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Drinking with a very long proboscis: Functional morphology of orchid bee mouthparts (Euglossini, Apidae, Hymenoptera)

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ABSTRACT

Neotropical orchid bees (Euglossini) possess the longest proboscides among bees. In this study, we compared the feeding behavior and functional morphology of mouthparts in two similarly large-sized species of *Euglossa* that differ greatly in proboscis length. Feeding observations and experiments conducted under semi-natural conditions were combined with micro-morphological examination using LM, SEM and micro CT techniques. The morphometric comparison showed that only the components of the mouthparts that form the food tube differ in length, while the proximal components, which are responsible for proboscis movements, are similar in size. This study represents the first documentation of lapping behaviour in Euglossini. We demonstrate that *Euglossa* bees use a lapping-sucking mode of feeding to take up small amounts of fluid, and a purely suctorial technique for larger fluid quantities. The mouthpart movements are largely similar to that in other long-tongued bees, except that the postmentum in *Euglossa* can be extended, greatly enhancing the protraction of the glossa. This results in a maximal functional length that is about 50% longer than the length of the food canal composing parts of the proboscis. The nectar uptake and the sensory equipment of the proboscis are discussed in context to flower probing.

1. Introduction

Bees (Apoidea) are among the most important flower-visiting insects and pollinators of Angiosperms and are informally divided into “short-tongued” and “long-tongued” groups. Bee mouthparts form a proboscis, which is often elongated to access nectar from long-spurred flowers. Euglossini or orchid bees are a fascinating group of Neotropical long-tongued bees that have attracted wide attention among tropical flower-visiting insects due to their extraordinary relationship with orchids. They exhibit extraordinarily long proboscides that in some species attain twice the length of the body (Dressler, 1982). Male Euglossini are the exclusive pollinators of nearly 700 species of orchids (Dressler, 1982; Roubik and Hanson, 2004) and are known to collect fragrances and other odoriferous chemicals from flowers using their modified legs. These chemical substances are accumulated in a hind tibial pocket and subsequently the volatile components are emitted at mating sites presumably to attract females (Dressler, 1982; Eltz et al., 2005). In addition, males and females visit numerous flowers for nectar consumption. Using their long proboscides, these bees are able to exploit a wide array of deep tubular flowers that are inaccessible to other bees (Dressler, 1982). Moreover, male orchid bees fly astonishingly long distances (Pokorny et al., 2015) and require a continuous supply of nectar, which they obtain through their long proboscis from flowers with various spur lengths. Their unusual behavior of collecting fragrances has been frequently studied and much information has been compiled about the use of the male “perfume” for female attraction (Williams and Whitten, 1983; Eltz et al., 2007). However, their flower-visiting behavior to obtain nourishment and the functional morphology of their extremely long proboscides has received far less attention.

The principle composition of mouthparts in *Euglossa* is similar to that in other long-tongued bees, such as the western honeybee, *Apis mellifera* (Winston, 1979; Stell, 2012). Bee mouthparts consist of the short unpaired labrum, the paired biting mandibles, and the labiomaxillary complex, which is adapted for fluid-feeding (Plant

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and Paulus, 2015). The latter is a functional unit composed of the basally linked pair of maxillae and the unpaired labium (Snodgrass, 1956; Winston, 1991). The proboscis consists of (i) the elongated galeae of the maxillae and (ii) elongated labial palps, which together form the food tube that encompasses (iii) the glossa. The protractible glossa is the principal organ of the proboscis; it absorbs liquids and conveys them into the food tube by capillarity and lapping movements. Proximally, the food canal empties into the functional mouth, which leads to the cibariopharyngeal cavity (Snodgrass, 1956). The glossa resembles a hairy brush with numerous cuticle annules each bearing an array of microtrichia. The annuli alternate with soft membranous cuticle areas that provide flexibility.

In the resting position, the proboscis of the honeybee is folded Z-shaped and stored beneath the head, and the apical components of the food tube have disengaged from each other (Snodgrass, 1956). The base of the proboscis is composed of cardo and stipes of the paired maxilla, as well as the proximal parts of the labium, including the postmentum, which, in *Apis* and *Bombus*, is divided into mentum and lorum (Plant and Paulus, 1987). Extension of the proboscis into the feeding position results from turning the cardo against the head, which results in an extension of the articulation of cardo and stipes by external maxillary muscles (Snodgrass, 1956). In *Apis*, unfolding the maxillary parts is transmitted by the connecting lorum and mentum, which passively allow for the projection of the labial components (Snodgrass, 1956; Plant and Paulus, 1987, fig. 18). In Euglossini the postmentum is not divided into a separate lorum and mentum as in *Apis*. However, the analogous parts of the postmentum connect to the cardines and labium in much the same manner (Plant and Paulus, 1987).

When feeding, the proboscis is extended and the glossa performs repeated licking movements extending the wettable glossa tip out beyond the food tube and withdrawing it. In *Apis* and *Bombus* the maxillae and the prementum with the labial palpi remain relatively motionless during nectar uptake (Snodgrass, 1956; Harder, 1982; Yang et al., 2014; Wu et al., 2015). Protraction and retraction of the glossa are termed a licking cycle studied in honeybees (Simpson and Riedel, 1964; Kingsolver and Daniel, 1995; Yang et al., 2014; Wu et al., 2015). A licking cycle has three phases. In the first phase, the glossa is protracted, and nectar is loaded in between the erected glossal microtrichia by capillary action. In phase two, the glossa retracts into the food tube with the nectar adhering to it. In the third phase, nectar is unloaded from the glossa and transported to the actual mouth by suction. Expansion of the cibarial sucking pump creates a pressure gradient to imbibe fluid through the proboscis (Winston, 1991). The glossa retracts by a partial recoiling at the glossa base into the prementum (Snodgrass, 1956).

Suction feeding was documented in orchid bees by Borrell (2004) based on observations of *Euglossa imperialis*. Borrell (2004, 2006) proposed that orchid bees shifted from lapping-sucking to a purely suctorial mode of nectar uptake. However, since the same general morphology and functional mechanisms of feeding that have been observed in other long-tongued Apidae appear to be present in the Euglossini (Plant and Paulus, 2015), we seek to examine the alleged absence of the lapping-sucking mode in orchid bees. Further, the functional morphology of the honeybee glossa has been subjected to recent biophysical examinations (Yang et al., 2014; Wu et al., 2015; Zhao et al., 2015), which permit comparisons regarding the function of the proboscis components in Euglossini.

The present study aims to investigate the mouthparts and drinking mechanism of *Euglossa* to gain a deeper insight into nectar-feeding techniques in extremely long-tongued orchid bees. Therefore, we compare the mouthpart morphology, mouthpart movements and feeding abilities of two congeneric species of *Euglossa*, which are similar in body size but differ significantly in proboscis length. Additionally, we examine the micro-morphology of the proboscis in context of nectar feeding.

2. Material & methods

2.1. Study site and studied species

Field work was conducted in February 2010 at the Tropical Research Station La Gamba, Puntarenas, Costa Rica (N 8°42′61′, W 83°12′97″; 70 m a.s.l.), adjacent to the Piedras Blancas National Park in the southwestern part of Costa Rica which is characterized by tropical lowland rainforest (Weissenhofer and Huber, 2001).

All studied bees were allured and captured in the Piedras Blancas National Park, where more than twenty species of Euglossini occur (Gruber et al., 2009). Two frequently encountered species, *Euglossa imperialis* (Cockerell, 1922) and *Euglossa championi* (Cheesman, 1929), were selected. They are similar in body size, but show great differences in proboscis length (Fig. 1A and B). Males of both species were attracted by fragrance baits using cineol, eugenol and methyl salicylate (Roubik and Hanson, 2004). Specimens were then determined under a stereomicroscope following the key in Roubik and Hanson (2004). Morphometric measurements were taken after the bees were immobilized in a refrigerator for 30 min. Fresh weight was taken with a digital pocket carat balance (Kern CM 50-C2N). Body length (anterior of the head to the posterior tip of the metasoma), head width (at the widest point, from eye to eye), thorax width (from outer edge to outer edge of tegulae) and proboscis length (in folded position, from the mandibles to flabellum) were measured using a digital caliper (Helios Digital Caliper Digi-Met 1220; 0.01 mm).

2.2. Feeding observations of orchid bees

After measurements, the feeding experiments were conducted using natural and artificial nectar sources in a mosquito tent. To ensure that the bees were fit for the experiments after being chilled for the biometrics, they were allowed to acclimatize for at least half an hour to regain flight ability.

Freshly cut flowers of *Calathea lutea* (Marantaceae) and *Stachytarpheta frantzii* (Verbenaceae) from the station's garden were presented as a bouquet of natural nectar sources for each test run. Nectar concentrations of natural and artificial sources were determined with a Zeiss refractometer. Nectar volume was measured in flowers with micro-capillary tubes. Corolla tube length was measured with a digital caliper.

Feeding observations of *E. imperialis* ($N = 11$) and *E. championi* ($N = 7$) were made using a Sony V50 digital video camera. Bees were presented with a 30% sucrose solution in artificial flowers, which previously had been tested for their attractiveness. The artificial feeding tube was transparent to permit observation of proboscis movements. Large amounts of sugar solution were offered using 200 μl micropipettes, whereas small drops offered in feeding trials had a volume of approximately 10 μl, roughly corresponding to the amount of nectar in a flower of *C. lutea*.

After the tests were carried out, all specimens were preserved in 70% ethanol. Each bee was labeled and stored separately. All specimens are held in the collection of the Department of Integrative Zoology (University of Vienna, Austria).

2.3. Microscopic examination and measurements of mouthparts

To measure the different parts of the proboscis, heads were dissected under a stereomicroscope to remove the mouthparts. The proboscides were placed on laminated graph paper and photographed with a Nikon D70 camera (Nikon 55 mm micro

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