenopteran proboscis is formed by constituent maxillary and labial components of the mouthpart that are linked basally. Characteristic for an elongated proboscis are a food canal, sealed by the elongated galeae and labial palps, a specialized tip region and a powerful suction pump in the head. In Apoidea, the food canal is assembled anew each time for feeding by unfolding the mouthparts, while the hairy glossa functions as a tongue (Grimaldi 1999, Borrell & Krenn 2006). The access to and sucking up of the concealed nectar of very long and slender corolla tubes is determined by the proboscoidal length (Zander 1946, Winston 1991, Proctor et al. 1996).

1.2 MOUTHPART MORPHOLOGY OF THE HONEYBEE

Like in most insects mouthparts of bees consist of four components: the unpaired labrum, the paired mandibles, the paired maxillae and the unpaired labium (Fig. 1).

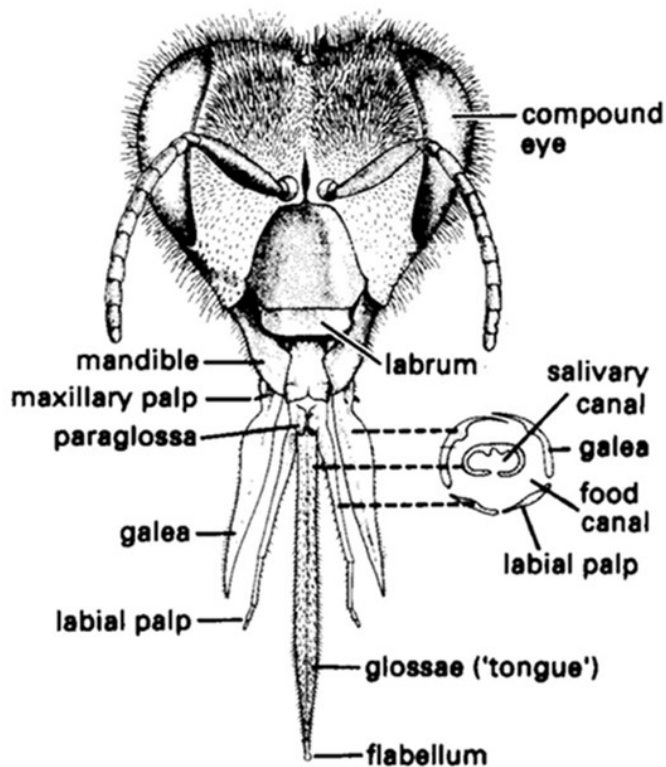


Fig. 1: Mouthparts of the honeybee referred to Gullan & Cranston (2010).

transporting it into the food canal by lapping movements. Proximally, the food canal empties into the functional mouth leading to the cibariopharyngeal suction organ (Snodgrass 1956, Michener & Brooks 1984, Gullan & Cranston 2010, Wu et al. 2015). The hairy glossa consists of hardened annules for strength alternating with soft membranous areas for flexibility (Winston 1991). In resting position the mouthparts are apart and the proboscis is folded backward beneath the head (Snodgrass 1956).

1.3 FOOD INTAKE AND CAPILLARY MECHANISMS

While feeding the maxillary parts of the proboscis and the labial palps remain relatively motionless and only the glossa with the flabellum performs licking movements. During extension, nectar adheres onto the glossa by capillarity because hairs expand outward. The glossa full of nectar is retracted while the hairs increase the resistance to fluid movement in the food canal (Kingsolver & Daniel 1995). This repeated extension and retraction of the glossa is called the licking cycle, consisting of three stages: 1) Glossal extension: Nectar is loaded onto the surface of the hairy glossa, followed by 2) Glossal retraction: Drawing of nectar into the food tube and finally 3) Unloading: Nectar is

The proboscis is formed by the maxillae and the labium and is adapted for lapping up fluid (Zander 1946, Winston 1991). The elongated parts of the proboscis (galeae, labial palps, glossa) come together building the labiomaxillary complex (LMC) by a basal sclerit, the lorum. The spatulate flabellum contains the opening for discharging salivary gland secretions (Snodgrass 1956). The principal organ of fluid loading is the long and flexible glossa. It is densely covered with erectile hairs forming a brush for adhering nectar and

removed from the glossa and transported into the mouth (Kingsolver & Daniel 1995). Movement of fluid is facilitated by back and forth glossal movement, capillary action, and pumping by the muscles of the cibarium which generate suction for ingestion (Winston 1991). The cibarium is developed into a highly efficient sucking pump surrounded by several pairs of dilator muscles (Snodgrass 1956, Winston 1991). Lateral muscles produce a powerful pump by the cibarium becoming a closed chamber connected to the food canal (Chapman 2013). A comparison of the protracted and retracted glossae of euglossine bees (*Euglossa imperialis* and *Euglossa championi*) showed that the functional length of the proboscis can be increased by protraction of the glossa (Gruber 2013) leading to the assumption that the feeding mechanism in euglossine bees is similar.

1.4 EUGLOSSINE BEES

Species of the genus *Euglossa* are of small to moderate size, brightly colored and encompass over 100 species of which 32 occur in Costa Rica. Orchid bees have a very fast flight and are important pollinators for a great variety of orchids. Male orchid bees collect perfumes from flowers and other sources, which they store in a bouquet of fragrances and later emit at mating sites (Dressler 1982, Eltz et al. 2005). A specific orchid species may attract only males of one single bee species, thus the relationship between the orchid flower and the euglossine bee is often highly specific (Williams & Whitten 1983). Orchid bees feed on nectar and their proboscis at least reaches to the base of the metasoma in resting position or in some representatives lengths of twice the body length (Roubik & Hanson 2004). These long tongues may be a result of mouthpart evolution to gain access to a wider variety of nectar resources. The morphology of the proboscis influences the visit of long-tubed flowers by the flower handling time, the quantity of nectar they must consume to fuel their activities, and the sugar concentration of the nectar they can consume (Kevan & Baker 1983, Borrell 2003).

1.5 AIM OF THE PRESENT STUDY

The two species *Euglossa imperialis* and *Euglossa championi* (Fig. 2) investigated in this study are widely distributed in the Piedras Blancas National Park in the Golfo Dulce region in Costa Rica. Both *Euglossa* species are relatively similar in body size but show a great difference in proboscis length. Based on morphometric data of the body and the proboscis showing similar basic structures of the proboscis but a striking difference in the much

longer distal parts (Gruber 2013), the present study aims to compare details of the morphometry and micromorphology of the proboscis of these two closely related species, and supports the presumption of adaptation to deeply concealed corollas. The comparison of a longer with a shorter proboscis within one genus should allow conclusion of allometric relation and the costs and benefits of an extremely elongated proboscis.

2 MATERIAL AND METHODS

2.1 STUDIED SPECIES

Male individuals of the two analyzed species *Euglossa imperialis* and *Euglossa championi* (Fig. 2) were collected by Maria Helene Gruber in the Piedras Blancas National Park in the Golfo Dulce region in Costa Rica in 2010. Because of their perfume collecting behavior male orchid bees can be easily attracted by fragrance baits (Rasmussen 2009). Orchid bees were attracted by fragrance baits (cineol, eugenol, methyl salicylate), captured and fixed in 70% ethanol (Gruber 2013). Due to the fact that females do not show this behavior, only male bees were analysed in this study.

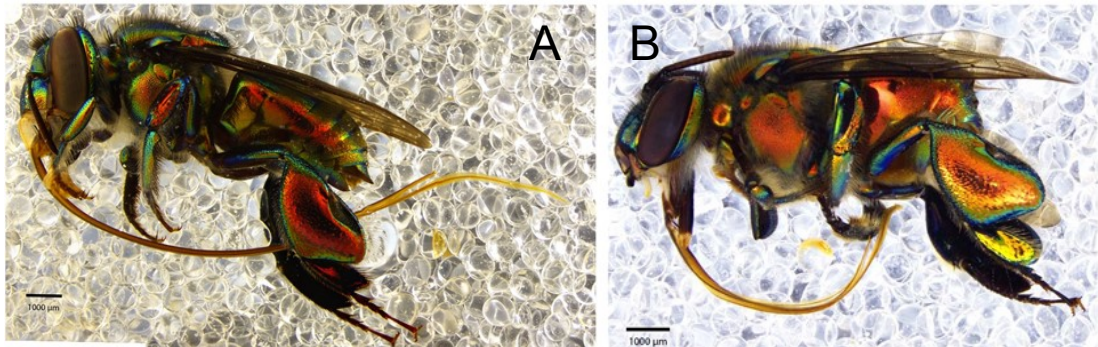


Fig. 2: Examined orchid bees. *Euglossa imperialis* (COCKERELL 1922) (A) has a similar body size but a much longer proboscis than *Euglossa championi* (CHEESMAN 1929) (B).

2.2 MICROSCOPIC OBSERVATIONS AND MEASUREMENTS

Mouthparts of six individuals of each species were removed and embedded in glycerin on microscope slides and covered with a coverslip. For measurements of mouthpart lengths as well as hair lengths supplements were examined with a Nikon Laborshot-2 and drawn with a 1.25x drawing tube. Mouthpart regions and hairs were each drawn three times with a measurement error of 0.28 % and 0.67 %, respectively, and a mean value was calculated. Sensilla of the glossae of six specimens of each species were counted using a light microscope.

Images of the whole bees and of the mouthparts were taken with a Nikon SMZ25 Stereomicroscope and the imaging program NIS elements software (Nikon Instruments, Japan), afterwards edited with Adobe Photoshop CS6. Light microscopy and Adobe Illustrator CS6 were used to clarify the investigation of shape and length of hairs on the elongated parts of the bee's proboscis (Fig. 3).

Therefore the elongated glossa was subdivided into a tip, middle and basic region (Fig. 3B). The tip region is the most distal region including the flabellum at the apex and the distal portion of the glossa which bears many long, broad and distant hairs. The middle region begins when the broad hairs become thin, shorter and more adjacent to the cuticle. The outward appearance of middle and basic region does not differ obviously, thus the basic region only describes the basic link of the glossa in the observations. Lengths of hairs are measured at ten positions evenly distributed along the tip region (1=near the flabellum; 10=near the basic line of tip region) (Fig. 3B). Noted were the averaged data in mm and the standard deviation.

For examination of the external surface scanning electron microscopy (SEM) was used. Therefor mouthparts were dehydrated in ethanol series (90%, 95%, 100%) each for 10 – 20 minutes and finally in 100 % acetone. After drying in a critical point dryer (Leica EM CPD300) mouthparts were fixed on stubs with graphit tape and conductive silver, and subsequently coated with gold in a sputtercoater (Joel JFC-2300 HR). Specimens were scanned with a Philips XL 30 ESEM and photos were edited with Adobe Photoshop CS6.

2.3 EXPERIMENTS ON NECTAR ADHESION

For studying nectar adhesion glossae of ten individuals of each species were removed and air dried. Dry glossae were weighed with a micro scale (Premium Mikrowaage Sartorius Lab Instruments, clearness of display 0.001 mg) and immersed in a 30 % sugar solution for 30 seconds. Afterwards they were weighed again. Weight data of the dry glossae were subtracted from the nectarous glossae to conclude on the amount of adhering sugar solution. Via the density of 1.1270 g/ml of a 30 % sugar solution (Holtzhauer 1997) it was possible to calculate the volume of the measured mass (Tab. 3). For comparison the nectarmass of both species was constituted in a box plot diagram generated with Sigma Plot 12.5. The measurement error was 3.27 %.

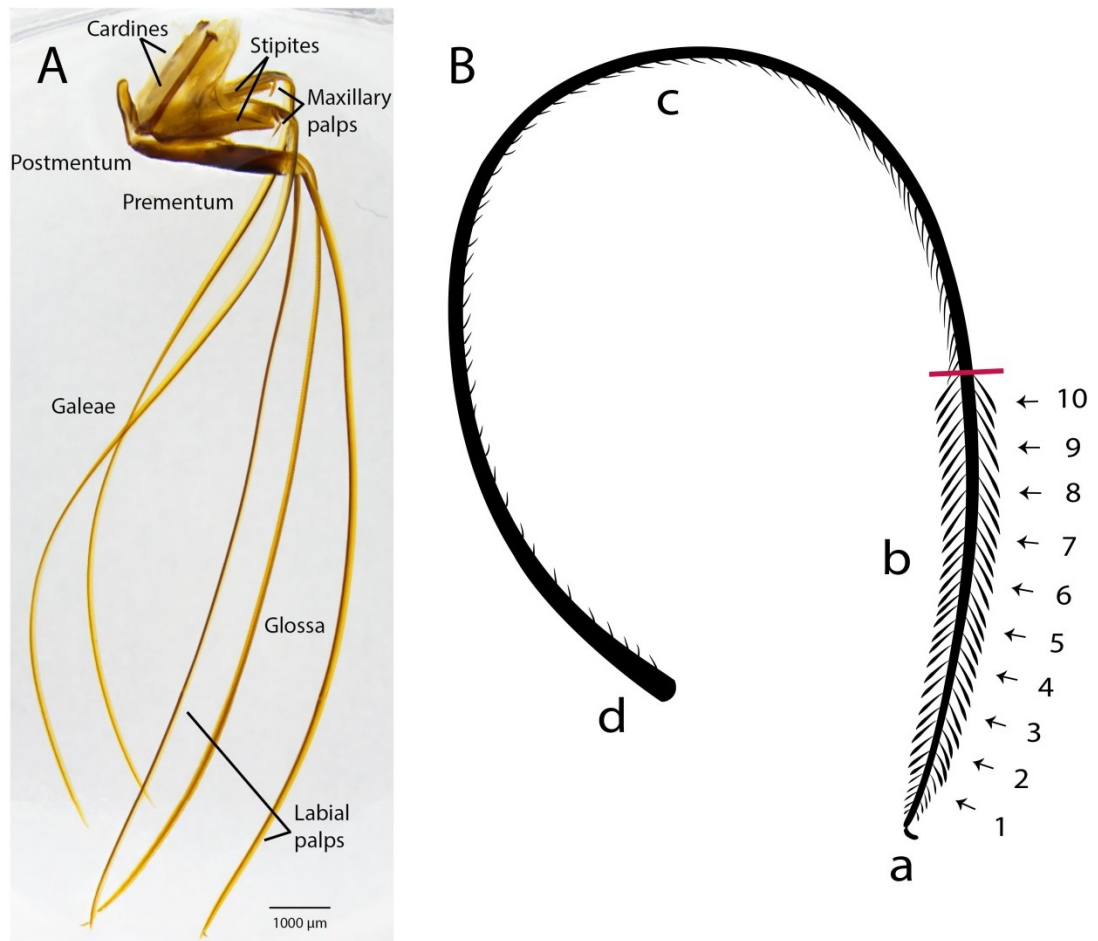


Fig. 3: Mouthpart components of the proboscis of *E. imperialis* (A). Schematic draw of the glossa (B) (a flabellum, b tip region, c middle region, d basic link). The bar marks the crossing of the tip to the middle region. The basic link forms the base of the glossa. Arrows show the ten positions where lengths of hairs were measured .

2.4 STATISTICS

Statistical analyses were conducted using IBM SPSS Statistics 21. For exploiting morphometric data the nonparametric Mann-Whitney-U-Test was used, while signification level was set at $p < 0.05$.

3 RESULTS

3.1 MORPHOMETRY OF THE PROBOSCIS

The morphometric measurements of the proboscides of six specimens of the two species showed significant differences in the length of the glossa, galeae and labial palps. The glossal length averaged 22.17 mm in *E. imperialis* and was twice as long as in *E. championi*. The same proportion was found in the length of the tip region (Tab. 1). The length of the apical flabellum of *E. imperialis* was 0.19 mm; it is significant longer than that of *E. championi* with 0.14 mm, however not twice as long. The total lengths of the tip region, the apical flabellum and the entire length of the glossa were greater in *E. imperialis*. However relative lengths were significantly greater in *E. championi* (Tab. 1). The measurements of hairs on the glossal tip region illustrated a significant difference between these two species (Tab. 1). The hairs were measured along the tip region from the distal tip to the basal limit and showed an increase along the length in both species (Fig. 4). Hair lengths increased from the apex to the middle region where the lengths abruptly decreased. The hairs of *E. imperialis* were significant longer than that of *E. championi* in all regions. The results of the hair measuring on the middle region and the basic link of the glossa showed a significant difference in the length of hairs (Tab. 1, Fig. 4). The hairs of the middle region of the glossa were smaller than at the tip region and the ones at the basic region were smaller than those in the middle. In either case the hairs of *E. imperialis* were little longer than that of *E. championi*, but not twice as long.

The glossa is composed of cuticle annuli where hairs extent outward and which are significantly greater in *E. imperialis* (Tab. 2). The length of annuli on the glossal tip region was 0.078 mm in *E. imperialis* and 0.059 mm in *E. championi*. Annuli of *E. championi* had 75.64 % the length of *E. imperialis*. Comparing the average lengths with the length of the entire glossa, there were 305 annuli on the glossa of *E. imperialis* and 199 annuli on the glossa of *E. championi*.

The microtrichia lengths on the galeae did not show a significant difference between the species, however that one of the labial palps were significant longer in *E. imperialis*. In the tip region of the labial palps of both species microtrichia were longer than in the mid region (Tab. 1). The comparison between the two species showed the almost same

microtrichia length on the galeae and ones significant longer on the labial palps of *E. imperialis* (Fig. 5).

Tab. 1: Measurements on proboscis of *E. imperialis* and *E. championi*. Morphometric data of the glossa, galeae and labial palps.

N = 6	Mean (mm) ± SD		Mann-Whitney-U-Test
	<i>E. imperialis</i>	<i>E. championi</i>	
<u>Glossa</u>			
Total length	22.17 ± 2.35	10.88 ± 0.87	Z = -2.882; P = 0.004
Tip region length	6.26 ± 0.30	3.84 ± 0.06	Z = -2.887; P = 0.004
Flabellum length	0.19 ± 0.03	0.14 ± 0.01	Z = -2.618; P = 0.009
Relative tip region length (% of glossa)	28.52	35.46	Z = -2.562; P = 0.010
Relative flabellum length (% of glossa)	0.88	1.25	Z = -2.882; P = 0.004
Relative flabellum length (% of tip region)	3.08	3.52	Z = -2.562; P = 0.010
Hair length tip region	0.33 ± 0.02	0.30 ± 0.02	Z = -2.937; P = 0.003
Hair length mid region	0.25 ± 0.03	0.19 ± 0.02	Z = -2.455; P = 0.014
Hair length basic link	0.19 ± 0.06	0.16 ± 0.03	
<u>Labial palps</u>			
Microtrichia length tip region	0.18 ± 0.03	0.15 ± 0.04	Z = -1.441; P = 0.150
Microtrichia length mid region	0.10 ± 0.03	0.08 ± 0.03	Z = -1.281; P = 0.2
<u>Galea</u>			
Microtrichia length tip region	0.21 ± 0.02	0.20 ± 0.01	Z = -0.641; P = 0.522

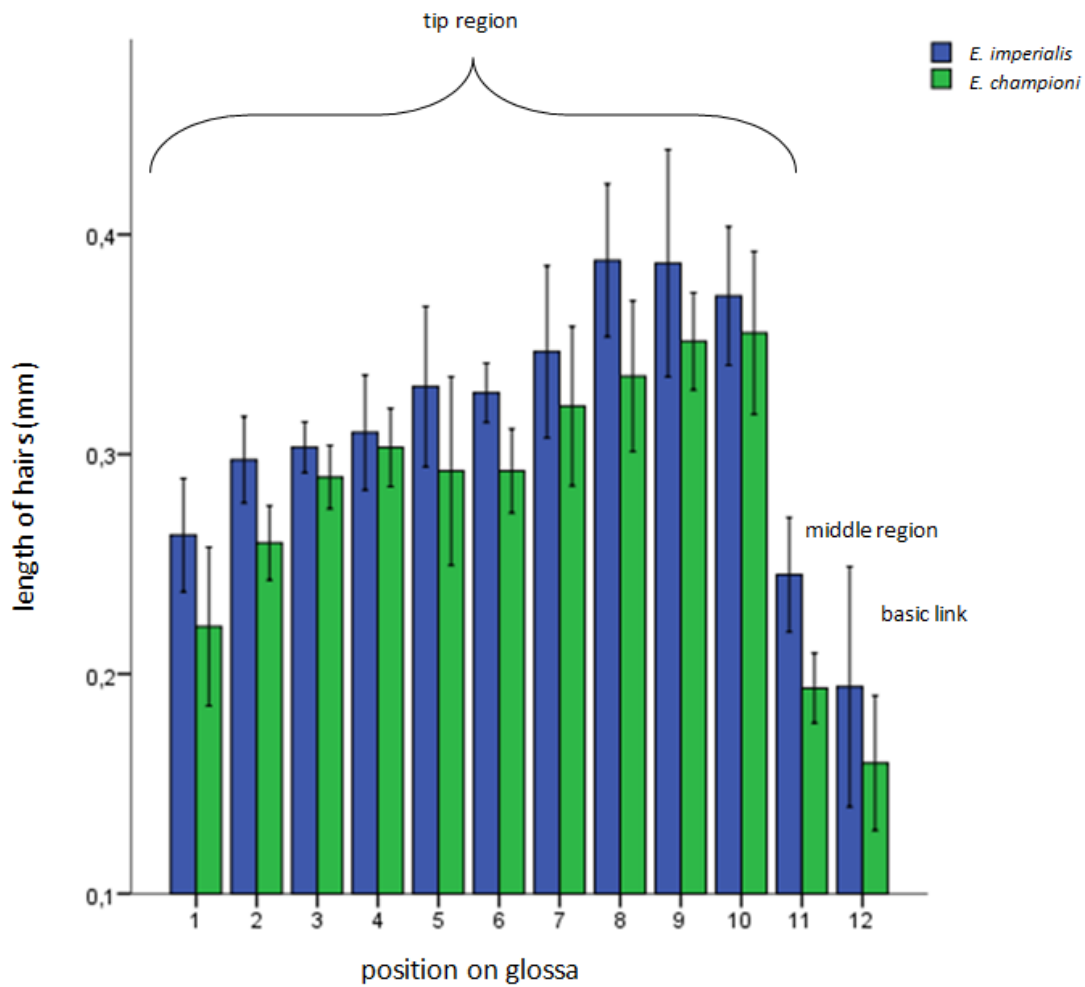


Fig. 4: Length of glossal hairs at different regions. The mean length of hairs was plotted in function of the distance from the tip. Hairs were measured at ten positions evenly along the glossal tip region and at the middle (11) and base (12). Hair lengths of *E. imperialis* in blue bars showed a greater length all over the tip region than *E. championi* (green bars). Error bars show standard deviation. N=6.

Tab. 2: Length of glossal annuli on the tip and the mid region.

N = 11	Median (mm) ± SD		Mann-Whitney-U-Test
	<i>E. imperialis</i>	<i>E. championi</i>	
Tip region	0.078 ± 0.004	0.059 ± 0.003	Z = -4.070; p < 0.001
Mid region	0.074 ± 0.004	0.059 ± 0.002	Z = -4.338; p < 0.001

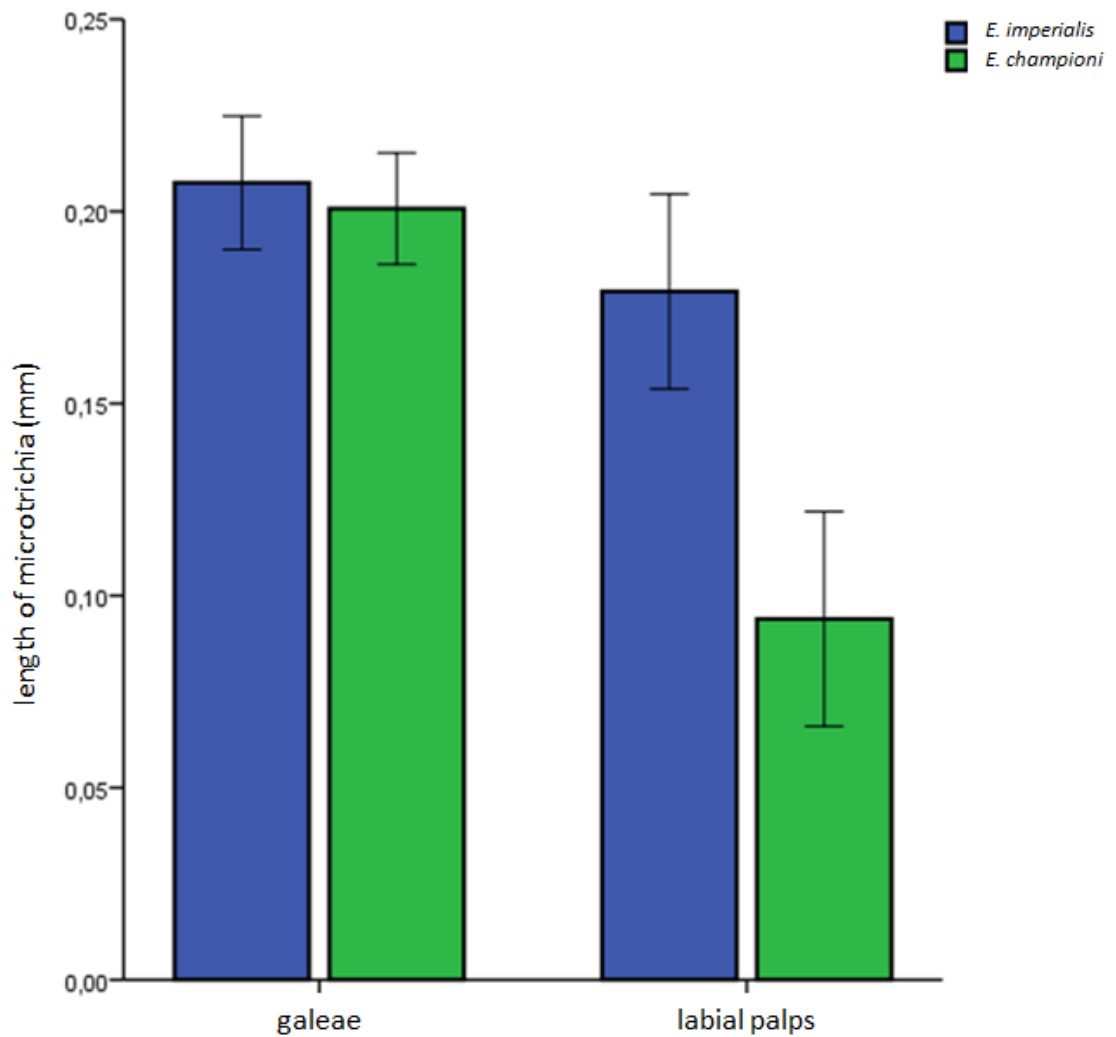


Fig. 5: Microtrichia length on tip region of galeae and labial palps. Differences between *E. imperialis* (blue) and *E. championi* (green) were highly significant in labial palps only. Error bars show standard deviation. N=6.

3.2 MICROMORPHOLOGY OF THE PROBOSCIS

Scanning electron microscopic investigations showed little differences in micromorphology between the extremely long proboscis of *E. imperialis* and that of *E. championi*. The glossa (tongue) is composed of annuli each bearing hairs and sensilla (Fig. 6, 7). In the proximal region microtrichia were consistently short and distant (Fig. 7D). The salivary canal of the glossa was ventral (Fig. 6E). At the dorsal site in the mid region of the glossa hairs were shorter and more adjacent than ventral around the salivary canal (Fig. 6D). The microtrichia at the ventral middle region were relatively short and thin and with split tips (Fig. 8B). The passage from the tip region to the middle region of the glossa was marked by the different appearance of the outstanding glossal hairs (Fig. 6F, 7F). In the

apical region hairs became broader and distant in both species (Fig. 6A, B, 7A, B). They were leaflike plain and long (up to 0.39 mm in *E. imperialis*) without split tips, but with regular standing sensilla on each annuli (Fig. 8A). The shape and arrangement of the cuticular hairs appeared similar in both species.

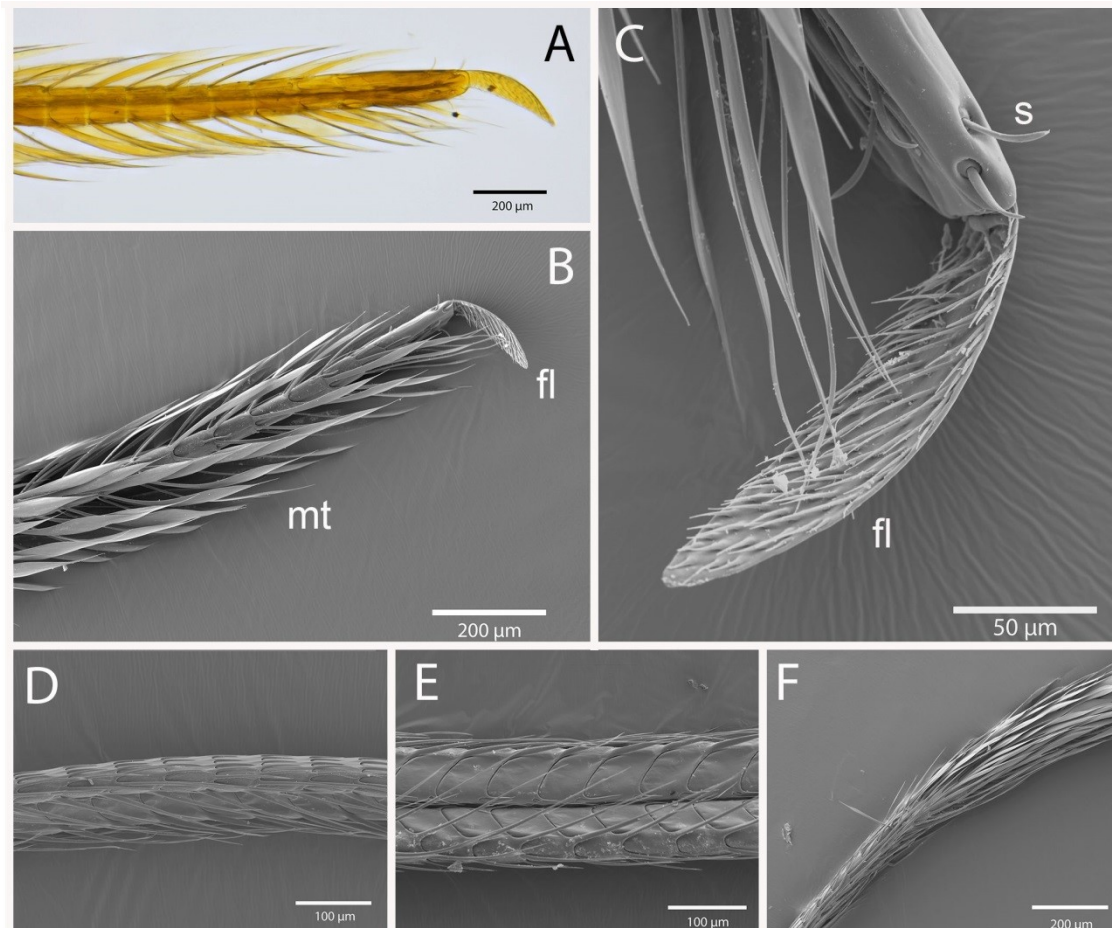


Fig. 6: Glossa of *E. imperialis*. Glossal tip region exhibited long flat hairs and the apical flabellum two visible sensilla (A, B, C). From tip to middle region hairs became shorter and fitted to the cuticula (F). In the middle region they were dorsal shorter than ventral near the salivary canal (D, E). fl (flabellum), mt (microtrichia), s (sensilla).

Number of sensilla at the tip region of *E. imperialis* (40 – 46, median 43) was greater than that of *E. championi* (32 – 37, median 36), counted from lateral side. *E. imperialis* overall had 63 sensilla on average on the glossa. 68.88 % of these sensilla were arranged at the tip region and 31.12 % at the middle and base. *E. championi* totally had 49 sensilla on average on the glossa; 71.41 % of them were at the tip region and 28.50 % were placed at the middle and basic region. Some glossae were straightened in a way to count sensilla from

the ventral site. Twice as many sensilla could be counted. *E. championi* had a portion of 79.92 % of *E. imperialis* at the tip region and 70.92 % at the middle and basic region.

The apical region of the glossa bore the flabellum and two sensilla outside the salivary canal near the tip (Fig. 6C). The same region of the glossal tip in *E. championi* bore three of the outstanding sensilla (Fig. 7C). These sensilla rose on each side of the salivary canal at the tip near the flabellum. The flabellum bore many little microtrichia at the ventral side, which were much smaller than the long leaflike hairs standing apart along the apical region (Fig 6C, 7C). On its dorsal side the flabellum was even and without hairs.

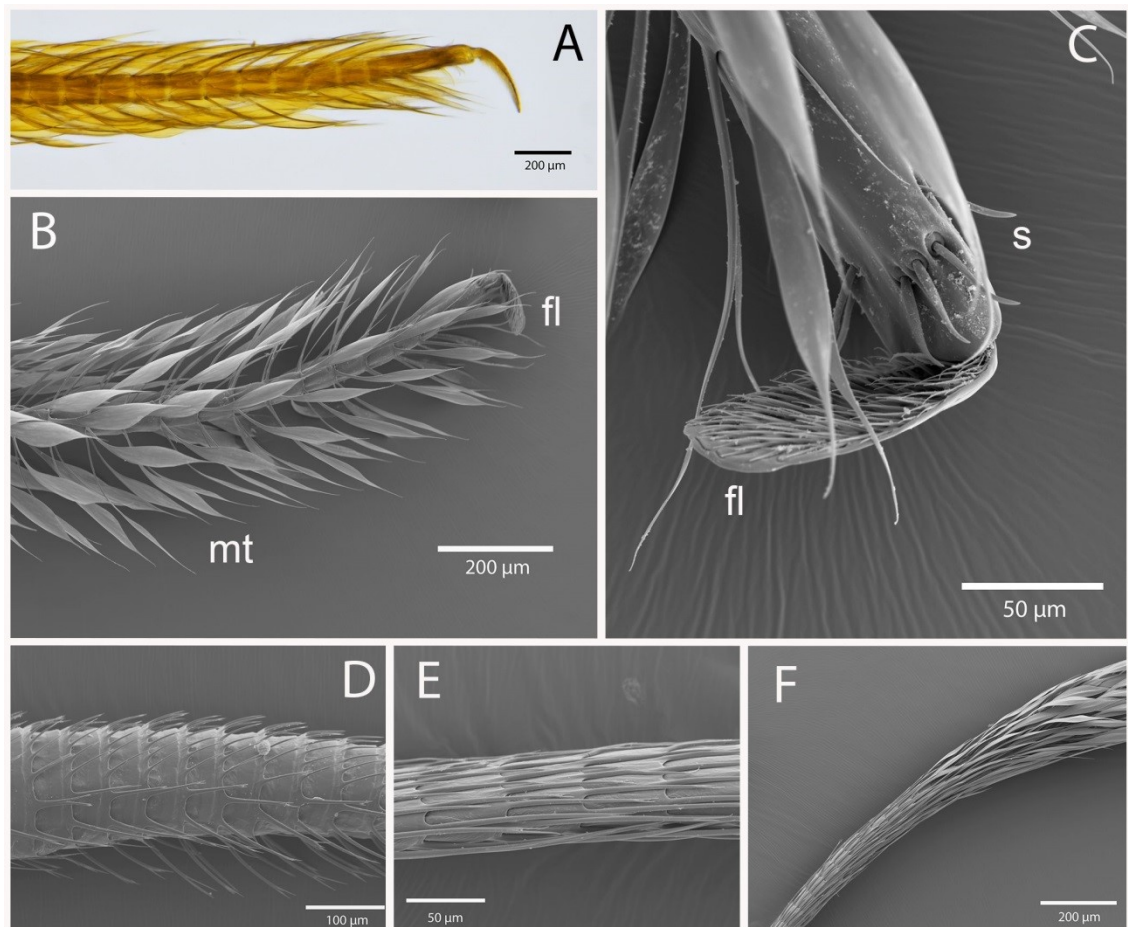


Fig. 7: Glossa of *E. championi*. Glossal tip region exhibits long flat hairs and the apical flabellum with three sensilla on each side (A, B, C). From tip to middle region hairs become shorter and fitted to the cuticula (F). In the middle region they are dorsal shorter than ventral near the salivary canal and basal they are thin and even distant (D, E). fl (flabellum), mt (microtrichia), s (sensilla).

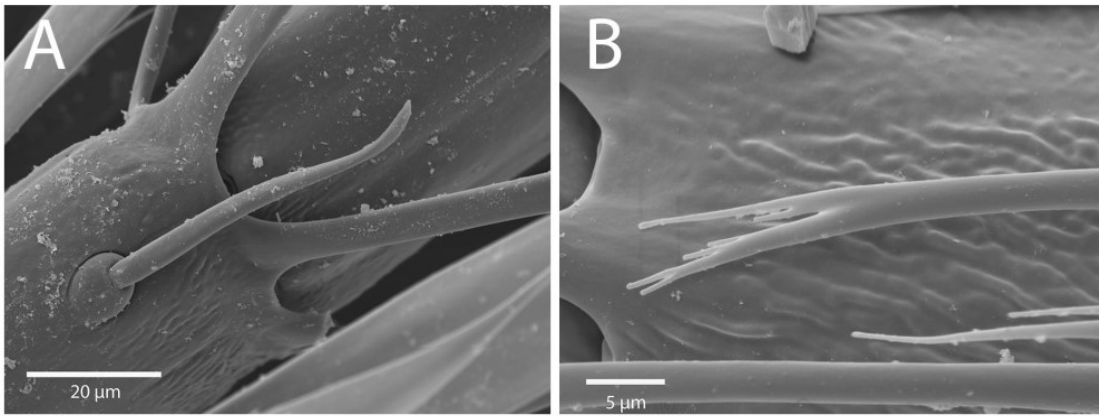


Fig. 8: Sensilla and microtrichia near the tip of the glossa of *E. imperialis*. The glossa bore sensilla (A) and microtrichia with splitted tips (B) in the tip region.

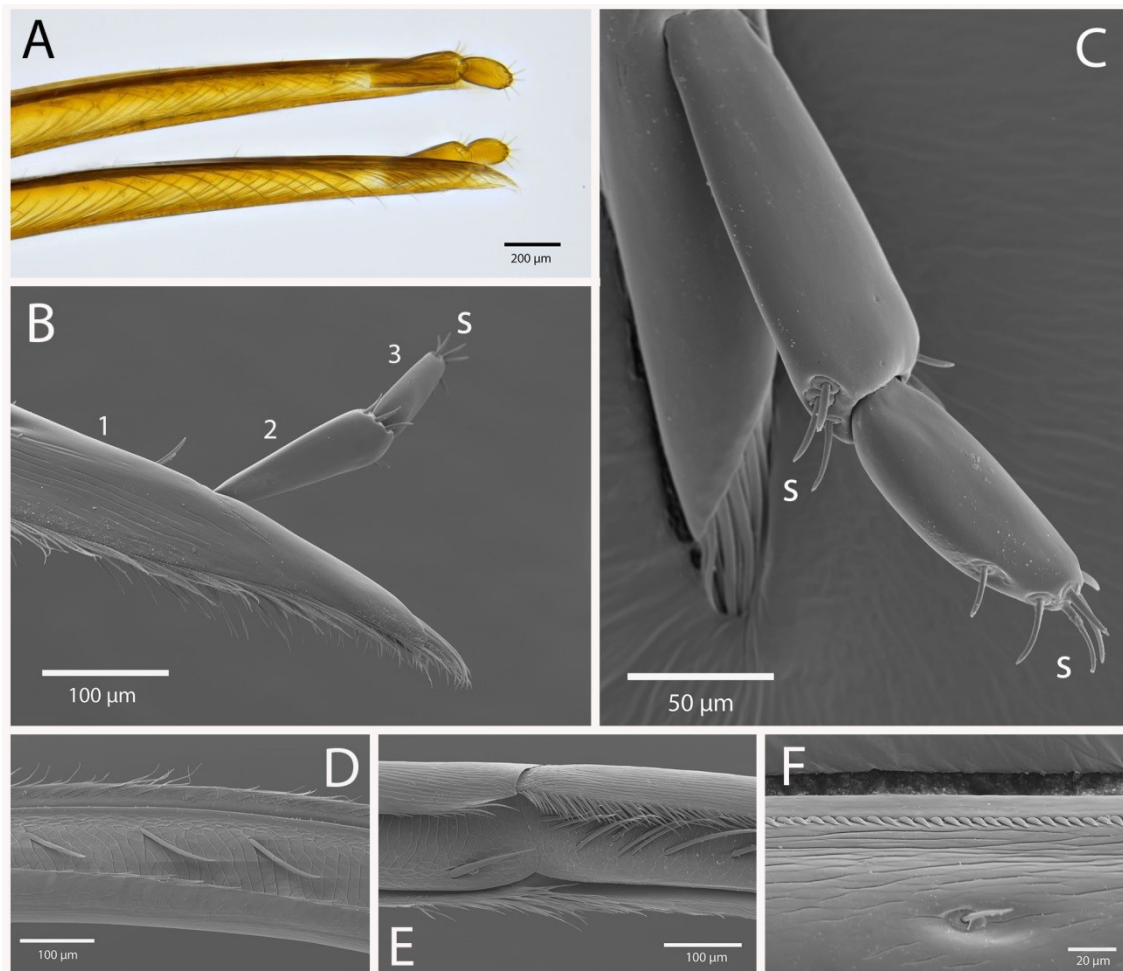


Fig. 9: Labial palps of *E. imperialis*. Light microscopic picture (A) and SEM pictures (B, C, D, E, F). Densely packed microtrichia at the tip of the first segment (B) and sensilla distal at the second and third segment (C). 1 (first segment), 2 (second segment), 3 (third segment), s (sensilla).

At the tip region of the labial palps of *E. imperialis* were many densely packed cuticular outgrowth, especially at the first segment (Fig. 9B). Distal of the second and third segment were sensilla located, six at each segment (Fig. 9C). The tip of the labial palps of *E. championi* appeared similar (Fig. 10). The distal parts of the second and third segment bore each six sensilla, as well (Fig. 10C). Labial palps of both species exhibited sensilla on the outside and long bristles inside the nutrition canal (Fig. 9E, 10D, E). Pointed cuticle structures were found at the dorsal edge of a labial palp (Fig. 9F, 10F).

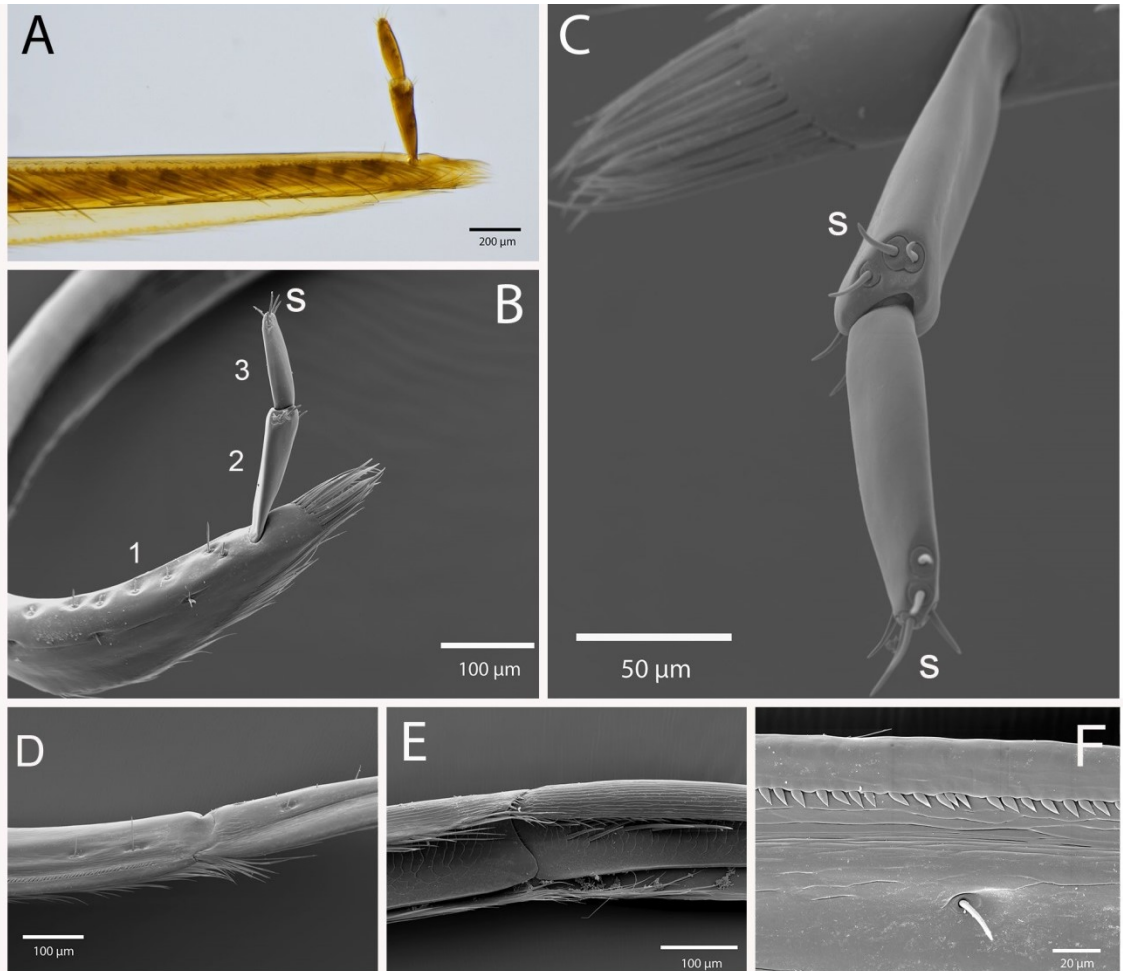


Fig. 10: Labial palps of *E. championi*. Labial palps on a light microscopic picture (A) and SEM pictures (B, C, D, E, F). Densely packed microtrichia at the first segment (B) and sensilla distal at the second and third segment (C). 1 (first segment), 2 (second segment), 3 (third segment), s (sensilla).

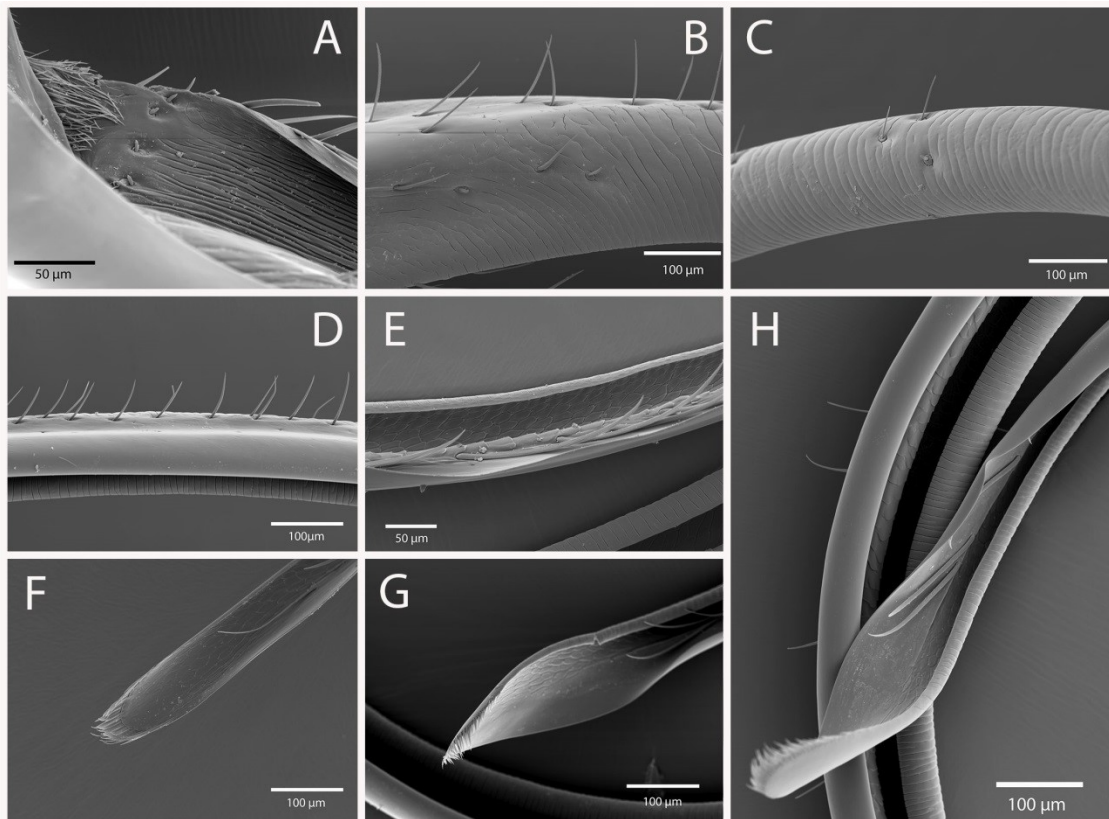


Fig. 11: Galea of *E. imperialis* and *E. championi*. The proximal part sustained a brush of microtrichia and a row of sensilla on the ventral (A) and few bristles on the dorsal site (B). Middle region showed cuticular rings (C) and medium sized bristles (D) and a view into the nutrition canal showed a strand with long microtrichia (E). Those reached to the tip region which contained a brush of microtrichia (F, G, H).

A dense cluster of microtrichia existed at the very tip of the galeae and a row of longer microtrichia grew some distant from the tip along the depression forming the nutrition canal until up to the base in both species (Fig. 11E, F, G, H). A view on the dorsal site of the base of the galea (Fig. 11B) and a ventral view into the nutrition canal (Fig. 11A) at the same height revealed a row of small sensilla at the base of the nutrition canal next to a cluster of bristles and few longer bristles stood on the outside. Long bristles were on the dorsal site in the middle region of the galea (Fig. 11D) and a strand of connected microtrichia inside the nutrition canal (Fig. 11E). The cuticular annuli were clearly to see (Fig. 11C).

3.3 NECTAR ADHESION

The significant difference between the weights of both species was detectable due to the differences in glossal lengths in *E. imperialis* and *E. championi*. *E. imperialis* had a glossal length almost twice as long as in *E. championi* and thus a glossal weight twice as high. Therefore the nectar mass that adhered to the glossa of *E. imperialis* was approximately twice as that in *E. championi* (Fig. 12). The same has been documented for the nectar amount. While *E. championi* had an average nectar amount of 0.05 mg, that one of *E. imperialis* was with 0.12 mg more than twice as big. The calculated volume for *E. championi* was 0.04 μl and for *E. imperialis* 0.11 μl . A significant difference between both species was recorded for all these listed data as well as for the difference between dry and nectarous glossa within each species (Tab. 3).

Tab. 3: Nectar amount on dry glossae.

N = 10	Median \pm SD		Mann-Whitney- U-Test
	<i>E. imperialis</i>	<i>E. championi</i>	
Length of glossa (mm)	23.20 \pm 1.20	11.75 \pm 1.28	Z = -3.800; P < 0.001
Dry glossa (mg)	0.11 \pm 0.02	0.05 \pm 0.01	Z = -3.782; P < 0.001
Wet glossa (mg)	0.23 \pm 0.08	0.11 \pm 0.05	Z = -3.214; P = 0.001
Nectar amount (mg)	0.12 \pm 0.08	0.05 \pm 0.04	Z = -2.192; P = 0.028

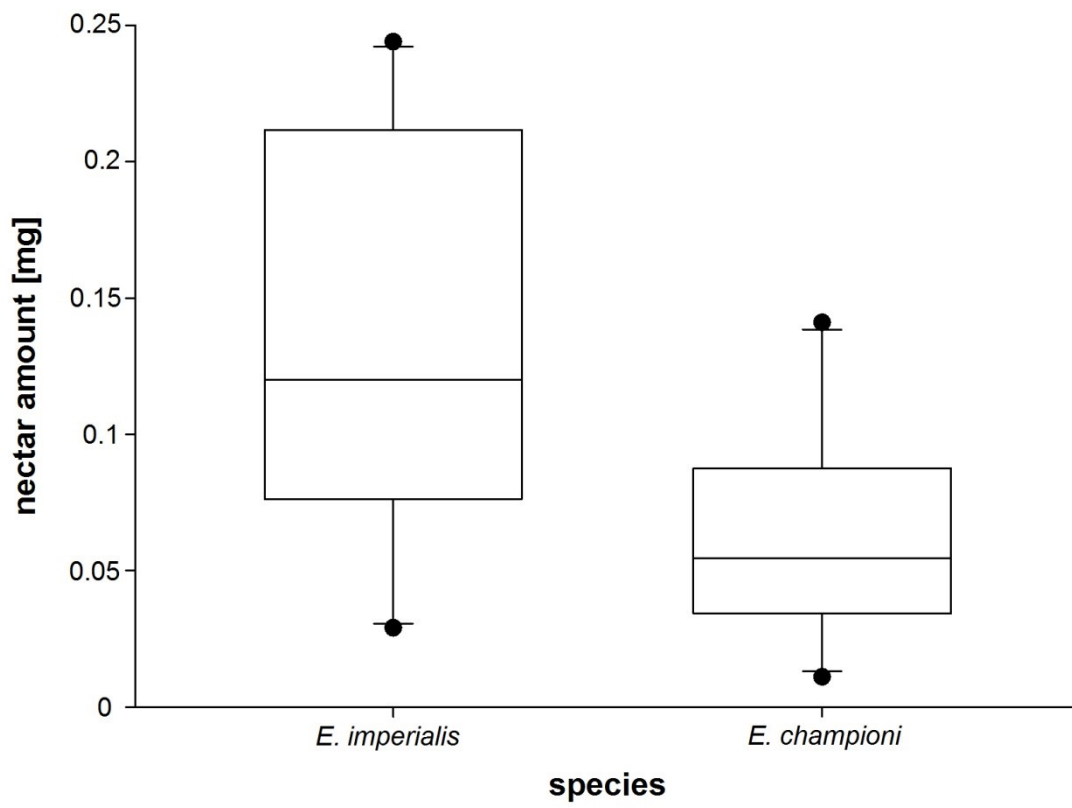


Fig. 12: Nectar mass of the glossa of *E. imperialis* and *E. championi*. Nectar mass adhered to the glossa after immersing with nectar.

4 DISCUSSION

4.1 MORPHOMETRY AND MICROMORPHOLOGY

Strikingly, the measurements conducted in this study, revealed a high difference in the length of the distal proboscis between the two observed species, this result is comparable to Gruber (2013). Morphometric measurements showed significant differences between the two species in the total glossal length and the length of glossal tip region. Both lengths were almost twice as long in *E. imperialis* compared to *E. championi*. In contrast, the length of the flabellum also differed significantly between the species; it was not of double size in *E. imperialis*. These differences in length were also found in relative lengths of the regions, which were greater in *E. championi* (Tab. 1).

The comparison of hair length on the glossal tip region showed an increase along the length from distal to the basal limit of the region demonstrating the hairs on *E. imperialis* were significantly longer as in *E. championi*. Consistently, the glossal hairs were longer in *E. imperialis* at the middle and basic region of the glossa (Tab. 1). Comparable to the finding of Wu et al. (2015) the average length of hairs increased from the proximal segment to the distal end of the glossa in the honeybee. However, within the glossal tip region hairs become shorter from basal to apical (Fig. 4). Unlike on the glossa and the labial palps, the lengths of microtrichia on the galeae did not differ significantly (Tab. 1). Reason for this non-allometric elongation could be traced back to the function of the mouthparts. The glossa is the distal part which has first contact with the fluid and if hairs are longer there is a greater capillary force for absorbing so maybe more nectar rises. The glossal hair length in *E. imperialis* is approximately three times the length of a honey bee (Wu et al. 2015).

The labial palps consisted of three segments and might function as caliper to measure the width of flower openings. Segments 2 and 3 of the labial palps bore many sensilla and might feel around for the depth and width of the flower calyx before inserting the proboscis and absorbing nectar. For the elongated proboscides of both species a elongation of the labial palps as well as the galeae was probably necessary to facilitate removal of nectar from a glossa that was too long to be retracted fully (Harder 1983). The galeae closed the nutrition canal and surrounded the glossa at the dorsal site and bore few sensilla and microtrichia since there were little differences in the lengths of galeal microtrichia.

The micromorphology of the glossal tip showed a difference in the number of sensilla distal near the flabellum (Fig. 6C, 7C). *E. imperialis* had two and *E. championi* had three sensilla at this position. These sensilla, like all the other sensilla found along the glossa, were sensilla chaetica and function as joined mechano-chemo-receptors or gustatory sensilla. This can be assumed because the gustatory sensilla on the mouthparts of the honeybee, studied by Galić (1971) and Whitehead and Larsen (1976), are sensilla chaetica and it is the only sensilla type found on the glossa of the honey bee. They extend on the chitinous annuli and among the sclerotized hairs of the glossa and are tip orientated the same way the hairs are. Here the sensilla were positioned the same way as described for the honey bee. The mean number of sensilla in *E. imperialis* on the whole glossa is 63 and in *E. championi* 49. The number of sensilla on the honey bee's glossa in comparison to the examined euglossine bees is 66 – 78 sensilla on average (Galić 1971). Both euglossine species had about 30% of all sensilla at the tip region. *E. championi* had 70-80 % the number of sensilla of *E. imperialis*. This number is not as much, as would be expected for a glossa with the double length.

4.2 NECTAR INTAKE

Due to the long glossa of *E. imperialis* the adsorbed volume of nectar differs significantly between the two species. In *E. imperialis* measured mass of nectar was almost twice as big (Tab. 3, Fig. 12), which can be explained by different glossal size. The much longer proboscis of *E. imperialis* recorded more total mass of nectar but less relative to the length. Gruber (2013) found that absorbed mean nectar volume of *E. imperialis* was 12.13 μl in 55.6 sec and that of *E. championi* was 1.7 μl in 11.6-39 sec. During feeding nectar is loaded between the hairs of the tip region, It can be conducted that in one lapping cycle *E. imperialis* can take up 0.11 μl and *E. championi* can take up 0.04 μl nectar onto the glossa. Compared to intake observations of Gruber (2010) and concluded from nectar adhering it can be calculated that 101 lapping cycles are necessary to take up a full meal of 12.13 μl in *E. imperialis*. This would be 1.8 licking cycles per second. *E. championi* takes 43 licking cycles for 1.7 μl nectar and between 1 and 4 licking cycles per second.

The hairs on the honeybee's glossa erect rhythmically and those on the basic segment erect earlier than those on the tip region. This phenomenon is described as asynchronous hair erection suggesting the honeybee having an optimal pattern for balancing nectar intake and viscous drag (Wu et al. 2015). A similar phenomenon is conjecturable in

Euglossa, since the same principle composition of mouthpart and similar vestiture on the glossa could be identified.

Accompanying with an extremely long proboscis there is a reduction in both energy intake rate and viscosity of nectar influenced by the sugar concentration. Intake rate declines as sucrose concentration increases. The feeding optimum of the suction feeding euglossine bee *Euglossa imperialis* falls below the optimum for bee taxa that lap measured as a function of sucrose concentration, nectar viscosity and ambient pressure (Borrell 2005, 2006, 2007). Gruber (2013) found that the sugar concentration of the nectar of *Calathea lutea* fed by *E. imperialis* was greater (39.4 %) than that of *Stachytarpheta frantzii* fed by *E. championi* (24 %). This is in contrast to the suggestion of Borrell (2005, 2006, 2007), after whom the nectar viscosity has to be low for optimal intake rate in extremely elongated proboscides. To achieve a higher digestive throughput a long proboscis allows a lower sugar concentration than that of lapping bees. He compared elongated proboscides with those of lapping bees but not elongated proboscides among themselves.

To optimize the energy rate of a low concentrated nectar solution, which could be taken up by long proboscides, male and female euglossine bees evolved a strategy for promoting evaporative water loss from nectar. Conveying drops of sugar solution to the base of the proboscis and manipulating them with rhythmic movements of the proboscis bees win a higher sugar concentration compared to that of the initially absorbed solution (Pokorny et al. 2014).

4.3 LONG-TONGUED FLOWER VISITING INSECTS

Comparing the proboscis of *Euglossa* with other insects with extremely elongated mouthparts the elongation of different mouthparts becomes apparent (Borrell & Krenn 2006). In long-tongued euglossine bees the galeae, labial palps and glossa are elongated, in extremely long-tongued butterflies (Riodinidae) the galeae form the food tube and in flies (Nemestrinidae) the prementum is elongated. The proboscis of the long-tongued flies may exceed double the body length (Karolyi et al. 2012), as well as the proboscis of *E. lycisca* (Riodinidae) is almost twice as long as the body (Bauder et al. 2011), which is comparable to the long-tongued *E. imperialis* with a proboscis reaching the doubled body size (Gruber 2013). In contrast to *Euglossa* in long-tongued Riodinidae there is no elongation of the tip region and a lower number of sensilla in extremely long tongued species (Bauder et al. 2013).

The comparison of the proboscis of long- and short-tongued Riodinidae (Lepidoptera), distinguished by flower handling time, size of the food canal and musculature of the suction pump (Bauder et al. 2011), demonstrated a non-allometric relationship for an extremely long proboscis (Bauder et al. 2013). For long-tongued insects a long flower handling time caused by a slow nectar intake and a longer manipulation time clearly presents a cost. But there are strategies for compensation. Suction time increases with proboscis length suggesting that a long proboscis facilitates drinking larger amounts of nectar from deep tubed flowers (Bauder et al. 2015).

Bumblebees with a long proboscis forage significantly faster than bees with shorter proboscis on flowers with long corolla tubes and bees with short proboscis prefer short corolla tubes (Inouye 1980). In most cases, bees visit flowers whose corolla tubes are shorter than the bee's proboscis (Harder 1982). So species with long glossae have access to nectar in a greater variety of flowers than those with shorter proboscides, and they are able to feed from a larger number of plant species (Harder 1985). Species with similar body length but twice as long proboscis can be expected to visit a greater number of flowers as proven for tropical Hesperidae (Bauder et al. 2015).

Results of the recent study support the idea of using proboscis length as a morphological indicator of resource utilization (Inouye 1980), as well as affecting differences in nectar uptake rate by morphological differences (Harder 1983). The morphometric comparison of the two related species illustrates an adaptation to nectar intake from extremely deep corolla tubes concerning only the distal parts of the proboscis. The extremely elongated proboscis of *E. imperialis* represents an optimal solution influencing the whole movement mechanism of the mouthparts. If body size and proboscis length of generalist nectar-feeding insects show an allometric relationship (Kunte 2007), the evolutionary development of the studied *Euglossa* species probably proceeded rapidly and competitively and does not lead back to allometric parameter. A discussion about costs and benefits of an extremely elongated proboscis has to be passed by flower visiting times, corolla depth and energy use related to the nectaramount.

5 ABSTRACT

Euglossa is the most species rich genus within the Euglossini and attracts the attention of many scientists because of their bright shiny and colorful appearance. There is still little known about the nectar feeding behavior, although their proboscides have extreme lengths. This study compares the mouthpart morphology of *Euglossa imperialis* with *Euglossa championi*. The lightmicroscopic and section electron microscopic (SEM) observations of the proboscis of two species within one genus that have almost the same body size but differ significantly in proboscis length show only the distal parts being clearly elongated in *E. imperialis*. Galeae, labial palps and glossa, forming the proboscis are almost double sized in *E. imperialis*, whereas the basal mouthparts are of same size. The glossa and glossal tip region are double the size in *E. imperialis*, the flabellum is larger but not twice as long. Broad and distant hairs at the apical region and thin adjacent microtrichia in the basal region are similar in both species. The total number of sensilla on the glossa of *E. imperialis* is greater, but not twice the number; the relative number and distribution is similar in both species. *E. championi* is able to take up nectar faster than *E. imperialis*. *E. imperialis* has a longer proboscis and is able to take up twice as much volume with one lapping cycle, whereas the number of licking cycles per second is alike, but the time needed on one flower is greater for *E. imperialis*. The morphometric comparison should allow conclusions of allometric relation and the consequences for an extremely elongated proboscis as an adaptation to nectar uptake. This study refers to the assumption that the extremely elongated proboscis of *E. imperialis* is an adaptation to long tubed flowers as alternative food source for stepping aside in the same habitat. Flower handling time, corolla depth of visited flowers and nectar gain should allow discussion about costs and benefits of an extremely elongated proboscis.

6 ZUSAMMENFASSUNG

Euglossa ist die artenreichste Gattung innerhalb der Euglossini und wird von mittelgroßen Arten mit glänzend bunter Körperoberfläche vertreten. Wenig untersucht wurden bislang die Nektaraufnahme und die Mundwerkzeuge, obwohl der Saugrüssel bei einigen Arten erstaunliche Längen aufweist. In dieser Arbeit wurde die Morphologie der Mundwerkzeuge von *Euglossa imperialis* und *Euglossa championi* vergleichend untersucht. Vertreter beider Arten besitzen etwa die gleiche Körpergröße, jedoch ist die Rüssellänge bei *E. imperialis* doppelt so groß wie die von *E. championi*. Der Vergleich der Mundwerkzeuge beider Arten mittels Lichtmikroskop und Rasterelektronenmikroskop (REM) zeigte, dass nur die distalen Teile, welche den Saugrüssel bilden, bei *E. imperialis* deutlich verlängert sind. Die basalen Teile hingegen, die für den Bewegungsmechanismus verantwortlich sind, weisen die gleiche Größe auf. Die Glossa und die Spitzenregion der Glossa sind bei *E. imperialis* doppelt so lang, das Flabellum dagegen ist bei *E. imperialis* länger, aber nicht doppelt so lang. Eine unterschiedliche Beborstung der Glossa durch blattartige Mikrotrichia (Haare) im distalen Abschnitt und dünnen Mikrotrichia an Mitte und Basisgelenk ist bei beiden Arten ähnlich. Die Anzahl der Sensillen auf der Glossa ist bei *E. imperialis* höher, die relative Anzahl und Verteilung der Sensillen auf der Glossa bei beiden Arten ähnlich. Nektarversuche zeigten, dass *E. championi* mit dem kürzeren Rüssel in der Lage ist, Flüssigkeiten schneller aufzunehmen, *E. imperialis* dagegen nimmt fast doppelt so viel Volumen auf und verweilt deutlich länger an der Blüte. Der funktionsmorphologische Vergleich dieser nah verwandten Arten zeigt, dass die Anpassung an Nektaraufnahmen aus besonders tiefen Blüten nur den distalen Abschnitt des Saugrüssels betrifft. Somit stellt der extrem lange Rüssel von *E. imperialis* eine sparsame Lösung dar, die nicht den gesamten Bewegungsmechanismus der Mundwerkzeuge beeinflusst. Die evolutive Entwicklung lief daher vermutlich rasch und „kostengünstig“ ab und ist nicht auf allometrische Körpergrößenänderungen zurückzuführen. Eine Diskussion der Kosten und Nutzen eines besonders langen Rüssels muss die Besuchszeiten an Blüten, die Blütentiefe und der Energieverbrauch in Relation zum möglichen Nektarertrag stellen.

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8 LIST OF FIGURES

Fig. 1: Mouthparts of the honeybee referred to Gullan & Cranston (2010).....	2
Fig. 2: Examined orchid bees.....	5
Fig. 3: Mouthpart components of the proboscis of <i>E. imperialis</i> (A).....	7
Fig. 4: Length of glossal hairs at different regions.....	10
Fig. 5: Microtrichia length on tip region of galeae and labial palps.....	11
Fig. 6: Glossa of <i>E. imperialis</i>	12
Fig. 7: Glossa of <i>E. championi</i>	13
Fig. 8: Sensilla and microtrichia near the tip of the glossa of <i>E. imperialis</i>	14
Fig. 9: Labial palps of <i>E. imperialis</i>	14
Fig. 10: Labial palps of <i>E. championi</i>	15
Fig. 11: Galea of <i>E. imperialis</i> and <i>E. championi</i>	16
Fig. 12: Nectar mass of the glossa of <i>E. imperialis</i> and <i>E. championi</i>	18

9 LIST OF TABLES

Tab. 1: Measurements on proboscis of <i>E. imperialis</i> and <i>E. championi</i>	9
Tab. 2: Length of glossal annuli on the tip and the mid region.....	10
Tab. 3: Nectar amount on dry glossae.	17

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