Sexual dimorphism in head structures of the weevil *Rhopalapion longirostre* (Olivier 1807) (Coleoptera: Curculionoidea): a response to ecological demands of egg deposition

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Extreme sexually dimorphic phenotypes are frequently attributed to strong sexual selection but they can also arise as a consequence of different ecological demands. The evolutionary emergence of elongated rostra was a key event in the adaptive radiation of weevils. Exaggerated female rostra evolved in numerous weevil taxa, enabling females to bore long channels for egg deposition into various parts of host plants. The investigated ecological scenario involves three species of brentid weevils, all associated with the same host plant, *Alcea rosea*. The present study reveals that: (1) *Rhopalapion longirostre* bores egg channels into the buds, and the female rostrum is twice as long and its surface is smoother than in the male; (2) *Alocentron curvirostre* and *Aspidapion validum* live on the same host plant but use the stems for egg deposition; in these species, female rostra are not exaggerated; (3) the females of all three species possess a stronger mandible musculature than males; (4) the elongated female snout of *R. longirostre* is a response to the requirements of boring egg channels of maximal depth into the buds of the host plant; and (5) female muscle strength is an adaptation to boring into hard plant tissues, irrespective of rostrum length. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **104**, 642–660.

ADDITIONAL KEYWORDS: adaptation – head morphology – mandible musculature – microCT – morphology – sexual dimorphic exaggeration – three-dimensional reconstruction.

INTRODUCTION

The superfamily Curculionoidea is a group of beetles characterized by an enormous diversity containing approximately 62 000 species belonging to 5800 genera (Kuschel, 1995). Their diversification is associated with niche shifts in host plants and larval habits. Anderson (1995) argued that the adaptive radiation of weevils was driven by two important factors; the close ecological association between the ancestors of Curculionoidea and ancient angiosperms (Farrell, 1998), and the evolutionary emergence of an elongated rostrum by which the females bore oviposition sites into the tissue or inner parts of the host plant. Regarding the elongated rostrum, major differences in the length between male and female weevils of certain species have been well known for almost two centuries. For example, Kirby & Spence (1826) mentioned this in their '*Introduction of Entomology*'. Darwin (1875, p. 208) remarked that the sexual dimorphism in weevil rostra, as well as many analogous examples, was insufficiently understood but that

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it must be seen in relation to 'different habits of life and not at all, or only indirectly, to the reproductive functions' (in the sense of sexual selection). That species of the genus *Curculio* attack a wide range of host plants is hypothesized to be a result of ecomorphological adaptations to oviposition site, and seed size has been postulated to be responsible for morphological changes in rostrum size (Hughes & Vogler, 2004). A recent series of studies (Toju & Sota, 2006a, b; Toju, 2009) show in great detail that, across different populations of the weevil species *Curculio camelliae* (Curculionidae), the relative female rostrum length correlates strongly with relative thickness of the pericarp of the fruits of its host plant, *Camellia japonica*. It was argued that natural selection drives the coevolutionary arms race between the weevil and its host plant.

Exaggerated female rostra are well documented within the Curculionidae (Menu, 1993; Toju & Sota, 2006a; Oberprieler, Marvaldi & Anderson, 2007). Elongated female rostra are scarcely reported for the Apioninae (Brentidae); however, one exception is *Antliarhinus zamiae*. Females use their extremely long rostra to chew through the cones of their host plants, species of the cycad genus *Encephalartos* (Zamiaceae), and deposit their eggs into the cycad's ovules (Donaldson, 1992; Oberprieler *et al.*, 2007: figs 13 and 14). The Brentidae originated in the mid-Cretaceous and their evolution parallels the rapid radiation of the Angiosperms (Oberprieler *et al.*, 2007; McKenna *et al.*, 2009). Palearctic genera of the Apioninae are characterized by attacking a particular part of their host plant for egg deposition (e.g. young stems, inflorescences, fruits, or immature seedpods). According to the site of egg deposition, larvae develop inside the host plant, and larval feeding habits and host tissue preferences have remained especially conservative in the various evolutionary lines of weevils (Marvaldi *et al.*, 2002).

We investigated three species of Apioninae, *Alocentron curvirostre* (Gyllenhal, 1833), *Aspidapion validum* (Germiny, 1917), and *Rhopalapion longirostre* (Olivier, 1807). The present study focuses mainly on *R. longirostre*, whereas the other two species serve for comparison. Phylogenetic relations among the Middle-European Apioniae are to date largely unknown. In their world catalogue of Curculionoidea, Alonso-Zarazaga & Lyal (1999) listed the genera *Aspidapion* and *Alocentron* within the tribe Aspidapiini, and *Rhopalapion* in the tribe Malvapiini. However, more detailed information and especially a molecular phylogeny of Malvaceae-associated weevils are still missing. All three species investigated in the present study are associated with the same host plant, *Alcea rosea* Linnaeus, 1758 (Malvaceae) (Die Käfer-Fauna Südwestdeutschlands, ARGE SWD, 2006). This plant species presumably originated in Anatolia (Hammer, 1994) and its distribution has expanded to include parts of Europe, North, and South America, mainly as a result of cultivation in gardens and horticultural use. Of the three investigated weevils, only *R. longirostre* clearly exhibits sexual dimorphism in terms of an exaggerated female rostrum. Sexually dimorphic rostra are generally rare in Middle-European Apioninae, which include 139 species belonging to 44 genera. In 113 of these species, there is no obvious sexual dimorphism in rostrum length; in 21 species, there is a slight dimorphism; in four species, the dimorphism is well pronounced, and only *R. longirostrae* shows an extremely dimorphic rostrum (Freude, Harde & Lohse, 1983). Furthermore, among the nine weevil species that are associated with Malvaceae, *R. longirostrae* is the only species that shows an obvious dimorphism. The uniqueness of this sexual dimorphism in *R. longirostrae* implies that a nondimorphic rostrum was the ancestral state, and the extreme elongation of the female rostrum represents an autapomorphic trait. We hypothesize that sexual dimorphism of the rostrum in *R. longirostre* is mainly caused by behavioural and ecological demands (i.e. sexually different tasks of copulation and egg deposition in interaction with the buds of the host plant, *A. rosea*). Furthermore, we assume that the internal and external head morphology reflects those different demands. By a detailed comparison of inner and outer head morphology of the three species, we seek to show the relationships between specific oviposition sites and morphological traits of the investigated weevils. Details on the biology of *R. longirostre* are provided in Behne (1998), Dieckmann (1977), Gønget (1997), Pupier (1997), Sprick, Winkelmann & Behne (2002), Wilhelm (2004), and Wilhelm *et al.* (2010).

MATERIAL AND METHODS
STUDY OBJECTS

Imagoes of *R. longirostre* (Olivier, 1807) (body length 2.4–3.4 mm, excluding rostrum; $\male\male$ $N = 106$, $\female\female$ $N = 84$) (Fig. 1A) were collected from seed capsules of their host plant, *A. rosea* L., from the garden terrace of the University of Vienna, Althanstrasse 14, in August 2008 and September 2009. Imagoes of *A. curvirostre* (Gyllenhal, 1833) (body length 2.9–3.4 mm; $\male\male$ $N = 26$, $\female\female$ $N = 17$) and *A. validum* (Germiny, 1817) (body length 3.2–4 mm; $\male\male$ $N = 20$, $\female\female$ $N = 23$) were collected from *A. rosea* in Tradigist, Lower Austria, in September 2010.

MACROPHOTOGRAPHY

Serial focus images of *R. longirostre* were taken with a Leica MZ16F stereomicroscope (Leica Microsys-
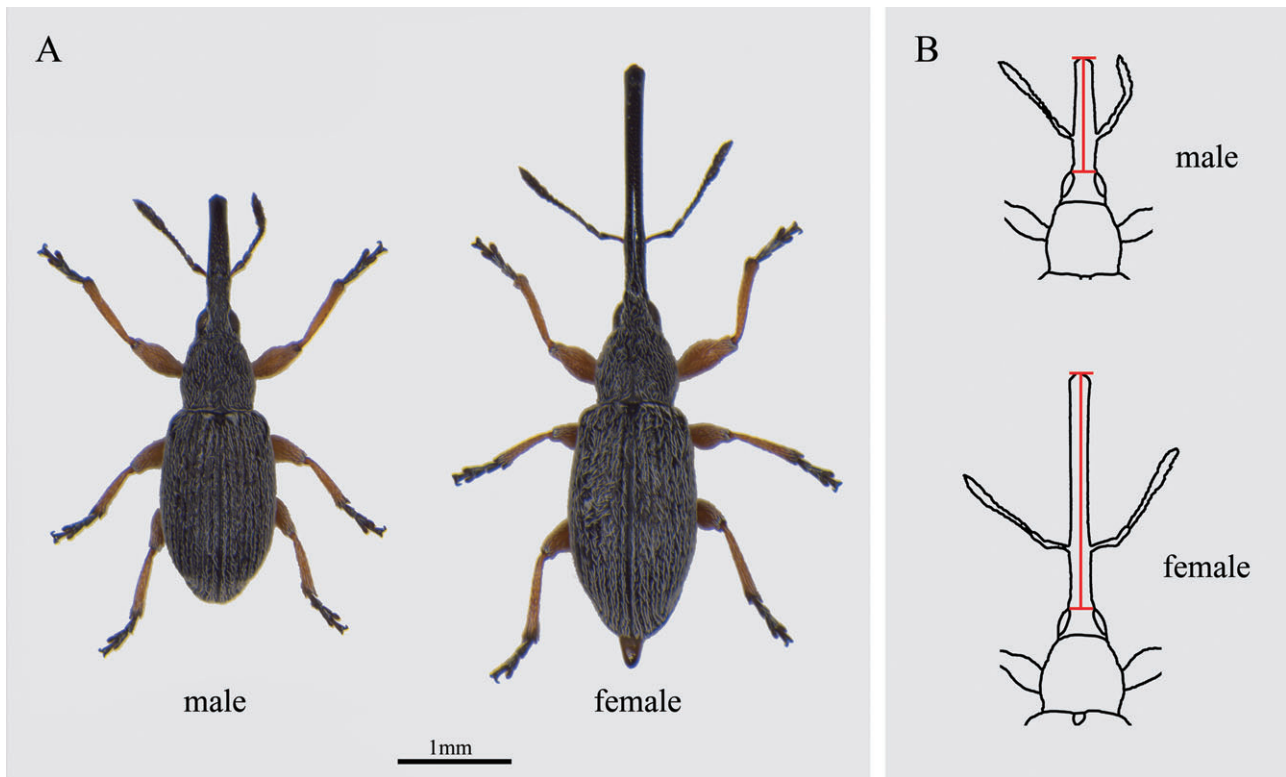
Figure 1. Microphotographs showing the sexual dimorphism in the rostra of male and female imagoes of *Rhopalapion longirostre*. A, the male rostrum is stout and roughly as long as the head plus pronotum. The female rostrum is roughly twice as long as that of the male. B, sections of measurements for measuring chord lengths of rostra with a measuring microscope.

tems) using a ProgRes C5 camera (Jenoptik), and stitched together using ADOBE PHOTOSHOP CS5 (Adobe Systems Inc.) focus-stacking tool.

SCANNING ELECTRON MICROSCOPY

Specimens of *R. longirostre* were dehydrated in a mixture of acidified dimethoxypropane and acetone, and chemically dried with hexamethyldisilazane. The specimens were gold-sputtered using Agar B7340 sputter coater (Agar Scientific Ltd) and photographed in a Philips XL2 scanning electron microscope (Philips Electronics).

MEASUREMENTS OF ROSTRUM LENGTH

Chord-lengths of rostra were measured for both sexes of all three species with a Nikon measuring microscope MM-40 as the distance from the tip of the rostrum (without mouthparts) to the anterior eye edge on the head capsule (Fig. 1B). x- and y-coordinates are given by a calibrated translation stage, and the z-coordinate is given based on the fine-focus position. Based on two measurements per specimen, the arithmetic mean was calculated.

SEMITHIN SERIAL SECTIONS

Male and female head capsules including the rostra were dissected, fixed in Bouin's solution, dehydrated with ethanol and acetone, and embedded in low-viscosity resin (Agar Scientific Ltd) using acetonitril as intermedium. Serial semithin sections (1 μm) were cut with a histo-diamond knife (Diatome AG) on an Ultra Cut-S microtome (Leica Microsystems). Sections were stained with methylene blue/azur II (Richardson, Jarett & Finke, 1960). Section images were taken on a Zeiss Axio Imager A1 (Carl Zeiss Micro-Imaging GmbH) microscope equipped with a ProgRes C14 camera (Jenoptik). Micrographs were edited and labelled using ADOBE PHOTOSHOP CS3 (Adobe Systems Inc).

X-RAY MICROTOMOGRAPHY (MICROCT)

Specimens were fixed in Bouin's solution, rinsed in 70% ethanol, and dissected by removing the abdomen to enable the contrast agent to soak through all tissues. After dehydration in a graded series of ethanol, specimens were stained using 1% iodine in absolute ethanol (I2E: 1% metallic iodine dissolved in 100% ethanol) for 24 h (Metscher, 2009b), and, after

Table 1. Mean ± SD chord length of rostrum within *Alocentron curvirostre*, *Aspidapion validum*, and *Rhopalapion longirostre*

	Alocentron curvirostre	Aspidapion validum	Rhopalapion longirostre
Females	*N* = 17	*N* = 23	*N* = 84
Chord length (mm)	1.270 ± 0.128	1.580 ± 0.292	2.150 ± 0.236
Males	*N* = 26	*N* = 20	*N* = 106
Chord length (mm)	1.147 ± 0.091	1.510 ± 0.391	1.050 ± 0.098
Differences of chord lengths (random permutational test)	*P* < 0.0001	*P* = 0.486	*P* < 0.0001

The last row shows the differences of the chord lengths between sexes in species, as tested by a random permutational test (Nemeschkal, 1999); 10 000 permutations each.

staining, rinsed in 100% ethanol for several hours to remove unbound iodine from the sample. Stained specimens were mounted in heat-sealed pipette tips (Metscher, 2009a) containing 100% ethanol. Samples were scanned using an Xradia MicroXCT (XRadia Inc.) with a tungsten X-ray source at a source voltage of 40 keV and a power of 8W, using a ×4 objective magnification and a 1024 × 1024 pixel detector. To increase image stack quality, we used the DRR function of the Xradia TXM CONTROLLER software (dynamic ring removal via random sample movement). For every scan, four projection images per angle were recorded resulting in total in 737 projections over a rotation of 184 degrees (–92° to +92°). Image stacks were reconstructed using the Xradia TXM RECONSTRUCTOR software 2 × 2 pixel binning (resulting in reconstructed volume images of approximately 512 × 512 × 512 voxels). For the male and female specimen of *R. longirostre*, three scans were recorded of overlapping regions along the rotation axis (tip of rostrum, middle of rostrum, head) and later stitched using the stitch plug-in embedded in the TXM CONTROLLER software. Finally, image stacks were exported in 8-bit TIFF format.

THREE-DIMENSIONAL RECONSTRUCTION AND RENDERING

Serial TIFF images were imported to the reconstruction software AMIRA, version 4.1 (Visage Imaging Inc.) and saved as an image stack in AMIRA mesh (.am) format. Morphological structures were segmented manually using mainly the brush tool of the AMIRA *Segmentation editor*. Based on segmented materials (cuticle, tentorium, mandible muscles and their tendons/apodemes), number of muscle fibres was counted for each muscle and the (voxel-based) volumes of muscles were extracted using the tool

Table 2. Differences of chord lengths between species in females, tested by a random permutational test (Nemeschkal, 1999) (10 000 permutations each)

	Alocentron curvirostre	Aspidapion validum	Rhopalapion longirostre
Alocentron curvirostre		*P* < 0.0001	*P* < 0.0001
Aspidapion validum			*P* < 0.0001
Rhopalapion longirostre			

Tissue statistics. In the same way, the overall head capsule volume was measured based on segmentation of the head capsule up to the anterior margin of the eyes, thus excluding the rostrum to give a valid reference head volume when comparing male and female relative muscle volumes. For visualization, triangle mesh surfaces were created using the tool *SurfaceGen*, and smoothed in several steps by alternately reducing number of triangles and using the tool *smooth surface*. After this, surface files were rendered with the AMIRA *Surface view* (*Direct normals* mode) using multiple viewers to combine solid and transparent surfaces in one rendering. For volume visualizations, we used the AMIRA *Voltex* tool, combining a greyscale colour look-up table (LUT) for the whole head rendering with a red tones LUT for segmented muscles.

STATISTICAL ANALYSIS

First, means and SDs of rostral chord lengths were calculated for both sexes of each weevil species. For testing differences between means of two sexes (Table 1) and two species (Table 2), a random permutational test (Manly, 2006; Roff, 2006) with 10 000 permutations each and based on the general linear model was applied. We preferred the randomization

approach because the normality assumption as required for a parametric *t*-test was not fulfilled in our data material.

Video sequences of live weevils were shot using a JVC GY-HD 111 camera equipped with a Nikon 60-mm macro objective on the garden terrace of the University of Vienna in June 2010 showing pre-copulation, egg channel boring, copulation, egg deposition, and post-copulation in *R. longirostre*. The video was recorded in HDV 720p progressive mode with a resolution of 1280×720 pixels and 25 frames per second. The final video clip was produced using PREMIERE PRO CS5 (Adobe Systems Inc.).

RESULTS
MORPHOLOGICAL COMPARISONS BETWEEN THE SEXES IN *R. LONGIROSTRE*

External head morphology
The remarkable external sexual dimorphism between the male and female rostra of *R. longirostre* is illustrated in Figure 1A. As in all Apioninae, the rostrum of *R. longirostre* is an elongation of the frontal part of the head. It projects forward, is slightly arched, and houses the mouthparts at the apex. Both the rostrum and main head capsule are well sclerotized.

The male rostrum is stout, densely covered with setae and minute pores, and it is approximately as long as the head plus pronotum. The female rostrum is much longer, thin, and smooth, with much fewer setae and pores (Fig. 2A, B). Comparing the absolute chord lengths of rostra, the mean rostrum length is twice as high in females (mean rostrum length = 2.15 mm; $N = 84$) than in males (mean rostrum length = 1.05 mm; $N = 106$). A random permutational test (Nemeschkal, 1999) shows a significant sexual dimorphism (10 000 iterations; $P < 0.0001$) (Table 1). The insertion of the antennae divides the rostrum into a pre-antennal and a post-antennal section, and it is the latter that is obviously elongated in the female (Figs 1, 2A, B). On the ventral post-antennal surface of the male rostrum are shallow grooves (Figs 2C, 3E). The post-antennal-section of the female rostrum is almost round in cross-section and ventral grooves are absent (Figs 2D, 3F).

In both sexes, the pre-antennal part of the rostrum forms two ventral grooves, which receive the antennae when they are folded back (e.g. when the weevil bores into plant tissue), and which are more prominent in the female's cross section (Fig. 3C, D). All Brentidae including Apioninae, to which *R. longirostre* belongs, have straight (orthocerous) antennae, whereas, in the Curculionidae, they are bent (geniculate) (Oberprieler *et al.*, 2007). In *R. longirostre*, they consist of 11 segments. The basal scapus articulates in the rostrum via a monocondylic hinge. The female scapus is longer than that of the male. Fully protruded, male antennae exceed beyond the tip of the rostrum (Figs 1, 2A); however, the female antennae do not extend as far as a result of the increased post-antennal length of her rostrum (Figs 1, 2B).

The mouthparts are situated at the apex of the prolonged rostrum and reveal no apparent sexual dimorphism (Fig. 2E, F). The apex of the rostrum is turned slightly downward. The mandibles are asymmetric and bear prominent teeth on their anterior margins. The maxillae occupy the longitudinal clefts at each side of the labium. The lacinia is a round lobe with long setae. Cardo, stipes, and galea are slightly curved inward and bear few thick setae. The maxillary palps insert between galea and lacinia; they are round and bear distally a number of short papilla-like setae.

Mandibular and maxillary musculature
The muscles of the mouthparts (mandibles and maxillae) originate on the inner walls of the head capsule and the tentorium (Fig. 3A, B); they insert at long, chitinous apodemes (referred to as 'tendons'), which necessarily run the full length of the rostrum to the

Figure 2. Scanning electron micrographs showing male and female rostra and mouthparts of *Rhopalapion longirostre*. A, lateral view of the stout male rostrum. Fully protruded, male antennae exceed beyond the tip of the rostrum. B, lateral view of the long and thin female rostrum. Because of the increased post-antennal length of the female rostrum, antennae cannot exceed beyond the tip of the rostrum. C, ventral view of the male rostrum. The male rostrum is densely covered with setae and minute pores. The antennae are folded back, and the two scapus-segments lie in the pre-antennal grooves. The male rostrum has also post-antennal grooves on the ventral surface. D, lateral view of the female rostrum. The female rostrum is much longer, thin and smooth and bears considerably fewer setae and pores. The left antenna is folded back; visible on the right side is a ventral groove that receives the scapus when folded back. Ventral post-antennal grooves are absent in the female. E, ventral view of male mouthparts, which are situated at the apex of the rostrum. The asymmetric mandibles work perpendicular to axis of the rostrum, whereas the maxillae operate in the longitudinal axis. F, ventral view of female mouthparts showing no obvious differences to that of the male.
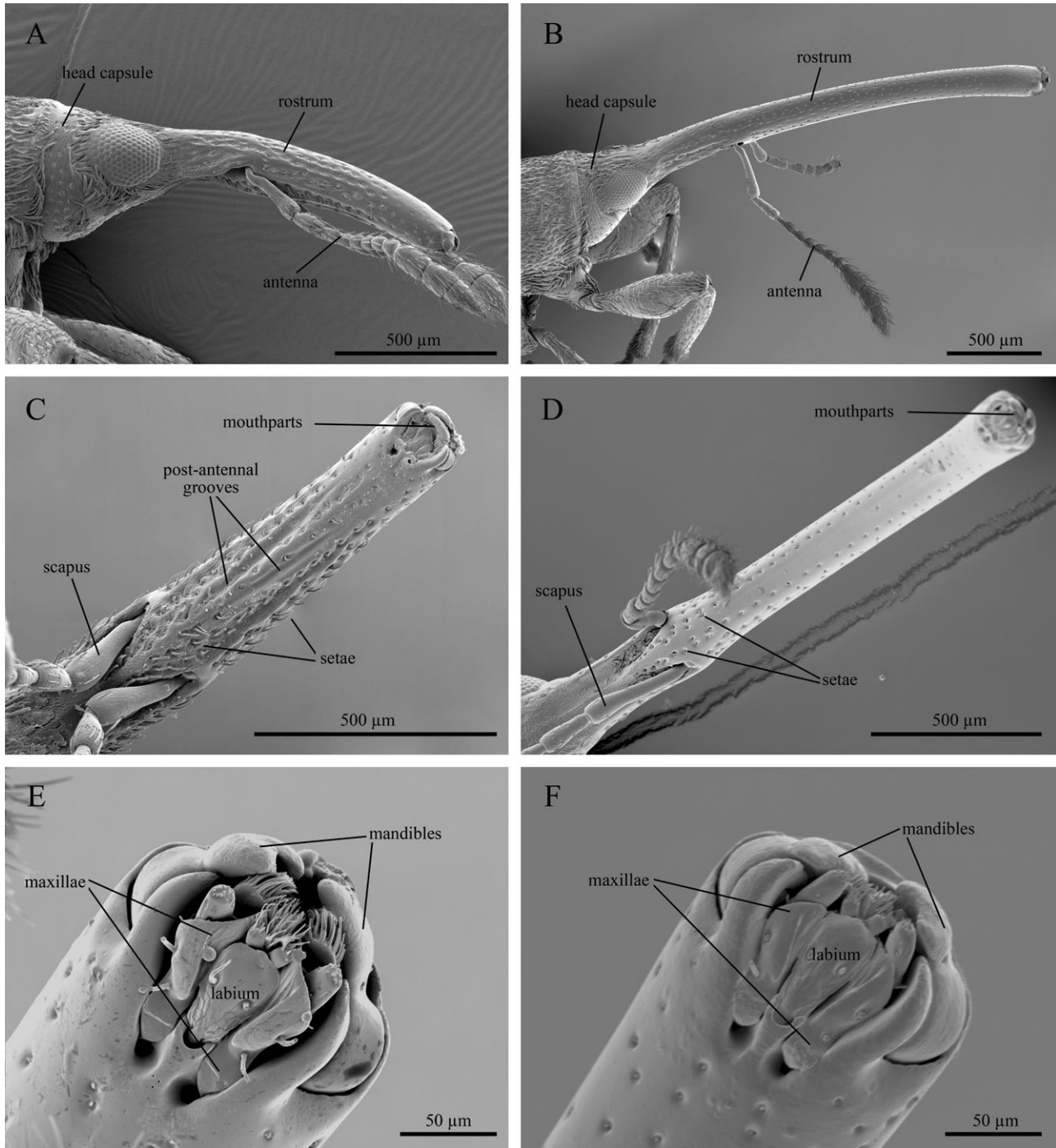
tip where the mouthparts are situated (Fig. 4A, B). Two large mandibular tendons (abductor and adductor tendons;) run ventrally on each side of the rostrum and are guided by sclerotized ridges (Fig. 3C, D). These tendons and their associated muscles are respectively responsible for closing and opening the mandible. In addition, there are three smaller maxillary tendons (lacinial flexor, promotor, and remotor tendons). Further details of the internal head morphology in weevils are provided in Dennell (1941) and Dönges (1954).

Comparison of the male and female mandibular muscles of *R. longirostre* reveals a distinct sexual dimorphism. In general, the mandibular adductor muscle is much larger than the mandibular abductor muscle (Fig. 4B, C). The female mandibular adduc-
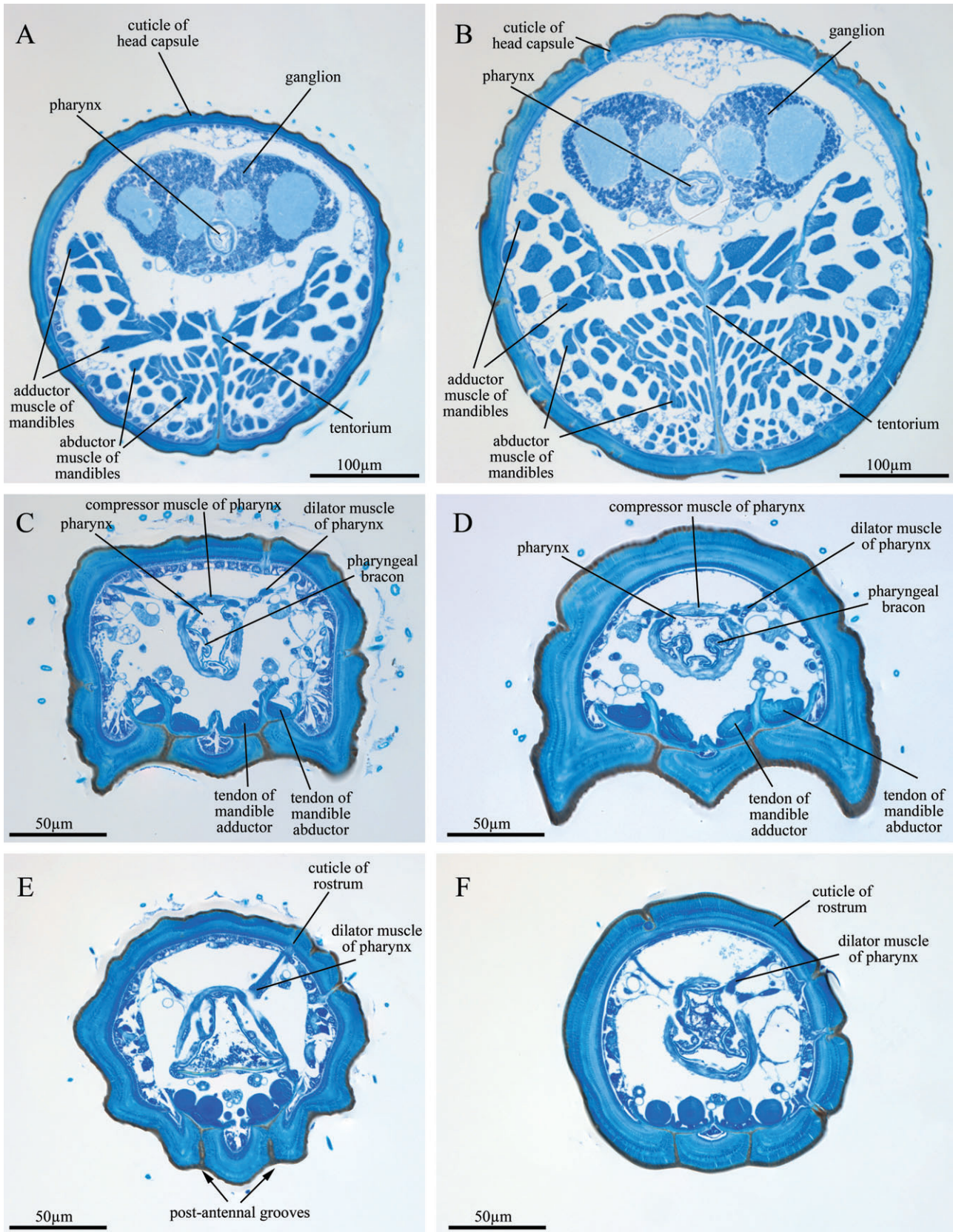
Figure 3. Microphotographs from semithin resin sections (1 μm section thickness, staining with methylene blue/azur II) showing inner head morphology of male and female *Rhopalapion longirostre*. A, cross section through the male head capsule showing the ganglion and the mandibular muscles originating from the walls of the head capsule, as well as from the tentorium. B, cross section through the female head capsule revealing a sexual dimorphism in terms of larger overall muscle size and higher number of muscle fibres in females. C, cross section through the male pre-antennal region of the rostrum showing the pharynx with its dilator and compressor muscles, as well as the large tendons of mandibular muscles. D, cross section through the female pre-antennal region of the rostrum showing inside a similar condition as in the male, whereas, ventrally, the prominent antennal grooves are visible. E, cross section through the male post-antennal region of the rostrum showing a surface that is characterized by notable grooves and number of setae. F, cross section through the female post-antennal region showing a rostrum that is very smooth and lacks notable grooves on its surface.

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tor muscle is significantly larger than that of the male and its origin site is considerably larger because it is dorsally extended at the lateral head capsule wall (Figs 4B, 5C) and medially extended at the posterior head capsule wall. The posterior wall of the head capsule also extends more medially, thus narrowing the horizontal diameter of the hole that connects the body cavity of head and thorax (Fig. 5A, C). Based on image segmentation and three-dimensional reconstruction, we counted, for the female specimen, 128 muscle fibres for the left mandible adductor muscle occupying 7.17% of the head volume (absolute volume of the female left mandibular adductor = 0.00566 mm^3, absolute volume of the female head capsule including cuticula = 0.07861 mm^3). In comparison, the investigated male specimen had only 109 muscle fibres occupying 4.4% of its head volume (absolute volume of the male left mandibular adductor = 0.00308 mm^3, absolute volume of the male head capsule including cuticula = 0.06956 mm^3). Extrapolating these numbers to both sides of the body results in a mandibular adductor that occupies 14.34% of the female head capsule and 8.8% of the male head capsule. These volumes allow an accurate relative comparison, although they do not represent absolute volumes in the living animals because fixation and dehydration may significantly cause shrinkage in muscle tissues.

Similarly, the mandibular abductor muscle is larger in the female than in the male. The dorsolateral origin of the ventrolateral muscle fibres is significantly extended (Figs 4C, 5B, D). In the female specimen, we counted 97 fibres for the left mandible abductor muscle occupying 3.7% of the head volume (absolute volume of the female left mandibular abductor = 0.00293 mm^3, absolute volume of the female head capsule including cuticula = 0.07861 mm^3). In comparison, the male specimen had only 69 muscle fibres occupying 2.34% of its head volume (absolute volume of the male left mandibular abductor = 0.00164 mm^3, absolute volume of the male head capsule including cuticula = 0.06956 mm^3). Extrapolating these numbers for the entire head

results in a mandibular abductor that occupies 7.2% of the female head capsule and 4.68% of the male head capsule. Again, these numbers do not represent absolute volumes in the living animal. In total, female mandible muscles (sum of adductor and abductor volumes) occupy 21.54% of the total head capsule volume, whereas male mandible muscles occupy only 13.48%.

Pharyngeal musculature
Although muscles of the mouthparts are restricted to the head capsule, muscles for expansion and compression of the food channel are arranged serially along the whole length of the pharynx in the rostrum. The pharyngeal dilator muscles originate at the dorsal inner wall of the rostrum and insert at regular intervals to the roof of the pharynx (Fig. 3C–F); they expand the pharyngeal tube upon contraction. The compressor muscles of the pharynx (Fig. 3C, D) work antagonistically, resulting in a compression of the pharyngeal tube. Additionally, the pharyngeal bracons (Fig. 3C, D) prevent the reflux of ingested material. A clear sexual dimorphism is also observable in the number of pharyngeal dilator muscles. Based on the same reconstructions that were used for analysis of mandibular muscles, we counted 186 pharyngeal dilators in the female and only 124 dilators in the male (for one side of the body).

SEXUAL DIMORPHISM IN *A. CURVIROSTRE* AND *A. VALIDUM*

Rostrum length
For comparative purposes, imagoes of *A. curvirostre* (Fig. 6A) and *A. validum* (Fig. 6D) were examined. When comparing the rostra of the sexes of one and the same species, in contrast to *R. longirostre*, these species show no obvious external sexual dimorphism in the length of their rostra. *Aspidapion curvirostre* is the smaller species, with a mean absolute rostrum length of 1.147 mm ($N = 26$) in the male and a mean female rostrum length of 1.27 mm ($N = 17$) (Table 1). Imagoes of *A. validum* are
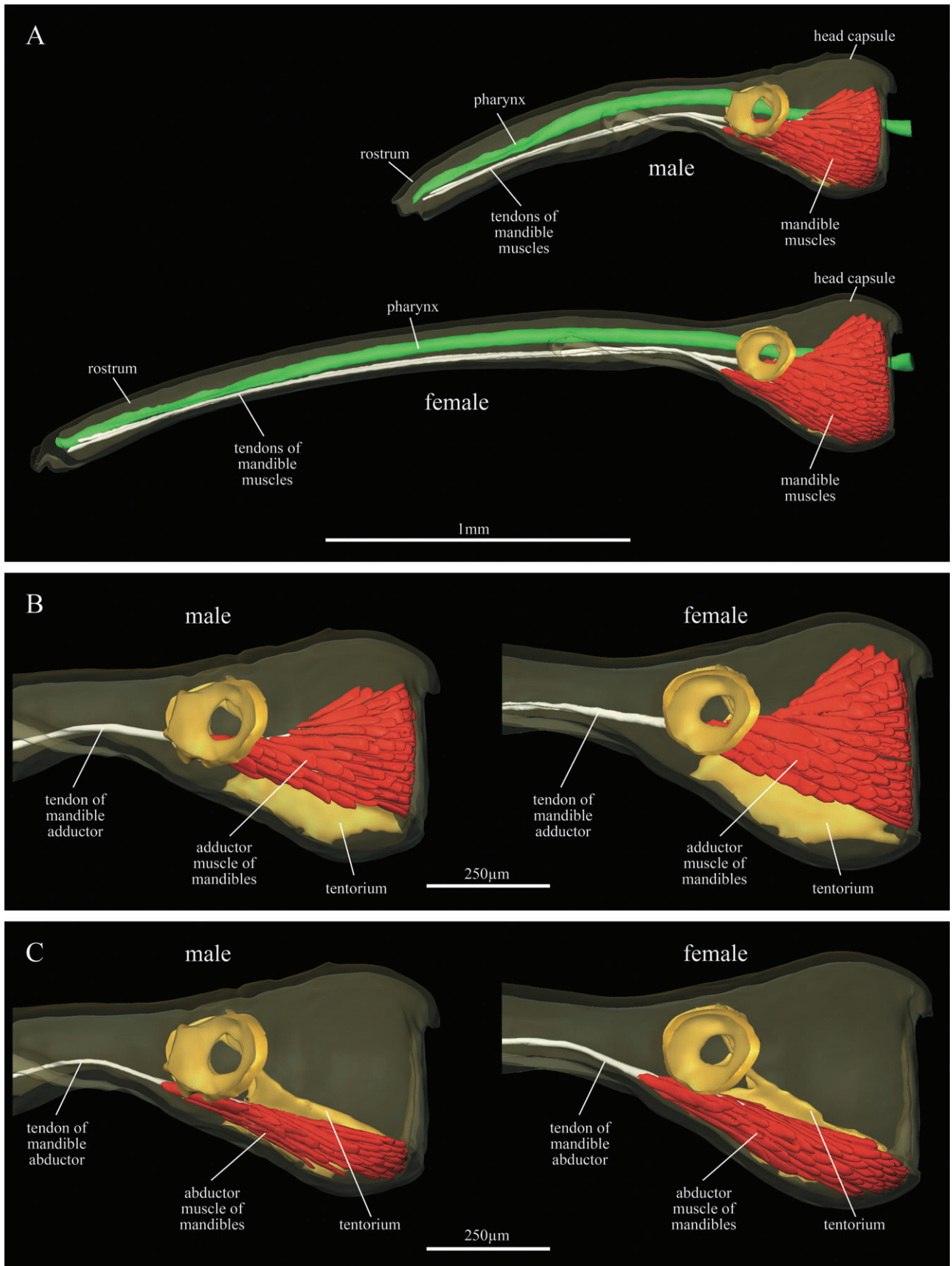
Figure 4. Three-dimensional reconstructions of the head of male and female *Rhopalapion longirostre* showing the head capsule and rostrum, pharynx, tentorium, the mandibular muscles and their tendons. A, lateral view of the whole head again reveals the striking dimorphism in the shape of the head concerning the length of the rostrum, which is significantly enlarged in the female. In addition, the female mandibular muscles are significantly larger than that of the male. B, lateral view of the male and female mandibular adductor muscles showing that the female mandibular adductor is significantly larger than that of the male, with an enlargement of muscle fibre origin site to more dorsal parts of the lateral head capsule wall. C, lateral view of the male and female mandibular abductor muscles showing that the female mandibular abductor is larger than that of the male.

generally larger, with a mean rostrum length of 1.51 mm ($N = 20$) in the male and a mean female rostrum length of 1.58 mm ($N = 23$) (Table 1). Comparing the absolute female rostrum lengths of all three investigated species using a random permutational test revealed significant differences between the females of all species (Table 2). Testing for sexual dimorphism in absolute rostrum length using a random permutational test revealed a significant sexual dimorphism in *A. curvirostre* (10 000 iterations; $P < 0.0001$) but not in *A. validum* (10 000 iterations; $P = 0.486$) (Table 1).

Mandibular musculature
Aspidapion curvirostre shows a sexual dimorphism in mandibular muscles, which are larger in the female. Similar to *R. longirostre*, we find instances of sexual dimorphism in this species in the enlargement of muscle fibre origins to more dorsal parts of the lateral head capsule (Fig. 6B, C). However, the sexual dimorphism in the mandibular muscles is less pronounced than in *R. longirostre*, where enlargement of fibre origins also involves other parts of the head capsule (Figs 4, 5). In *A. validum*, a slight sexual dimorphism is indicated concerning the cross sections of single muscle fibres. The thickness of the fibres in the female are comparable to that in *R. longirostre* and *A. curvirostre* (Fig. 6B, E); however, in the male, they are much thinner and amount to a much smaller total cross sectional area (Fig. 6E, F).

MATING AND EGG DEPOSITION IN *R. LONGIROSTRE*
Videos (see Supporting information, Videos S1, S2, S3, S4) show the behavioural sequence of egg channel boring, copulation, and egg deposition in detail [for higher quality, see external permalinks for the whole movie: standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645)]. The most important events are: (1) after finding an appropriate bud the female weevil, with the male on her back, bores an egg channel into it (Fig. 7A; see also Supporting information, Video S1); (2) she folds her antennae back completely, thus enabling her to insert the rostrum very

deeply through the two layers of the bud sepals (Fig. 7B; see also Supporting information, Video S1); (3) as soon as the female is willing to copulate, several other males attempt to hinder copulation by using their stout rostra as a lever (Fig. 7C; see also Supporting information, Video S2); and (4) after copulation, the female finishes egg channel boring, with her rostrum in a position maximally inserted into bud tissue up to the anterior eye margin. After removing her rostrum out of the borehole, she turns around and inserts her pygidium into the egg channel to deposit eggs (Fig. 7D; see also Supporting information, Video S4).

DISCUSSION

SEXUAL DIMORPHISM IN ROSTRUM LENGTH: A FEMALE RESPONSE TO EGG CHANNEL BORING?

We have shown explicitly that the female of *R. longirostre* uses her elongated rostrum for boring an egg channel through the two layers of sepals (see Supporting information, Videos S1–4). The weevil feeds monophagously on its host plant, together with two closely-related species of Brentidae: *A. curvirostre* and *A. validum* (G. Wilhelm, pers. observ.). Weevil communities on *A. rosea* may differ in various regions in Middle Europe (Kozlowski & Knutelski, 2003). Both sexes of all three species consume leaf parts of the host plant but occupy different parts of the plant for development. Although *A. curvirostre* and *A. validum* develop in the stems of *A. rosea* (Fig. 8), *R. longirostre* exclusively chooses the buds for development (Die Käfer-Fauna Südwestdeutschlands, ARGE SWD, 2006; see Supporting information, Videos S1, S2, S3, S4; Fig. 7). We argue that the exaggerated relative female rostrum length in *R. longirostre* is an adaptation to bore egg channels into buds, which is based on two important facts. First, the obvious sexual dimorphism and the behavioural observation demonstrate its function in reproduction during copulation and egg deposition. Second, the female rostrum is not exaggerated at all in the two related species that bore into the stems but not into buds.

In all three species, the females bore egg channels into the selected plant parts, although why should a
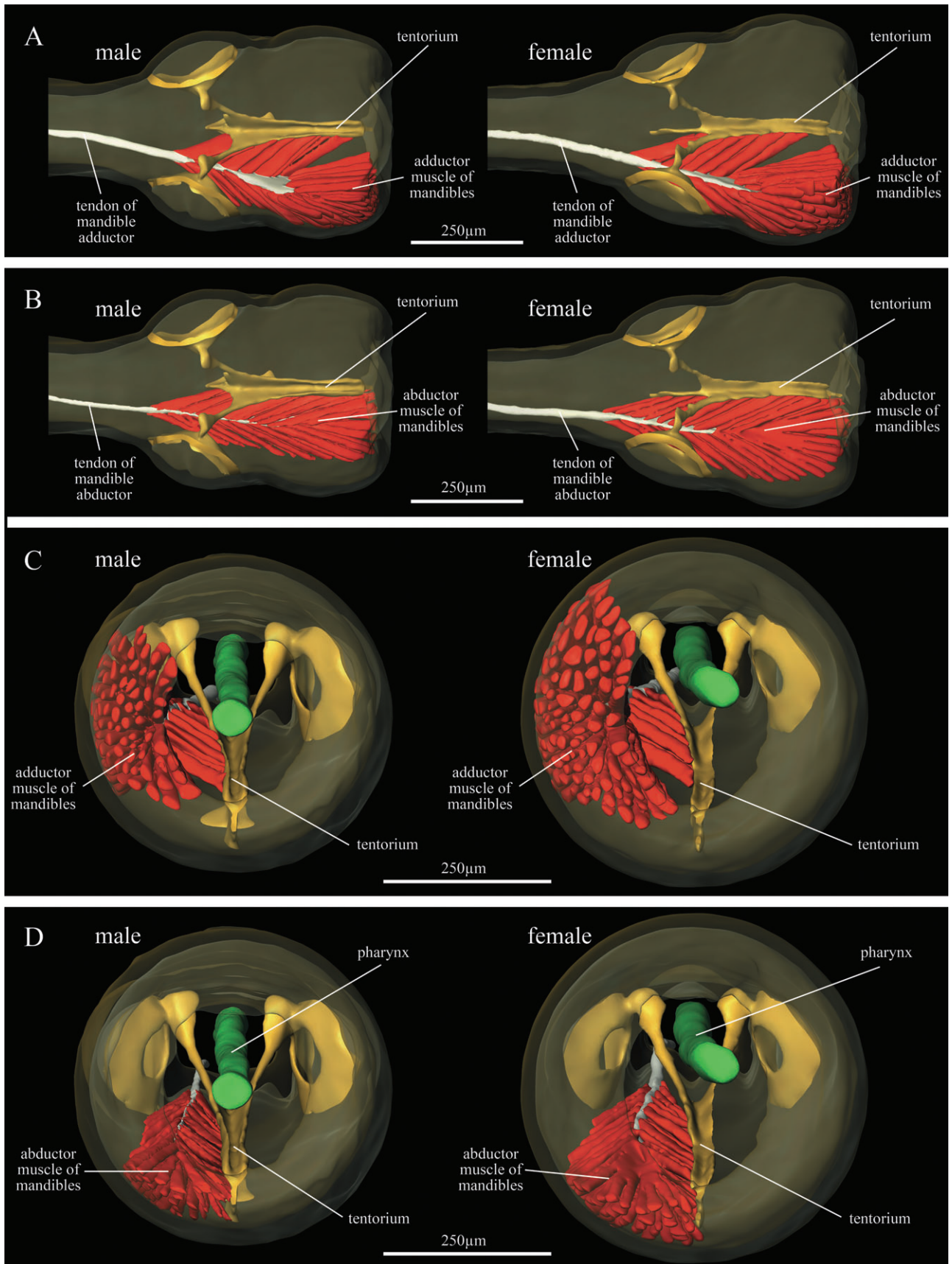
Figure 5. Three-dimensional reconstructions of the head of male and female *Rhopalapion longirostre* showing the head capsule and rostrum, pharynx, tentorium, the mandibular muscles and their tendons. A, dorsal view of the male and female mandibular adductor muscles showing that the female mandibular adductor is significantly larger than that of the male, with an extension of muscle fibre origins to more median parts of the posterior head capsule wall. B, dorsal view of the male and female mandibular abductor muscles showing that the female mandibular abductor is larger than that of the male. C, posterior view of the male and female mandibular adductor muscles showing that the female mandibular adductor is significantly larger than that of the male, with an enlargement of muscle fibre origin sites to more dorsal parts of the lateral head capsule wall and to more median parts of the posterior head capsule wall. The muscle medially occupies more space and thus narrows the internal horizontal diameter of the head cavity. D, posterior view of the male and female mandibular abductor muscles showing that the female mandibular abductor is larger than that of the male and has a dorsolateral enlargement of the ventrolateral muscle fibre origin site.

long rostrum be necessary to bore into *A. rosea* buds? The buds of the long inflorescences of *A. rosea* are surrounded by outer and inner sepals bearing a thick cover of stiff hairs, which protects the pollen grains. The mechanical requirement for boring through such a thick layer is an elongated boring tool (Fig. 7A). The smooth rostrum surface and the mouthparts at the tip (Fig. 2B, D, F) are excellent tools to drill a long borehole through the thick sepals. When boring, the female partly rotates her head making the process more efficient (see Supporting information, Video S1), similar to a drill used in geological boring. The mandibles at the tip of the rostrum correspond with the drill bit, the elongated snout with the drilling rod, and the excavated plant tissue is transported through the pharynx with the drilling core. By contrast to the long and smooth female rostrum, the male rostrum lacks these adaptations for boring deeply into bud tissue. It is stout, and its surface is structured with grooves and ridges, which provide a higher mechanical stability. The male does not bore into flower buds but feeds on plant tissue. Presumably, males use their rostrum as a tool to disturb other males during copulation in attempts to displace them (see Supporting information, Video S2), as described for other weevil species (Yoshitake & Kawashima, 2004).

SEXUAL DIMORPHISMS IN MANDIBLE MUSCULATURE

We argue that the increased length and smoother surface of female rostra are adaptations to boring in specific plant tissues. However, sexual dimorphism is not restricted to external characters but also concerns the musculature of mouthparts. In *R. longirostre*, we reveal a clear dimorphism in mandible musculature. Both mandible adductor and abductor occupy significantly larger volumes within the head capsule of females. Two alternate hypotheses can be formulated: (1) a long rostrum needs a larger muscle volume to ensure the same bite-force than a short rostrum; (2) muscle volumes do not coincide with

rostrum length. The larger volume yields a stronger bite force, whereas twice the rostrum length has little to no impact on power transmission from muscles to mandible via the apodemes. We favour the second explanation because the sex differences in adductor (i.e. relative muscle volume of 14.34% of the female and 8.8% of the male head capsule volume) and abductor (i.e. relative muscle volume of 7.2% of the female and 4.68% of the male head capsule volume) muscle volume are so clear that an explanation solely based on lower power transmission in longer apodemes is not sufficient. Chitinous insect apodemes are stiff organs with a tensile modulus approaching that of bone (Young's modulus for locust apodemes $= 1.2 \times 10^{10}$; Young's modulus for bone $= 1.7 \times 10^{10}$) (McNeill Alexander, 1983). Observations in *A. curvirostre* and *A. validum* support this interpretation because they exhibit no obvious sexual dimorphism in rostrum length but notable differences in mandible musculature.

Some females of *A. validum* have a stronger (i.e. thicker) mandible musculature than males. Additionally, one of three investigated female specimens of *A. validum* showed an enlargement of muscle origin extending to more dorsal parts of the lateral head capsule wall. Based on comparative morphology, we conclude that sexual dimorphism in mandible muscles is less pronounced in *A. validum* and *A. curvirostre* than in *R. longirostre*. Our volume measurements and observations based on microCT images and serial sections leave no doubt concerning the sexual dimorphism in mandible muscles in this species (Figs 4, 5). Given the small sample size ($N = 6$ in *A. validum*, $N = 2$ in *A. curvirostre*), we cannot give a confident estimation of intrasexual variability in either of the two species. Consequently, we cannot exclude overlap of muscle size range between the sexes. Nevertheless, we repeatedly found the tendency of larger muscles in females than in males. Assuming that muscle size in both species is larger in females than in males, we can conclude that larger muscle volumes and stronger bite forces are
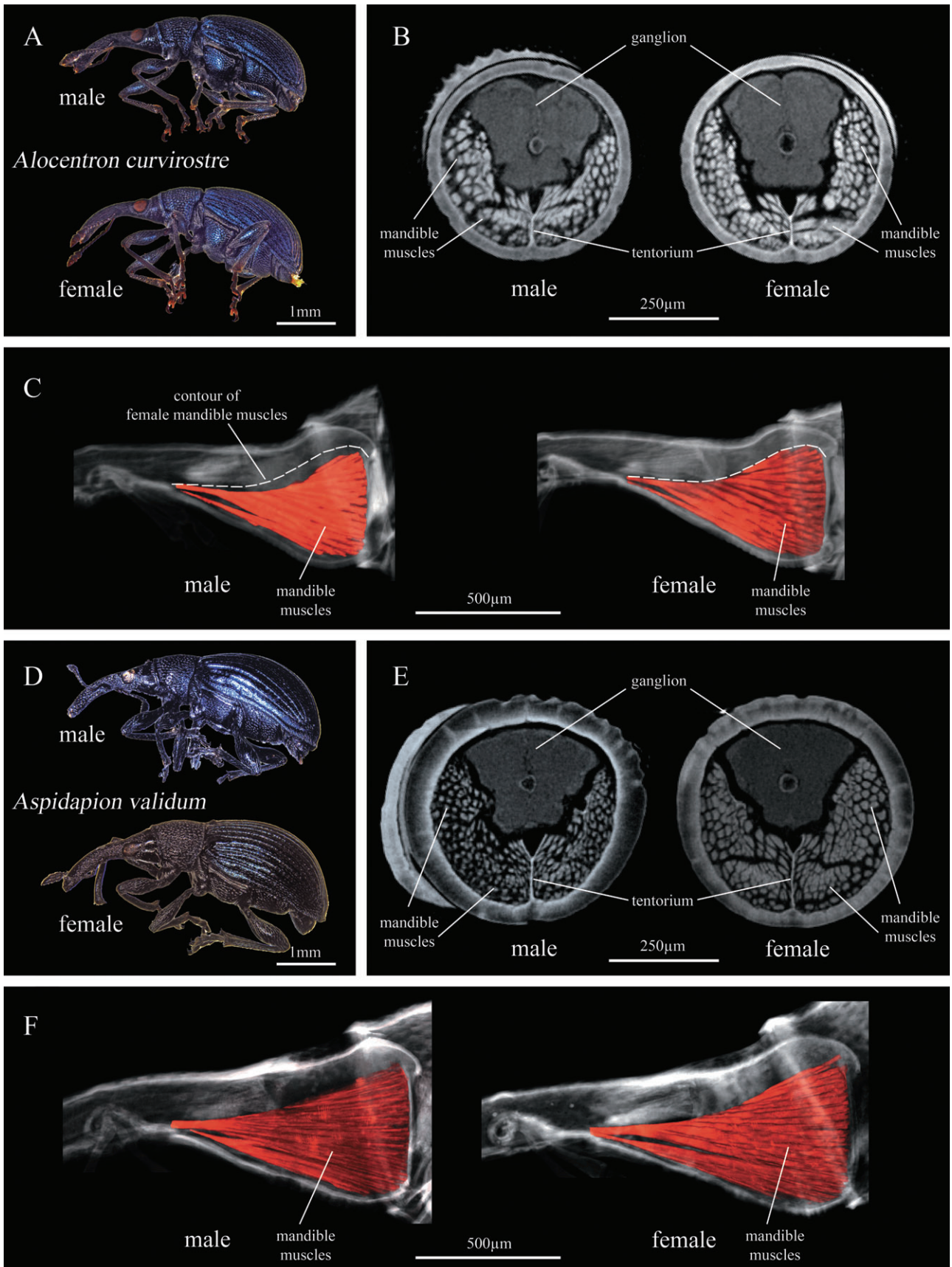
Figure 6. Sexual dimorphism in the mandibular musculature of *Alocentron curvirostre* and *Aspidapion validum*. A, microphotographs showing male and female imagoes of *A. curvirostre*. B, virtual sections through the male and female head capsules of *A. curvirostre* from a X-ray microtomography (microCT) based image stack showing the ganglion and the mandibular muscles originating from the walls of the head capsule, as well as from the tentorium. The fibre thickness in mandibular muscles of both sexes is similar but the overall muscle is larger in the female. C, lateral view of volume renderings of the male and female head capsules of *A. curvirostre* from a microCT based image stack showing sexual dimorphism in mandibular muscles. The female mandibular muscles are larger than that of the male, and the muscle fibre origin sites are expanded to more dorsal parts of the lateral head capsule wall. D, microphotographs showing male and female imagoes of *A. validum*. E, virtual sections through the male and female head capsules of *A. validum* from a microCT based image stack showing the ganglion and the mandibular muscles originating from the walls of the head capsule, as well as from the tentorium. These sections reveal a sexual dimorphism in mandibular muscles, in that the cross section area of single muscle fibres is larger in the female than in the male. F, lateral view of volume renderings of the male and female head capsules of *A. validum* from a microCT based image stack showing very similar contours of male and female mandibular muscles but thinner muscle fibres in the male.

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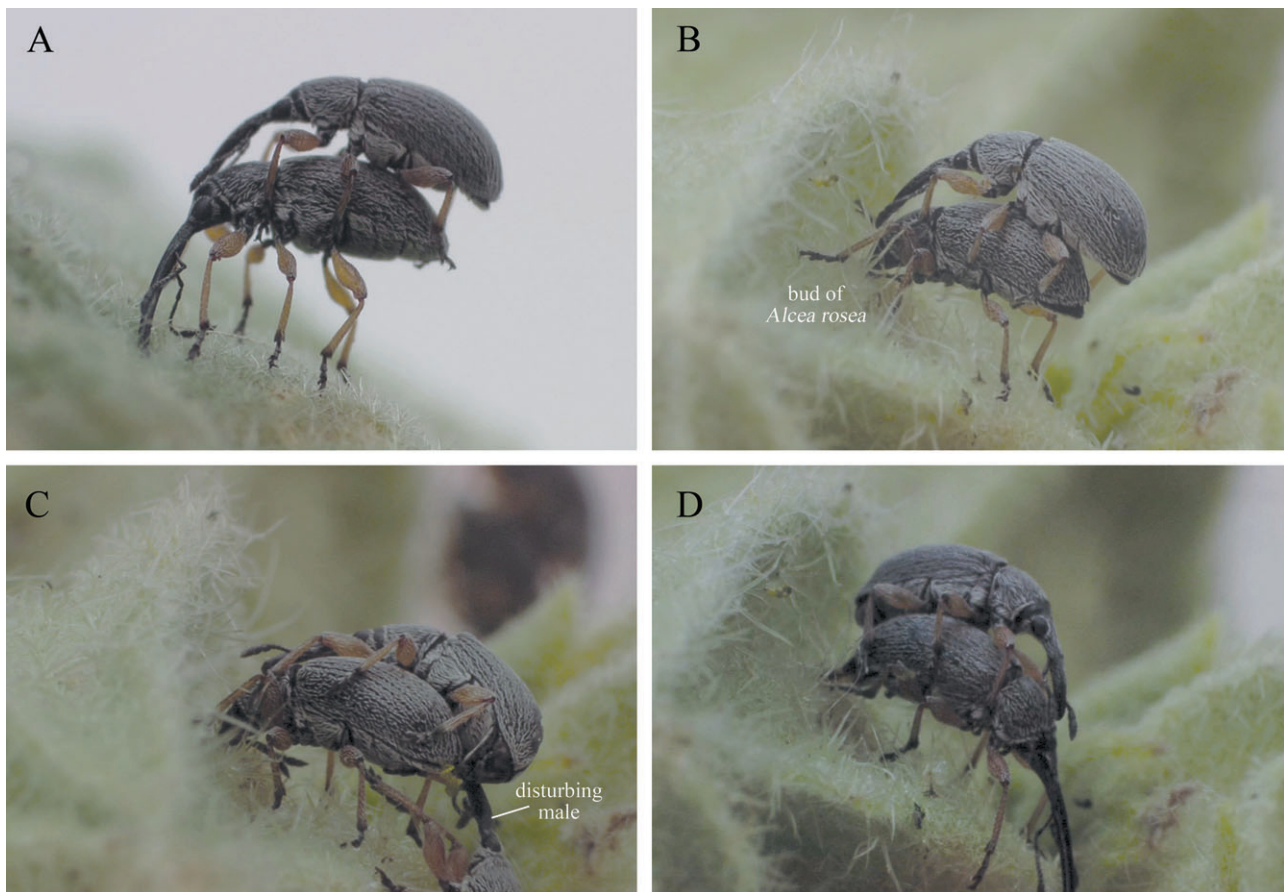
Figure 7. Behaviour of *Rhopalapion longirostre*, male and female during egg channel boring, copulation and egg deposition (see also Supporting information, Videos S1, S2, S3, S4). A, the female is looking for an appropriate site in a bud of *Alcea rosea*. B, with the male on her back, she bores a deep channel into the bud. C, a second male tries to disturb copulation using his stout rostrum. D, the female turns around and deposits her eggs into the bored channel.

biomechanical adaptations to bore into hard materials, such as the buds and stems of *A. rosea* that are much harder than the plant's leaves, on which both sexes feed. In this interpretation, adaptations in muscle size do not necessarily coincide with increasing rostrum length, which can be explained in terms of required depth of the bore channel for different parts of the plant.
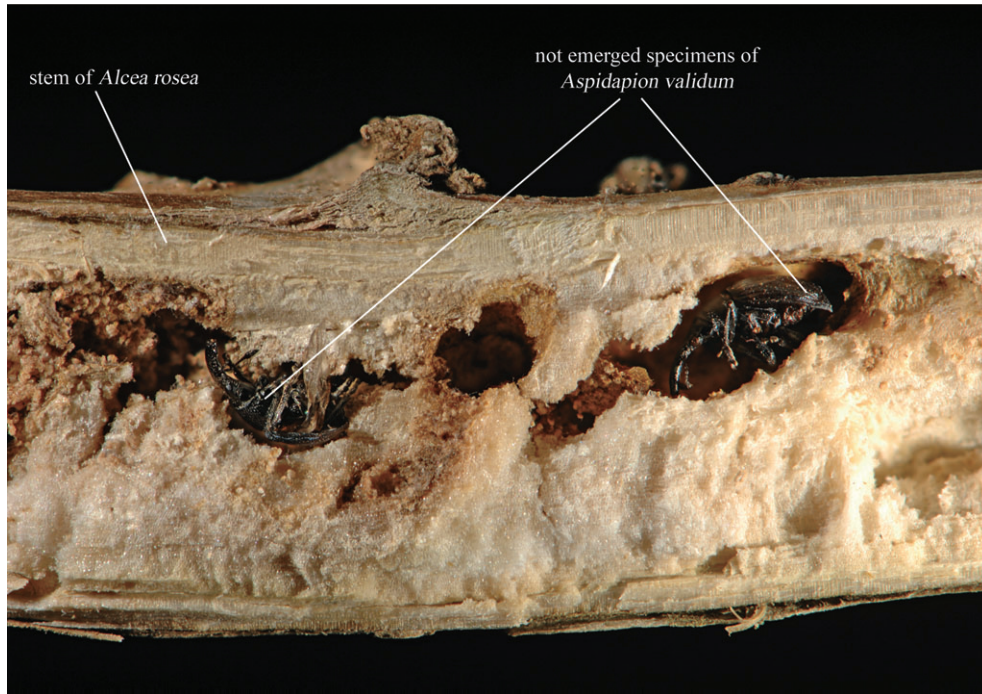
Figure 8. Unemerged adults of *Aspidapion validum* found in a pith of a stem of *Alcea rosea*.

THE ROLE OF THE ANTENNA IN EGG CHANNEL BORING

Oberprieler *et al*. (2007) argued that the orthocerous antennae of the Apioninae are disadvantageous in an evolutionary context because they are straight and cannot be folded back easily. They considered this as the reason why the diversity of species having orthocerous antennae is significantly lower compared to the diversity of Curculionidae, which possess geniculate antennae. Although this argument may apply to many Apioninae species, it does not hold true for the female of *R. longirostre* because the basal scapi of the orthocerous antennae are elongated, and the antennae can be folded back completely (Figs 2C, D, 7B; see also Supporting information, Video S3). The prominent pre-antennal grooves receive the antennae during the boring process, which enables the female to insert the rostrum as far as the anterior margin of her eyes (see Supporting information, Video S3).

ECOLOGICAL DEMANDS: FACTORS LEADING TO SEXUAL DIMORPHISM

In the extinct Huia bird (*Heteralocha acutirostris*) from New Zealand, the beak of the male was short, slightly arched downward and robust, as in woodpeckers, whereas the female's beak was finer, longer, and curved downward, as in hummingbirds (Buller, 1872–1873; Darwin, 1875, pp. 208, 209). Whereas the male used its bill to chisel into outer layers of decaying wood or live trees, the female's bill reached areas inaccessible to the male, such as the burrows of insect larvae into the wood of live trees. The divergence between the bills represents an example of niche differentiation by which the intraspecific competition between the sexes was reduced and allowed the Huia to extend their food sources (Moorhouse, 1996). Mentioning this example, Darwin noted that in 'some few birds the beak of the male differs from that of the female', although he also stated that 'differences of structure between the two sexes in relation to different habits of life are generally confined to the lower animals'. Concerning weevils, he wrote, 'In some weevil-beetles . . . there is a great difference between the male and female in the length of the rostrum or snout; but the meaning of this . . . is not at all understood' (Darwin, 1875, p. 208). In the Curculionidae, the existence of an elongated female rostrum is a well-known phenomenon. For example, in a thoroughly analyzed system involving the weevil predator *Curculio camelliae* and its host plant *Camellia japonica* (Toju & Sota, 2006a), the significance of the long rostrum is interpreted as an adaptive key trait in a coevolutionary arms race. The females bore channels through the thick pericarps using their rostra and deposit eggs with their ovipositor into the fruits of *C. japonica* and the developing larvae infest the seeds. The last-larval instars escape from the fruits and overwinter in the soil under trees (Saito &

Suzuki, 1982). Imagoes of both sexes are equipped with long rostra; the female rostrum is twice as long as that of the male (Toju, 2008). The development of a thick pericarp in *C. japonica* is seen as a significant selective factor resulting from predation by *C. camelliae*. One outcome of the coevolutionary arms race is the evolution of the longest rostrum in the genus *Curculio* (Toju & Sota, 2009).

In our focal study object, *R. longirostre* (Brentidae: Apioninae), there is a conspicuous sexual dimorphism in rostrum length similar to that in the genus *Curculio*. The female rostrum in *R. longirostre* is the longest in Middle-European species of Apioninae (Kozlowski & Knutelski, 2003), and it is more than twice as long as the stout male rostrum (Figs 1, 2). Thus, we must address the following question: what kinds of demands are predominately responsible for the emergence of sexual dimorphism in this species?

ADAPTATION OF ROSTRUM LENGTH IN *R. LONGIROSTRE*

The interaction between the host plant and weevil parasite investigated in the present study is very complex, and we propose that a coevolutionary arms race is not necessarily involved in the emergence of the elongated rostrum of *R. longirostre*. This is mainly based on features of its life history that differ significantly from *C. camelliae*. *Rhopalapion longirostre* bores into buds but not into fruits. The inflorescences of *A. rosea* contain approximately 40–60 buds in various stages of maturity that ripen in a proximo-distal fashion. To oviposit, females of *R. longirostre* tend to choose buds at a certain stage of maturity (i.e. which are ripe and contain well-developed pollen grains and ovules that will blossom in approximately 2 weeks). Choosing the appropriate stage of bud development ensures that there will be enough time for larval development (Wilhelm, 2004). Generally, when the proximal buds of an inflorescence have ripened and blossomed, the distally located buds are at an earlier stage of development (i.e. smaller and unripe). *Alcea rosea* is a perennial plant; thus, in the first year, it develops small inflorescences with few tiny buds, and the ripe seed capsules contain approximately 30 single seed chambers. During the second and third years, the inflorescences attain a height of approximately 1.5–2 m with more and larger buds having approximately 35–40 seed chambers in each seed capsule (G. Wilhelm, pers. observ.).

This leads to a certain set of circumstances in an *A. rosea* population: when females search for boring localities, there are plants of different ages and thus differently sized buds at the preferred stage of maturity. In addition, size variability among buds within the same plant contributes to total size variability in buds. Several days after the female bores an egg channel through the double layers of bud sepals and deposits three to four eggs into the inner bud, the larvae begin to hatch. They gnaw feeding channels through the pollen grains in the direction of the plant's ovules. By contrast to the behaviour of *Curculio* sp. (Saito & Suzuki, 1982), the last-larval instar enters one of the seed chambers in the seed capsule and consumes the entire ripe seed. Before pupation, the larva bites a hole into the wall of the seed chamber to ease its emergence as an adult from the dry chamber.

One crucial question remains to be addressed. How does this relate to fitness and how did natural selection favour elongated rostra in the evolution of the sexual dimorphism in *R. longirostre*? We hypothesize that elongated rostra were favoured by natural selection for two reasons. First, a longer rostrum enables the weevil to bore through the sepals of larger buds, and larger buds possess, on average, larger seeds. Because larger seeds contain more food resources, this yields a fitness advantage for larvae that develop in larger seeds. Second, a larger rostrum enables deeper penetration into the pollen grains, and eggs that are deposited deeper inside the bud are better protected against environmental stress.

Given the fact that different sizes of buds are available for females to deposit their eggs, and accepting the hypothesis that an elongated rostrum yields a fitness advantage by providing better conditions for larval development, it becomes apparent that natural selection favours an elongated rostrum. Larger rostra enable weevils to bore into larger buds, thereby increasing their reproductive fitness. To explain an adaptive scenario, we need not to assume a coevolutionary arms race because different sized buds are available as boring localities. Weevil adaptation can proceed, in principle, without a direct response of the host plant in terms of thicker sepals. In our investigated animal–plant interaction, this represents a plausible scenario because the success of the plant is guaranteed by temporal succession of buds (for a similar ecological scenario, see Nuismer & Ridenhour, 2008; in their system, seed parasitism of larvae not only causes a reduction in host plant fitness, but also contributes to phenotypic selection for flowering time and floral display size). The weevils use a temporal window for their development, and the time periods before and after weevil activity ensure ample opportunity for the successful production of plant seeds (Wilhelm *et al.*, 2010). Based on our present data, we cannot exclude the possibility that an arms race occurs, although, if it does (which can only be tested by an investigation on different populations including measurements on the thickness of sepals), we conclude that it is much less effective than in the *C. camelliae* system because the successful strategy of temporal bud

succession may weaken selective pressure on sepal thickness.

RISKS AND BENEFITS ARISING FROM AN ELONGATED ROSTRUM IN *R. LONGIROSTRE*

We have argued that natural selection favours rostrum elongation, although the elongated rostrum of females also bears a high risk when metamorphosed weevils attempt to leave their site of pupal development. The exit from the dry seed chambers after pupation and development to adult weevils is problematic, particularly for the females. In a mortality study (G. Wilhelm, unpubl. data), 205 dry seed capsules were examined using logistic regression, randomization tests, and bootstrapping (Nemeschkal, 1999). This preliminary study showed that there were significantly more dead females having broken rostra in seed chambers than males ($P = 0.0468$, permutational test: 10 000 iterations, one-sided tested). The extraordinarily long female rostrum is obviously disadvantageous for escaping. Mortality during escaping may counteract selection for rostrum elongation, thus placing a limit on rostrum exaggeration.

Despite the risks in escaping seed chambers, development inside the bud is presumably an advantage for *R. longirostre* because the larvae of *R. longirostre* feed on plant parts with high energy density (i.e. pollen grains). The last instar enters a chamber of the seed capsule and consumes the seed, which is stuffed with cyclopropenoid fatty acids and main fatty acids typical for Malvaceae (Asparagin, Betain and Lecithin) (Bruchhausen, Hager & Blumer-Schwinum, 1993). We argue that the benefits arising from development in seed chambers significantly exceed the drawbacks of having to escape from them, thus rendering *R. longirostre* a successful weevil species with high local abundances.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Supporting video 1. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 1: Pre-copulation and egg channel boring. A female weevil with a male on her back searches for an appropriate locality to bore an egg channel into a bud of *Alcea rosea*. Searching, pre-copulation, and egg channel boring may take up to several hours (in this clip, only a few minutes of this phase are shown). The female folds her antennae back completely, thus enabling her to insert the rostrum very deep into plant tissue (2:26). During pre-copulation, the male stimulates the female with the tarsae of his third pair of legs (02:35). This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

Supporting video 2. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 2: Copulation. Once the female is willing to copulate, she lowers her pygidium (00:09) to receive the male's aedeagus (00:15). At this point, several males may attempt to disturb copulation using their stout rostra. The male is able to fend off the other competitive males and successfully achieves copulation (02:04). This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra. univie.ac.at/o:102645).

Supporting video 3. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 3: Post-copulation and egg deposition (Part 1). After copulation, the female finishes construction of the borehole with her rostrum maximally inserted into bud tissue up to the anterior eye margin (00:30), whereas the male continues to stimulate her pygidium (00:43). This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra. univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

Supporting video 4. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 3: Post-copulation and egg deposition (Part 2). The female removes the rostrum from the borehole (00:06), turns around (00:23), and inserts her pygidium to deposit three of four eggs into the egg channel (01:00); egg deposition can take up to 20 min. During post-copulation, the male remains on the female's back even after she has departed from the bud (02:00); post-copulation may take up to 1 h. This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

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