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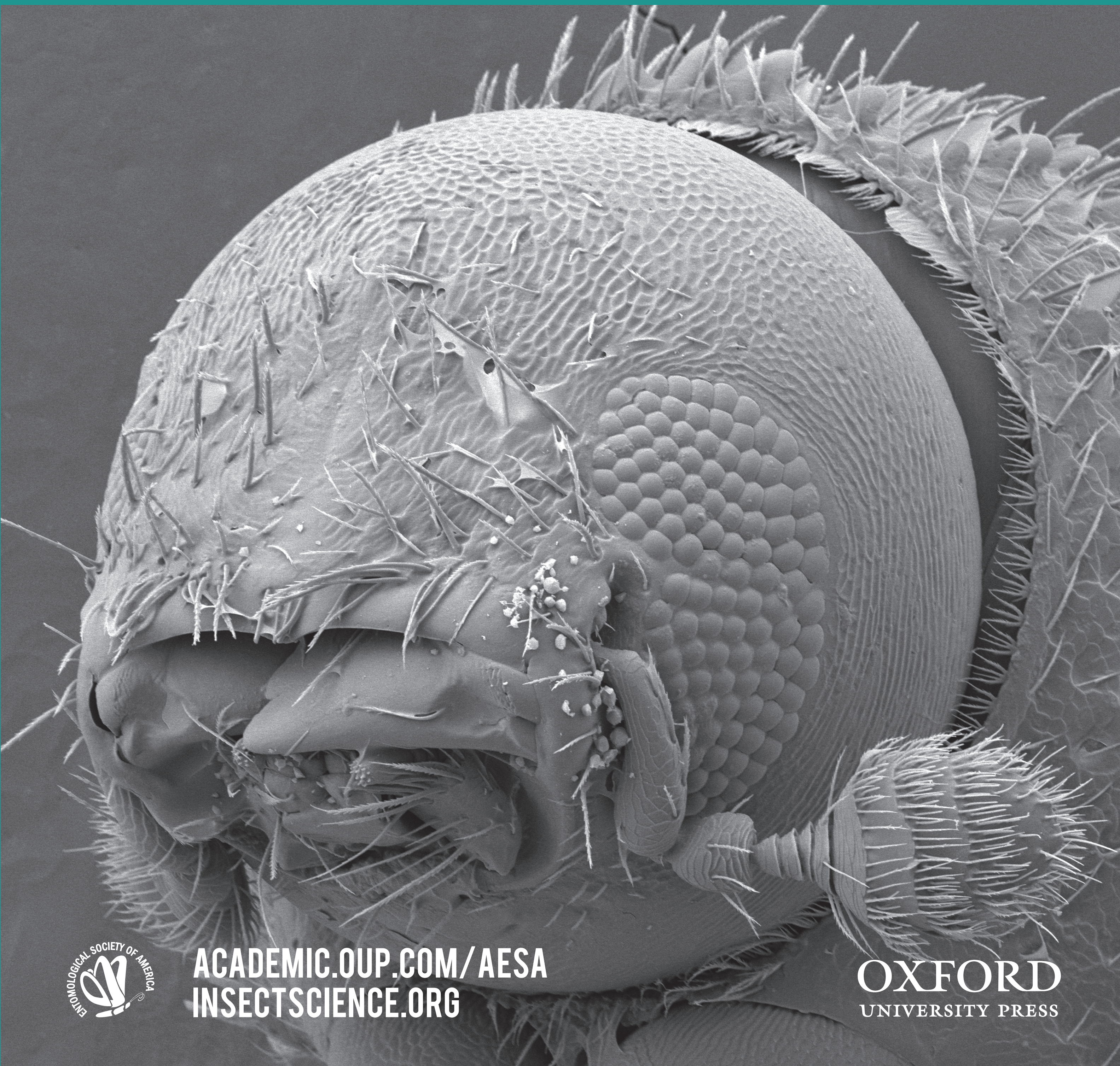

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Research

Mouthpart Structure and Elemental Composition of the Mandibles in the Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae)

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Abstract

The various parts of the mouth in the coffee berry borer, *Hypothenemus hampei* (Ferrari), have been visualized and identified using scanning electron microscopy. The labial and maxillary palpi are three jointed and connected by a membrane that allows for telescoping. The maxillary palpi contain two types of sensilla (basiconic and campaniform) within an apical cuticular depression in the third segment of the palpus. The sides of the third segment of the maxillary palpus exhibits rod-shaped depressions, known as sensilla digitiformia. Several cuticular elements were detected in the mandibles, including Al, C, Ca, Cl, Mg, Na, O, P, and Zn. Zinc, a heavy metal, was only detected in the incisors and could provide abrasion resistance.

Key words: bark beetle, broca del café, *Coffea*, Scolytinae, sensory

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is a small bark beetle endemic to Africa and nowadays present in most coffee-producing countries (Vega et al. 2015). The insect feeds exclusively on the two coffee beans inside the coffee berry, and the mechanisms used to breakdown caffeine are mediated by the gut microbiota (Ceja-Navarro et al. 2015). In Brazil, the world's top coffee producer, yearly losses caused by the insect have been estimated at US$215–358 million (Oliveira et al. 2013).

Even though the coffee berry borer has been studied for >100 yr (Infante et al. 2014, Pérez et al 2015), very little is known about its mouthpart structure and elemental composition of the mandibles. Why is it important to know about the coffee berry borer mouthparts? One reason is that it provides information on the basic biology of the insect, including its gustatory (or chemosensory) receptors. For example, insect mouthparts (labial and maxillary palpi) include chemoreceptors in the form of terminal uniporous sensilla enclosing sensory neurons, used to determine whether specific food chemicals are phagostimulants or deterrents (Chapman 2003). Terminal pore sensilla, which may also be located in other parts of the insect (e.g., legs), can also respond to odors (Chapman 2003).

Another reason is that heavily sclerotized mandibles are important in the mastication of food (Hillerton et al. 1982), and in the case of the coffee berry borer, strong mandibles resistant to abrasion are needed to construct galleries within the coffee seed, where the females oviposit, and to feed on the seed. Several studies have reported on the elemental analysis of insect mandibles and on the detection of zinc (a heavy metal), which provides hardness and abrasion resistance to the incisors, i.e., the cutting edges of the mandibles (Hillerton and Vincent 1982; Hillerton et al. 1982; Edwards et al. 1993; Schofield et al. 2002; Schofield 2005; Cribb et al. 2008a, b; Andersen 2010).

In this paper, we identify the different structures in the mouth of the coffee berry borer and report on the elemental analysis of the mandibles.

Materials and Methods

The mouthparts of adult males and females were photographed using low-temperature scanning electron microscopy (LTSEM) as described in Vega et al. (2014). Elemental analysis of male and female mandibles was determined using SEM and energy dispersive X-ray spectroscopy (EDS), which is used to determine the dry

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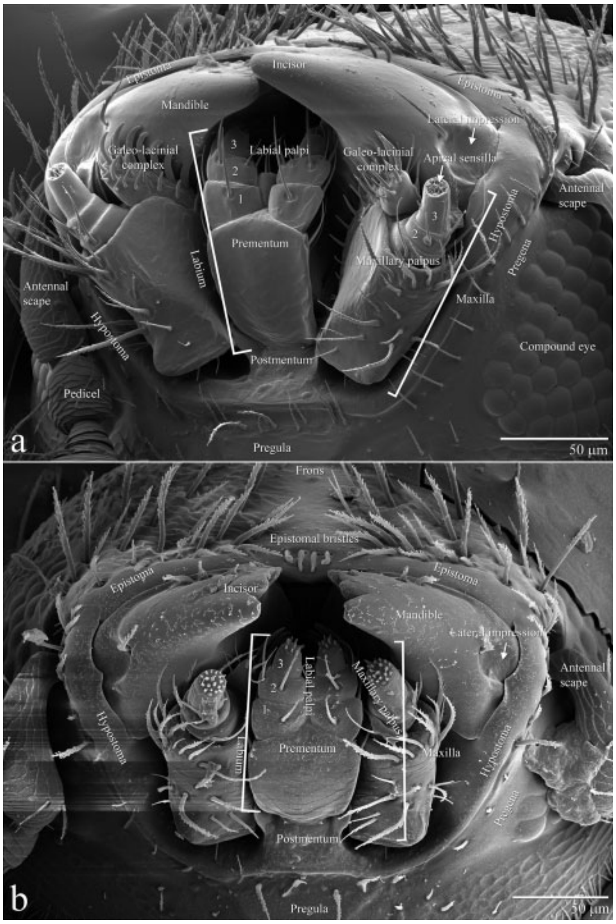

Fig. 1. Mouthparts of a female (**a**) and a male (**b**) coffee berry borer.

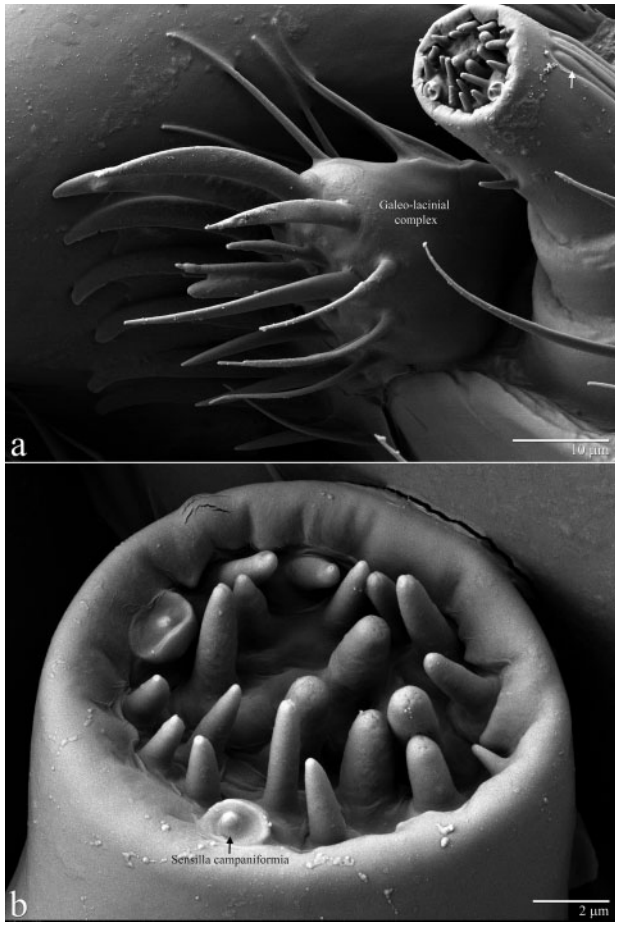

Fig. 2. (**a**) Galeo-lacinial complex and three-segmented maxillary palpus showing *sensilla digitiformia* (arrow) and the apical cuticular depression containing apical sensilla. (**b**) Detail of apical sensilla showing sensillia basiconia and two sensillia campaniformia.

weight percent of each element at the sampled point. Mandibles were carbon coated, then imaged with a backscatter detector and analyzed at 15kV in a Zeiss EVO 60 Extended Pressure SEM (Carl Zeiss AG, Switzerland). Point analyses were performed for both ventral and dorsal sides of the mandibles in female and male insects and EDS mapping performed for common elements present in the basal and incisor regions of only the female mandible to display element spatial distribution. Point analysis was performed with a spectrum collection time of 30 s, and EDS mapping was performed with a dwell time of 500 µs. There were five replicates for the dorsal and five different replicates for the ventral side of female mandibles, each with one to four subsamples for each the base and the incisors. There were three replicates for the dorsal and three different replicates for the ventral side of male mandibles, each with two to four subsamples for each the base and the incisors.

Confocal microscopy was used for visualizing the ventral side of the male and female labium, utilizing a Zeiss laser scanning confocal microscope (LSM 710; Carl Zeiss AG, Switzerland) and collecting green and red spectra.

Results and Discussion

As expected, mouthparts for males are smaller than that for females (Fig. 1), based on the smaller size of male insects (Vega et al. 2015). The mandibles have been referred to as the first pair of jaws, with the maxilla referred to as the second pair of jaws (Metcalf et al. 1962). The lacinia (Figs. 1 and 2) is "the cutting or chewing part of the maxilla and is often furnished with teeth and spines" (Comstock 1948). It is used to hold food in place and to help masticate it, i.e., break it into smaller pieces before being ingested. The mandibles and maxillae operate in a transverse form, i.e., from side to side (Metcalf et al. 1962).

The labial and maxillary palpi in the coffee berry borer are three jointed (Figs. 1–5), as has also been reported for the genus *Dendroctonus* (Hopkins 1909), and "are connected by a flexible membrane which allows for a certain amount of telescoping" (Hopkins 1909), as seen for the coffee berry borer maxillary palpi while feeding on a coffee seed (Fig. 4). The maxillary palpi (Figs. 1–5) are sensory organs containing two types of sensilla within an apical cuticular depression in the third segment of the palpus (Fig. 3). Most of the sensilla are basiconic and two are campaniform (Fig. 2). There are 20 basiconic sensilla in females, (we didn't examine them in males), as has also been reported for both sexes in *Ips acuminatus* (Moon et al. 2014). As discussed above, the sensilla in the labial and maxillary palpi serve as chemoreceptors to determine if the food is a phagostimulant or a deterrent (Chapman 2003). The sides of the third segment of the maxillary palpus exhibits rod-shaped depressions, also seen in *I. acuminatus* (see Fig. 4C in Moon et al. 2014). These depressions are known as *sensilla digitiformia*, and have been reported in maxillary palpi of larval Carabidae (Giglio et al. 2003) and the

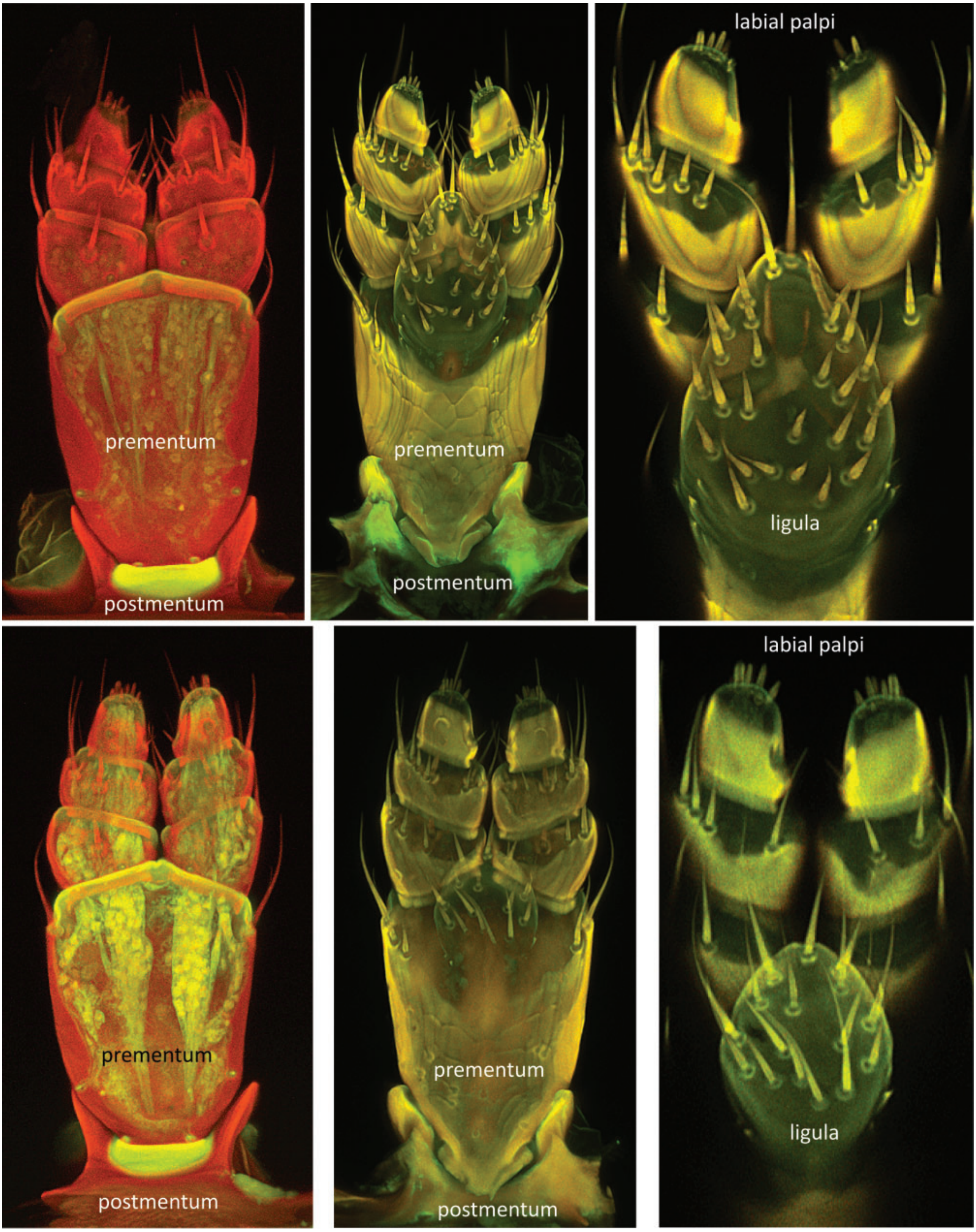

Fig. 3. Details of female (top) and male labium (bottom). First image is dorsal side; second image shows ventral side; third image shows details of ventral side, focusing on the ligula.

labial palpi of larval Elateridae (Zacharuk et al. 1977). They have been reported to serve as mechanoreceptors, chemoreceptors, and possibly in hygroreception (Giglio et al. 2003).

Several cuticular elements were detected in the mandibles (Fig. 6), including C, O, Mg, P, Cl, Ca, and Zn (Table 1; Figs. 7 and 8). Zinc and Cl were only detected in the incisors, while Mg and Ca were only detected in the mandibular base (Figs. 7 and 8). Although P also was detected in small traces in the incisors, it is largely present in the mandibular base. Interestingly, the elevated heavy metal composition (i.e., Zn) in the incisors also is visible when imaging with the backscatter detector of the SEM (Figs. 7 and 8).

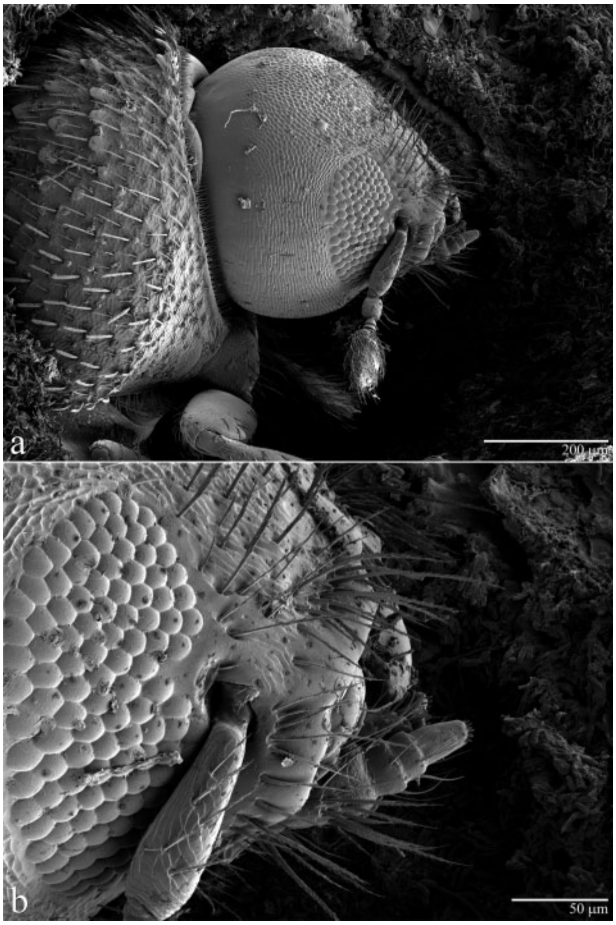

Fig. 4. Female coffee berry borer inside a gallery in a coffee seed (**a**) with detail of extended maxillary palpus toward the seed it is feeding on (**b**).

When Zn was present, Cl was also present. This type of metal–halogen enrichment has also been reported in the fangs of marine worms and spiders (Schofield 2005). In total, 136 species in five insect orders have been shown to contain Zn in areas that come in contact with the environment (e.g., mandibles, ovipositors, etc.; Schofield 2001). For example, Hillerton and Vincent (1982) reported large quantities of Zn in the incisors of locust mandibles. They also found Zn was present in the incisors of 20 species of insects in the Orthoptera (5 species), Phasmida (2), Lepidoptera (6), Hymenoptera (5), and Coleoptera (2) but was not detectable in 11 species of Coleoptera, four species of Dictyoptera, and one species of Dermaptera. Other elements found in locust mandibles (Hillerton and Vincent 1982) as well as all other species within the orders listed above were Si, P, S, Cl, K, and Ca. In a study examining the presence of Zn and Mn in the cutting edges of 54 Coleopteran species, Zn was detected in 18 species in the families Dermestidae, Anobiidae, Ptinidae, Cerambycidae, and Bruchidae, and in the bark beetle *Dendroctonus micans* Kugelann (Hillerton et al. 1984). Thus, the coffee berry borer is the second bark beetle shown to contain Zn in the mandibles.

Metal enrichment could help define insect life history traits, as has been speculated for mandibles in leaf-cutting ants (Schofield et al. 2002), drywood termites (Kalotermitidae; Cribb et al. 2008b), various stored-product pests (Hillerton et al. 1984, Morgan et al. 2003), and as shown for ovipositors in gall-associated Hymenoptera (Polidori et al. 2013). It is plausible that for bark beetles in general, which have to bore into hard substrates (bark of trees; seeds), metal enrichment of the mandibles will be shown to be a common trait.

We were unable to locate information on strength and toughness (*sensu* Pittia et al. 2007) of coffee beans excised from field-collected coffee berries at different stages of development. Nevertheless, at harvest time coffee beans have 45–55% moisture (Brando 2004, Gautz et al. 2008) and based on our field experience, are quite flexible and not overly hard. Therefore, it is possible that Zn enrichment of the coffee berry borer incisors is necessary to reduce abrasion, or that it is an evolutionary relic from what might have been a previous life history involving boring into bark, as done by 179 species of *Hypothenemus* (Vega et al. 2015). The only other *Hypothenemus* species feeding on seeds is *H. obscurus*, which feeds on various seeds and fruits, including nutmeg, macadamia nuts, cocoa, tamarind, longan, etc. (Vega et al. 2015).

Table 1 Elemental analysis (% ± SE) for the dorsal and ventral base of the mandible and the dorsal and central incisor region of female and male coffee berry borers

Section analyzed:	% C	%O	%Mg	%P	%Cl	%Ca	%Zn
Dorsal female base	73.7 ± 1.19	23.8 ± 1.73	0.44 ± 0.14	2.0 ± 0.42		2.0 ± 0.47	
Ventral female base	72.5 ± 2.35	19.1 ± 1.54	0.61 ± 0.15	2.3 ± 0.68		6.2 ± 1.91	
Dorsal female incisors	66.8 ± 0.49	27.0 ± 0.88		0.18 ± 0.05	0.74 ± 0.53		5.4 ± 0.5
Ventral female incisors	68.2 ± 1.21	23.5 ± 1.78		0.19 ± 0.02	0.80 ± 0.16		9.3 ± 2.95
Dorsal male base	74.1 ± 2.24	16.4 ± 3.12	0.72 ± 0.25	3.5 ± 1.01		5.4 ± 1.76	
Ventral male base	71.3 ± 1.34	21.6 ± 1.49	0.87 ± 0.17	3.4 ± 0.66		4.5 ± 1.12	
Dorsal male incisors	68.3 ± 2.64	13.2 ± 2.20		0.29 ± 0.05	0.72 ± 0.16		17.6 ± 4.31
Ventral male incisors	66.5 ± 2.65	23.4 ± 2.22		0.27 ± 0.08	0.36 ± 0.11		7.6 ± 1.92

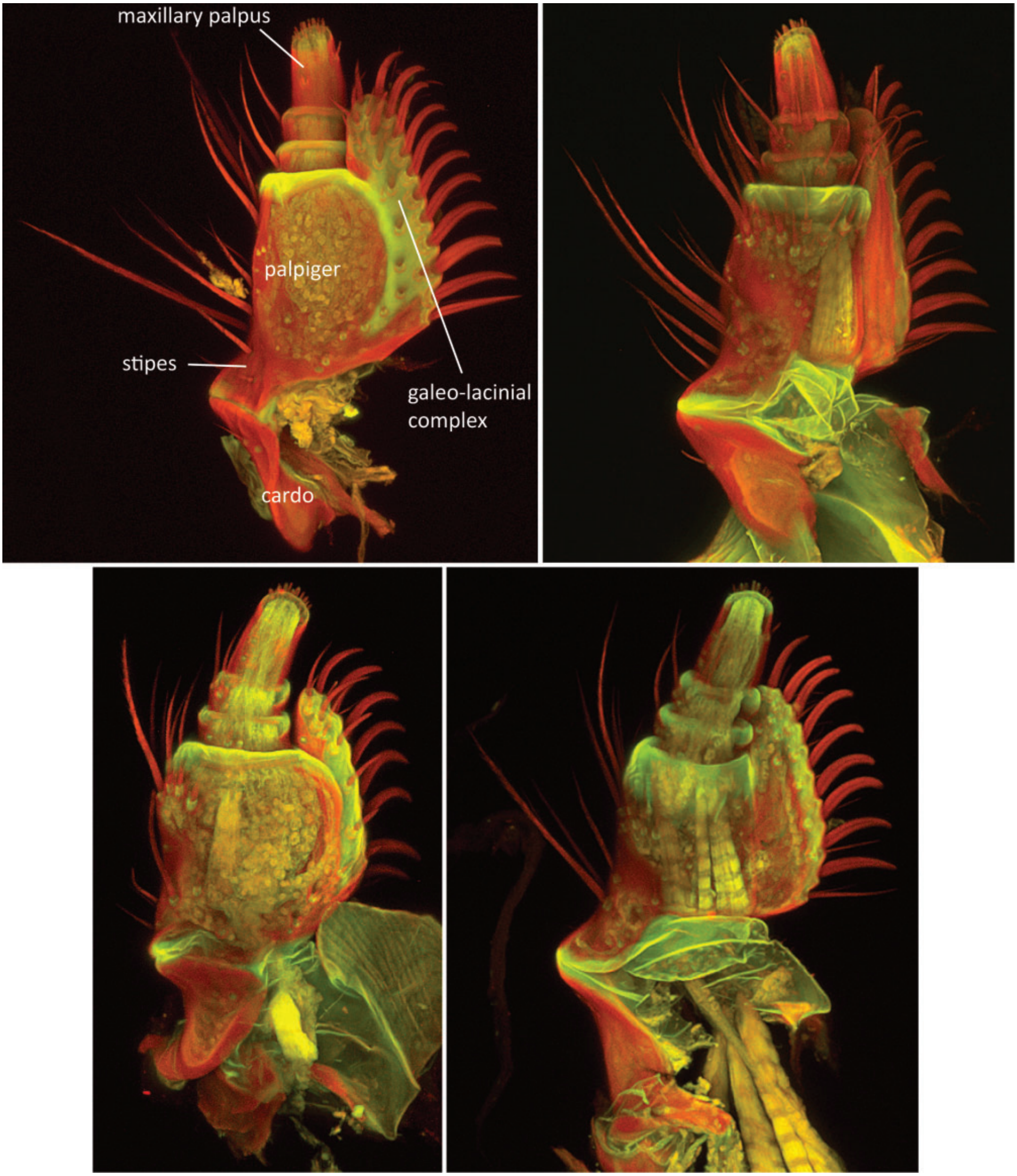

Fig 5. Details of maxilla for female (top) and male (bottom), showing maxillary palpus, palpiger, stipes, galeo-lacinial complex, and cardo. Left photo is ventral side and right photo is dorsal side.

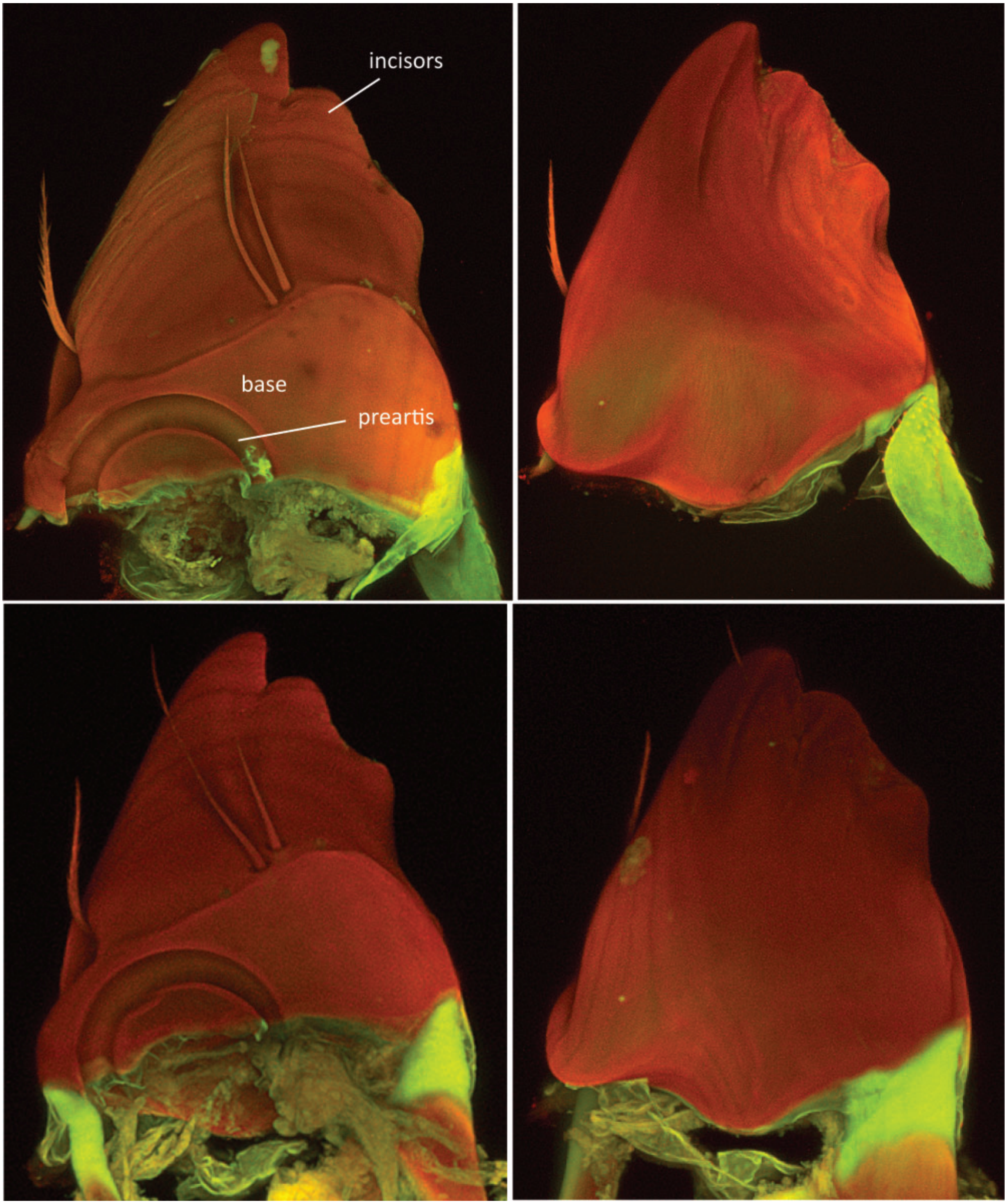

Fig. 6. Details of mandibles for female (top) and male (bottom), showing incisors, base of the mandible, and preartis. Left photos are for dorsal side and right photos are for ventral side.

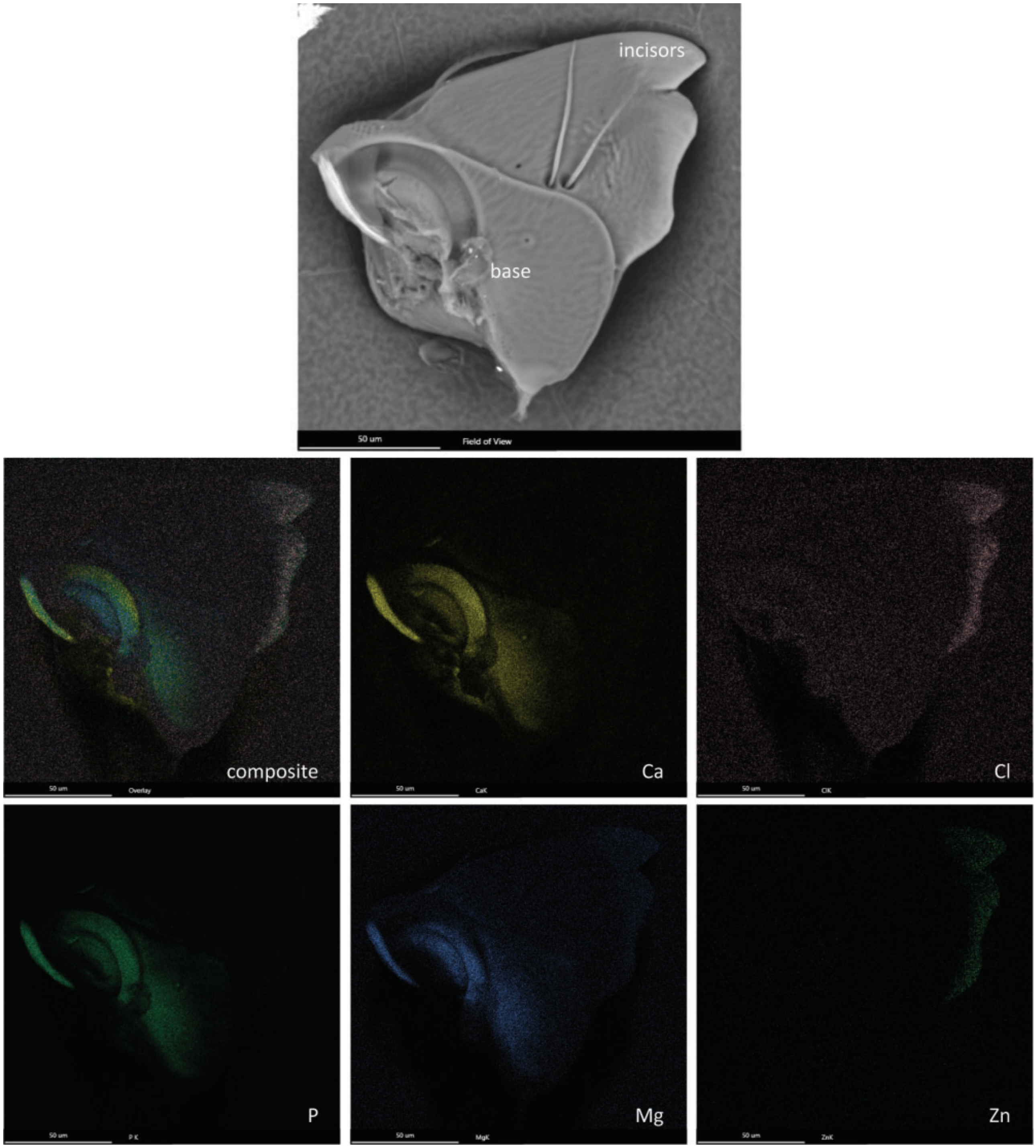

Fig. 7. Dorsal surface of female mandible, showing an EDS composite followed by individual results of elemental mapping for Ca, Cl, P, Mg, and Zn.

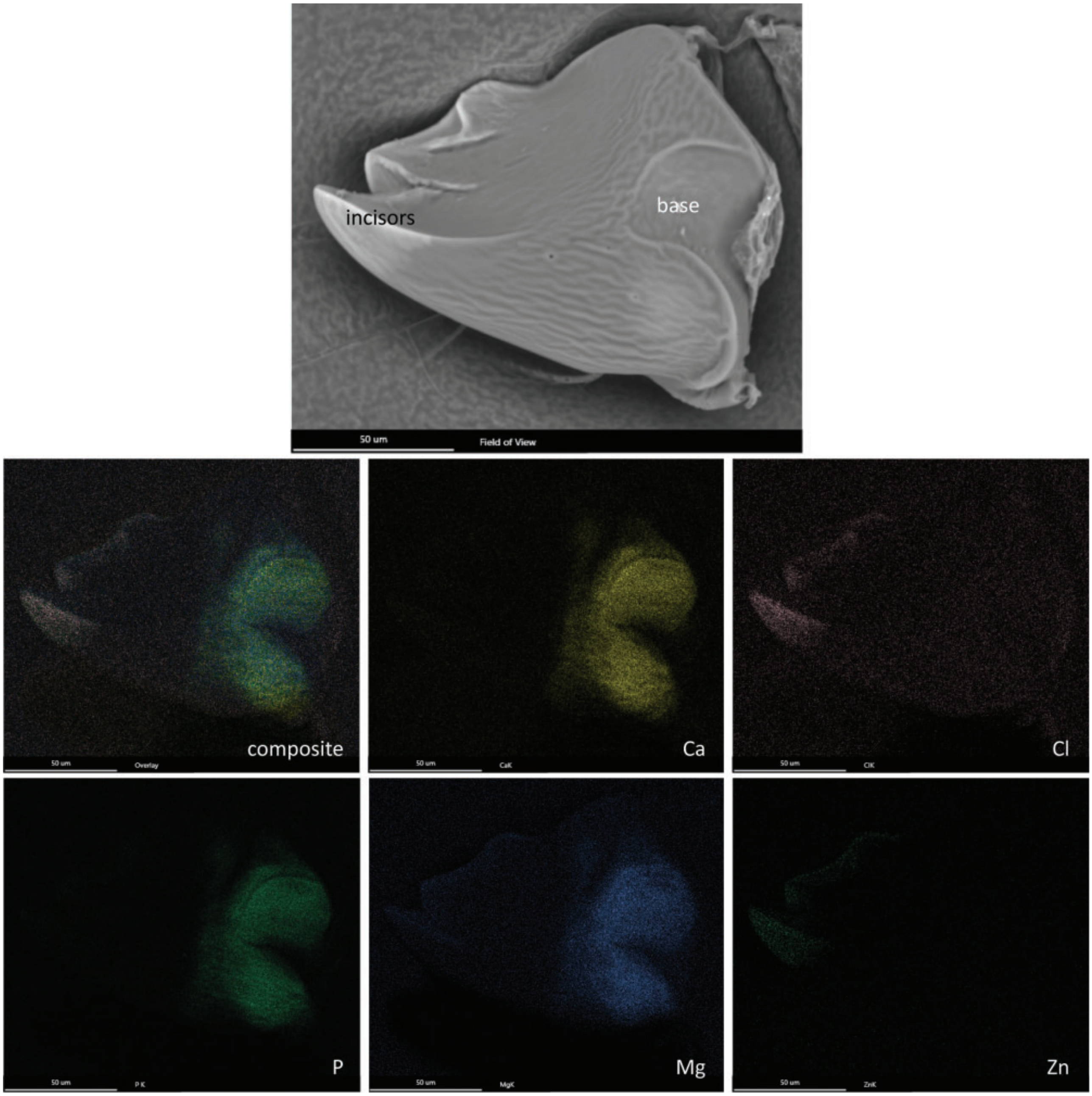

Fig. 8. Ventral surface of female mandible, showing an EDS composite followed by individual results of elemental mapping for Ca, Cl, P, Mg, and Zn.

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