The Coleopterists Bulletin, 44(1):37–49. 1990.

A SCANNING ELECTRON MICROSCOPIC VIEW OF THE FINAL LARVAL INSTAR OF *ZABROTES SUBFASCIATUS* (COLEOPTERA: BRUCHIDAE: AMBLYCERINAE)

GARY S. PFAFFENBERGER

Department of Life Sciences, Eastern New Mexico University, Portales, New Mexico 88130, U.S.A.

ABSTRACT

The final larval instar of *Zabrotes subfasciatus* (Boh.) is described and illustrated using scanning electron micrographs. Discrepancies, particularly relating to smaller anatomical structures, are evident through the comparisons of this and previous descriptions. Discussions of the functional morphology of the decurved labral spines and the apodous condition are also furnished.

The Mexican bean weevil is a cosmopolitan pest of many domesticated and non-domesticated plants (Center and Johnson 1974; Jarry and Bonet 1981; Johnson 1985; Pfaffenberger, in press). Therefore much has been written about its biology (Pfaffenberger and Johnson 1976) and distribution (Decelle 1975). Plants of future economic value to man, that are also infested by *Zabrotes subfasciatus* (Boh.), have likewise been reported (Johnson 1985; Singh *et al.* 1979).

Studies of adults have also shown that males live longer than females (Sharma *et al.* 1979), that longevity varies inversely with temperatures above 25°C (Sharma *et al.* 1979), and that qualitative and quantitative changes in tissue proteins (Sharma and Sharma 1979*a*) and lipids (Sharma and Sharma 1979*b*) diminish as adults age.

A plethora of information has been written about the morphology (Singh 1978; Vats 1972), systematics, and economic impact of adults of this species while comparatively little has been written about other equally important life stages. To demonstrate the usefulness of non-adult traits, characteristics of the pupal window (part of seed testa), have been used to distinguish this species from *Acanthoscelides obtectus* (Say) which also may infest similar, commercially important legumes (McFarlane and Wearing 1967).

Larval morphology, as determined by light microscopy, has been reported (Arora 1978; Pfaffenberger, in press; Pfaffenberger and Johnson 1976). However, this paper presents for the first time a critical SEM examination of the final instar larva. Such information will be useful in corroborating the accuracy of previous descriptions (Arora 1964–69, 1978; Zacher 1930).

MATERIALS AND METHODS

Specimens were obtained from stock cultures maintained at Eastern New Mexico University. Specimens were prepared for examination by subjecting them to a dehydration series of 90%, 95%, and absolute alcohol. Specimens were then critical point dried, coated with gold-palladium and examined using a ISI-100B SEM at an accelerated voltage of 15 KV.

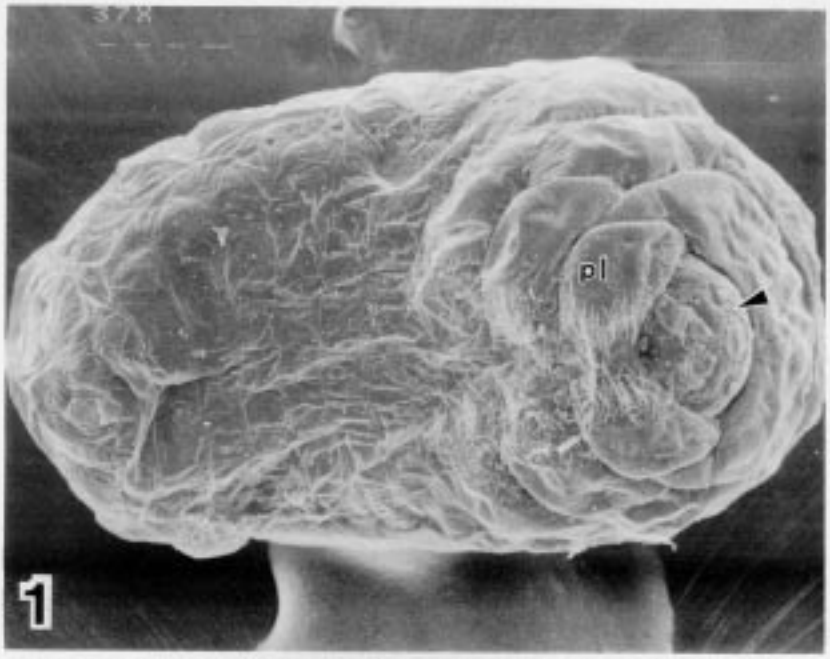


Fig. 1. *Zabrotes subfasciatus*, final instar habitus, ventral view; legs absent; prothoracic lobe (pl); dart marks head capsule.

Final Instar Larva

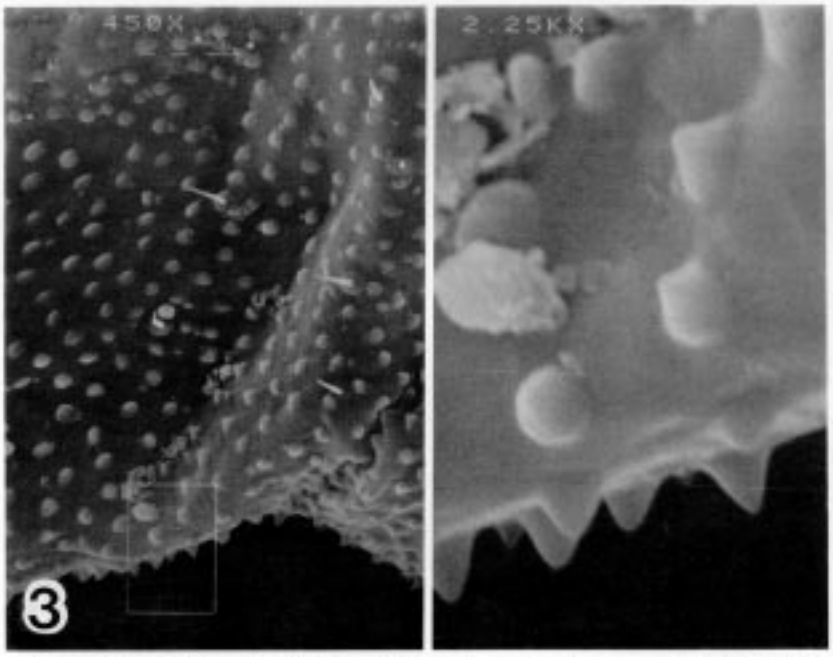
Description. *Body* (Fig. 1): 1.7 mm wide by 3.6 mm long, cyphosomatic, width greatest in metathoracic and first abdominal segments tapering to minute tenth abdominal segment (Figs. 1, 18). Integument with numerous, large tubercles, especially prominent over thoracic segments (Figs. 2–4) and tergal regions, becoming smaller, less numerous along posterior abdominal segments and pleural regions (Fig. 15), absent on tergites (Figs. 1, 15, 18). Abundant sensilla trichodea present on thoracic sternites (Figs. 1, 15), abdominal sternites with few sclerotized pointed projections. Tenth abdominal tergite (Fig. 18) with numerous sclerotized pointed projections, two short sensilla trichodea on tenth abdominal tergite and sternite.

Head (Figs. 1, 2): Retracted, mostly asetiferous. Five pairs of short sensilla trichodea present (see darts, Fig. 2), four in trapezoid arrangement above and between antennal bases and clypeus (Fig. 3), one posterolateral to bases of mandible and maxilla (Fig. 3). Mouth parts hypognathous.

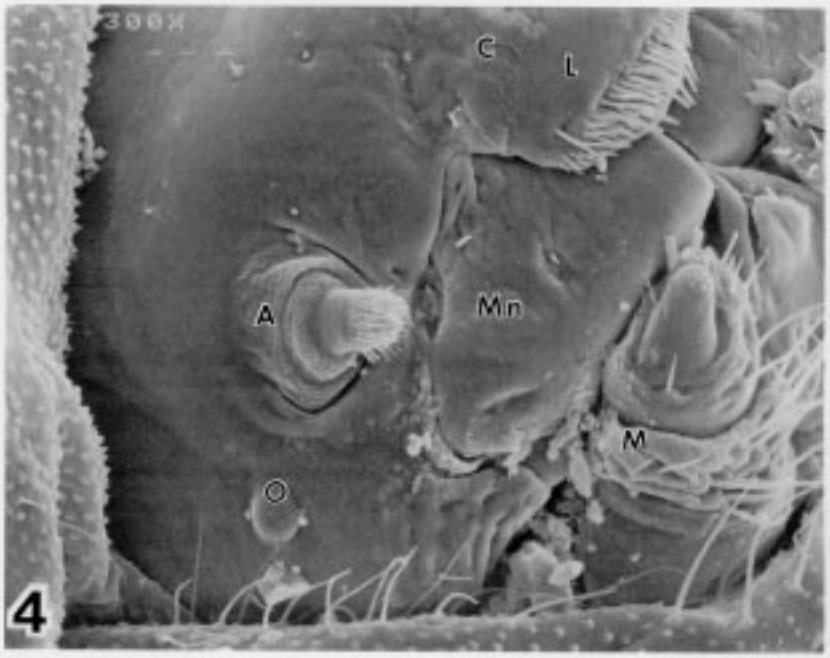
Ocelli (Figs. 2, 3, 5): Single pair, posterolateral to bases of antenna and mandible. Apparently two-facet type (Figs. 3, 5).

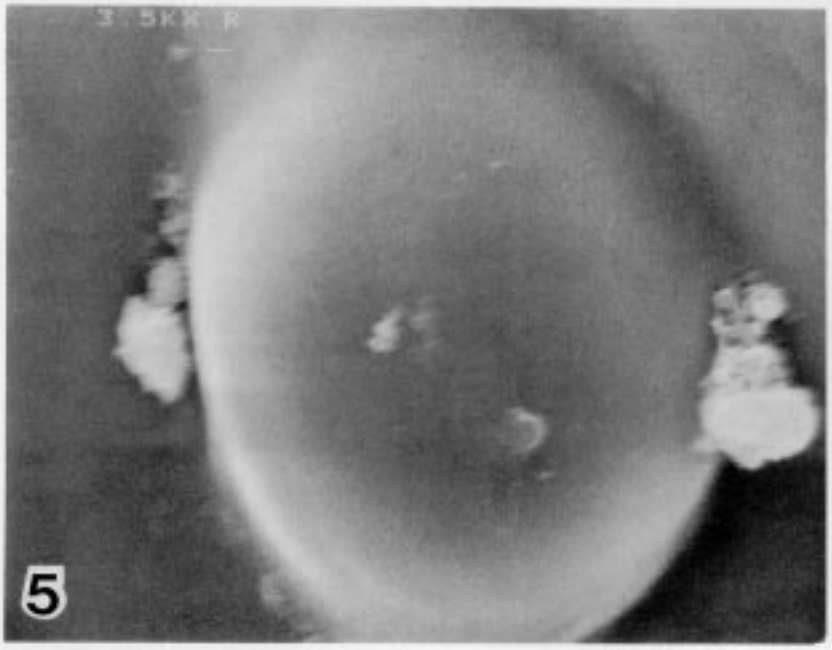
Antenna (Figs. 2, 3, 6, 7): Composed of three telescopic segments, distal segment with apicodorsal, elongate sensillum chaeticum (dart, Fig. 7). Two sensilla basiconica (large and small) present on distal end of apical segment. Sensilla chaeticum and basiconica protected by numerous, concentric rows of elongate microtrichia (Fig. 7).

Clypeolabrum (Figs. 2, 3): Clypeal portion (C, Figs. 2, 3) transversely elongate, sensillum trichodeum and subtending sensillum ampullaceum located posterolaterally, posterior border convex, concave anterior border. Labral portion nearly oval with convex anterior and posterior margins (Figs. 8, 9; L, Figs. 2, 3) with posteromedial sensillum ampullaceum (arrow, Fig. 8) and pair of elongate posterolateral sensilla trichodea (darts,

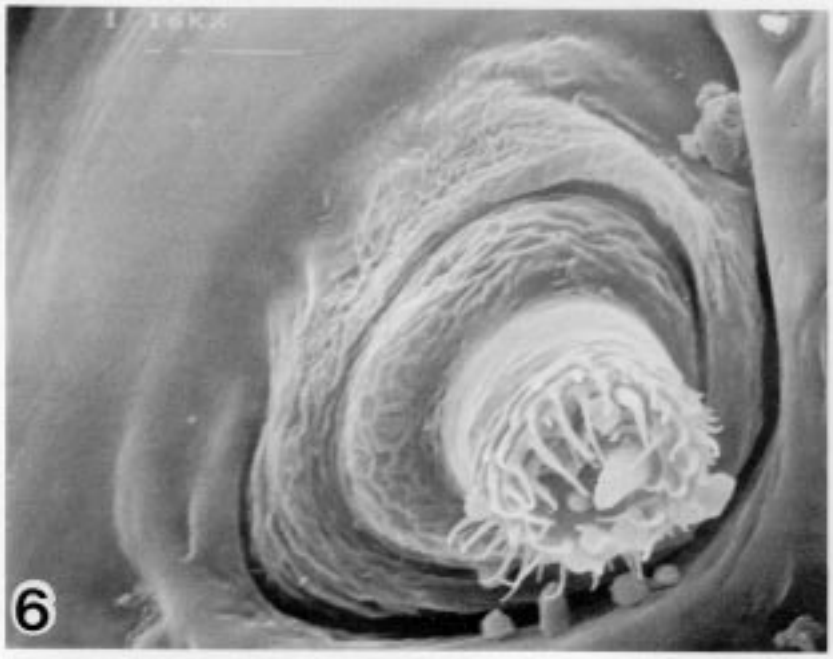


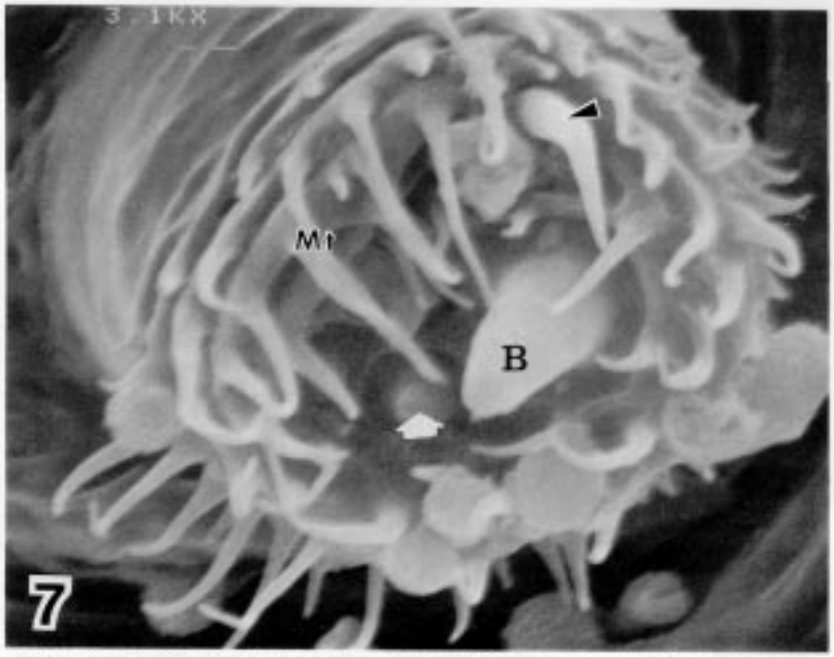
Figs. 2, 3. *Zabrotes subfasciatus*. 2, head capsule, enface view; antenna (A), clypeus (C), labium (La), labrum (L), maxilla (M), mandible (Mn), sensilla trichodea (darts). 3, tubercles on integument.



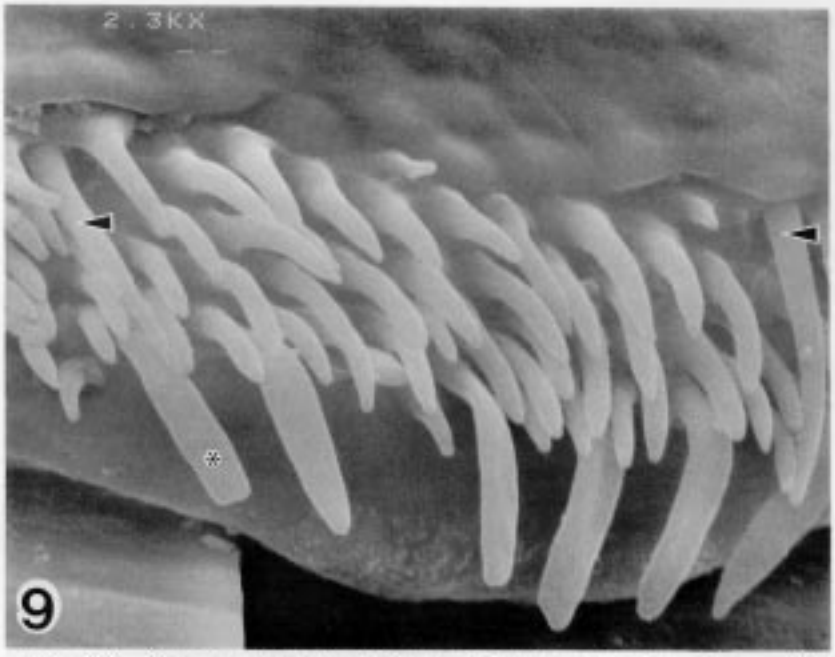


Figs. 4, 5. *Zabrotes subfasciatus*. 4, head capsule, lateral view; antenna (A), clypeus (C), labrum (L), maxilla (M), mandible (Mn), ocellus (O). 5, right ocellus.

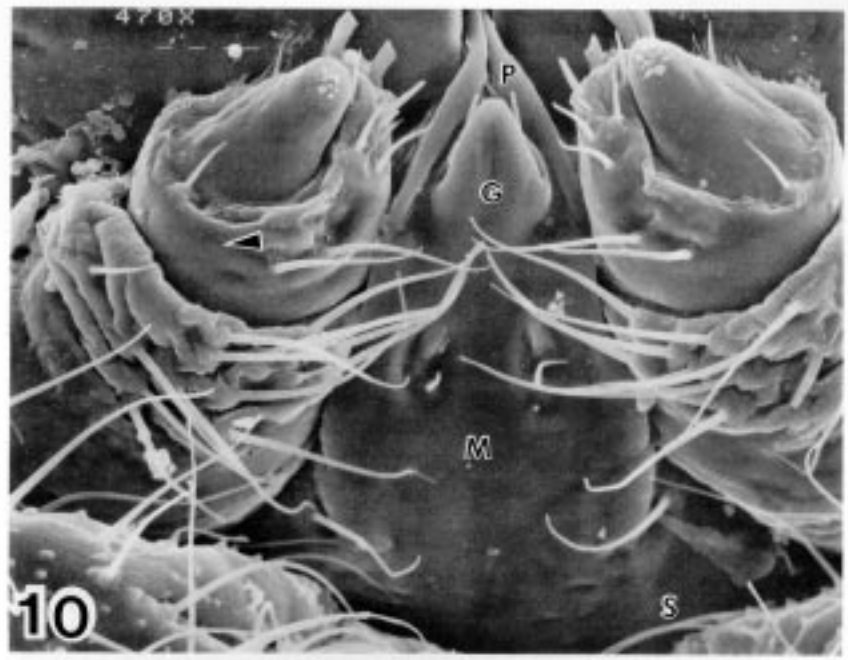


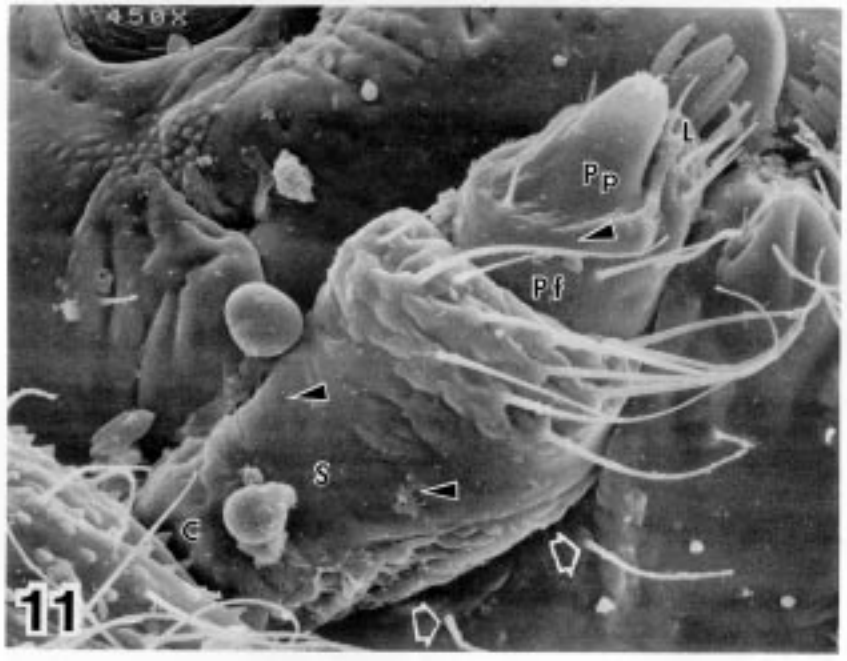


Figs. 6, 7. *Zabrotes subfasciatus*, antenna. 6, three segments. 7, apical segment; enlarged sensillum basiconicum (B), smaller sensillum basiconicum (arrow), sensillum chaeticum (dart), microtrichia (Mt).

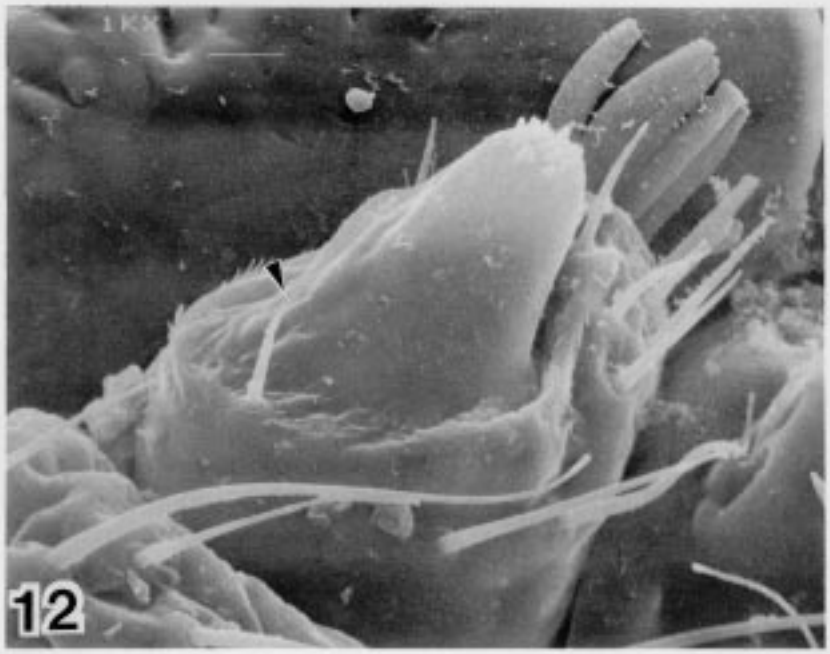


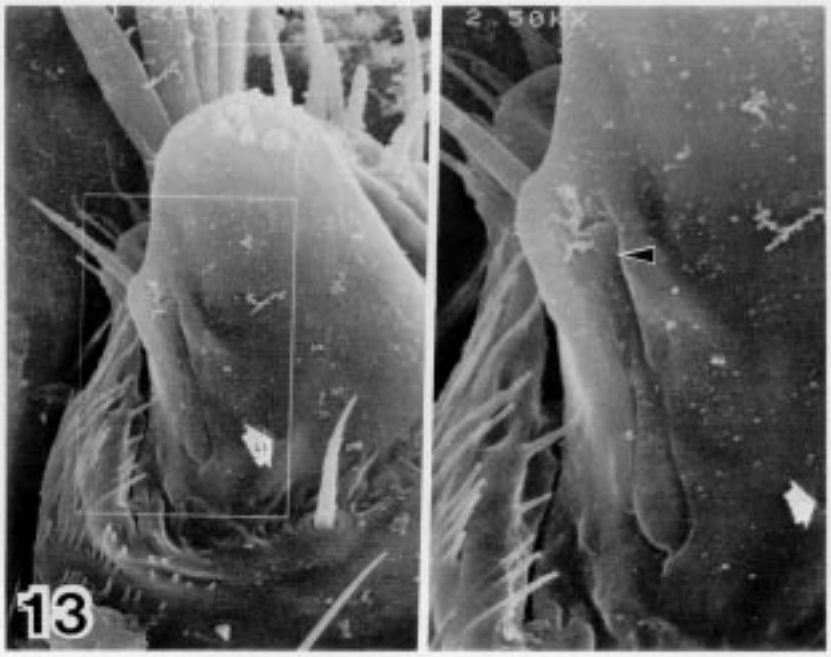
Figs. 8, 9. *Zabrotes subfasciatus*, labrum and labral margin, enface views; sensilla trichodea (darts), sensillum ampullaceum (arrow), decurved labral spines (*) among mat of microtrichia.





Figs. 10, 11. *Zabrotes subfasciatus*. 10, labium and neighboring maxilla, ventral view; glossa (G), mentum (M), paraglossa (P), submentum (S), sensillum ampullaceum (dart). 11, right maxilla, ventrolateral view; cardo (C), lacinia (L), palpifer (Pf), palpus (Pp), stipes (S), sensilla ampullacea (darts), sensilla trichodea (arrows).





Figs. 12, 13. *Zabrotes subfasciatus*, maxillary palpus; sensillum placodeum (dart) embedded in dorsal surface, sensilla ampullacea (arrow).

Fig. 14. *Zabrotes subfasciatus*, apex of maxillary palpus with sensilla basiconica.

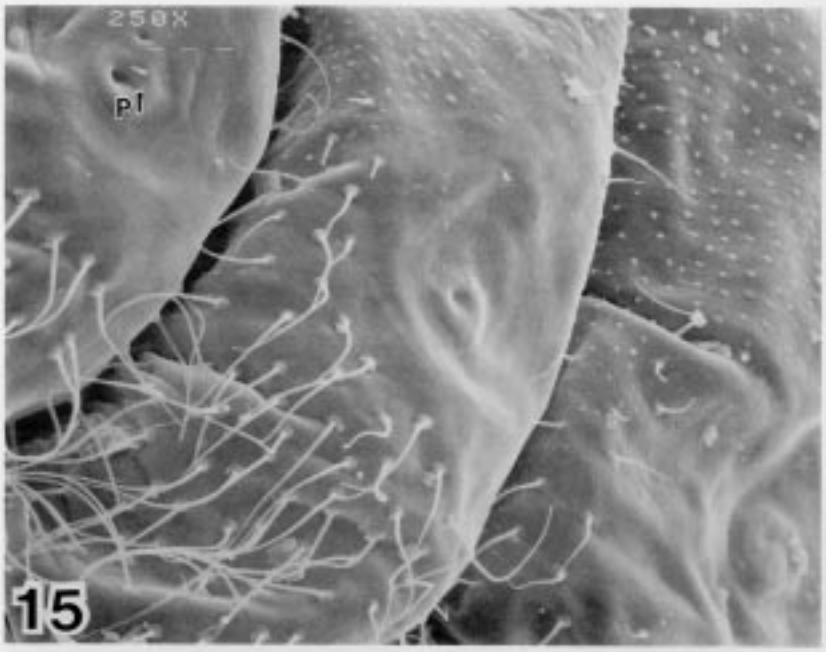
Fig. 8); six elongate, truncate, decurved spines arching over anterior margin (*, Figs. 8, 9), transversely elongate patch of microtrichia immediately posterior to spines obscuring pair of sensilla trichodea (dart, Figs. 8, 9).

Mandible (Mn, Fig. 2): Monocondylic, with awl-shaped chewing surface, middorso- and midanterolateral surfaces with sensillum trichodeum.

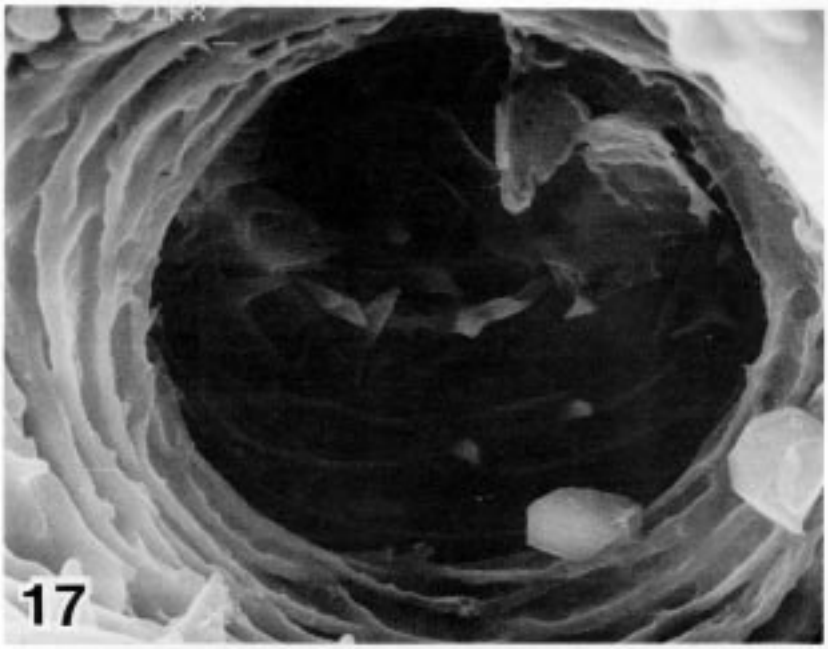
Maxilla (M, Fig. 2; Figs. 11, 12, 13): Cardo (C, Fig. 11) transversely elongate. Sclerite of stipes (S, Fig. 11) with two sensilla ampullacea (darts, Fig. 11), membranous stipes with 13–15 sensilla trichodea. Palpifer (Pf, Fig. 11) with one dorsomedial (Fig. 13), one short dorsolateral, two medioventral and one anterolateral sensilla trichodea (Figs. 11, 12); one sensillum ampullaceum located distoventrally (dart, Fig. 11); anterodorsal margin with numerous rows of microtrichia (Fig. 13). Lacinia (L, Fig. 11) with five distomedial, contiguous, truncate, spatula-shaped spines (Fig. 12); one tapered spine located ventrally to spatula-shaped spines (Figs. 11, 12); five sensilla trichodea located ventrolateral to spines and medioventral to palpifer (Pp, Fig. 11). Palpifer with dorsal, recessed sensillum placodeum (dart, Figs. 12, 13), one proximolateral sensillum ampullaceum near base of sensillum placodeum (arrow, Fig. 13), apical end with 12 variable sized sensilla basiconica, lateralmost one largest (Figs. 12, 14).

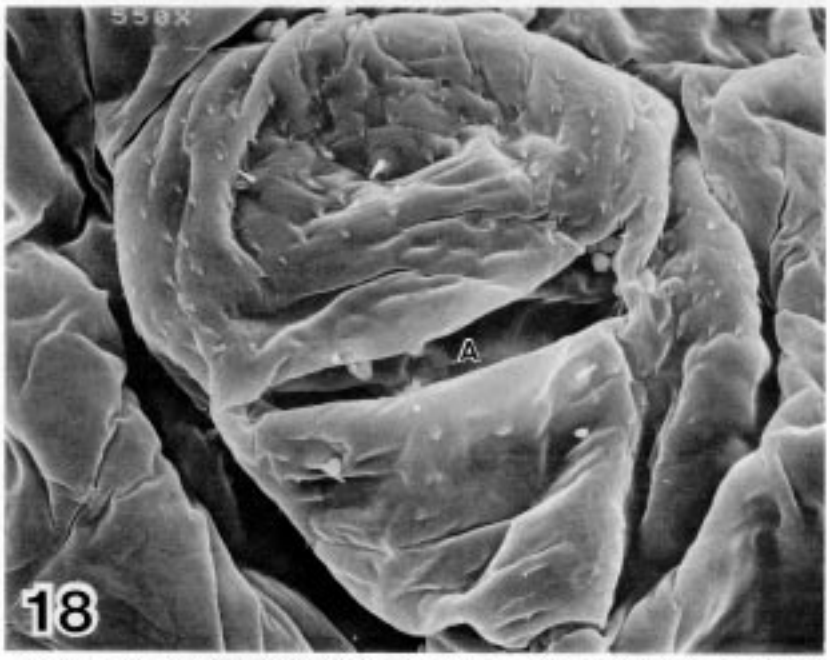
Labium (S, M, G, P, Fig. 10): Submentum (S, Fig. 10) transversely elongate with lateral pair of sensilla trichodea (arrow; Fig. 11), medial pair (fig. 34.774, Pfaffenberger, in press) not visible; mentum (M, Fig. 10) sclerotized, with membranous islets each bearing one sensillum trichodeum; glossa (G, Fig. 10) with anterolateral pair of sensilla trichodea; paraglossa (P, Fig. 10) narrow, elongated anteriorly.

Leg (Figs. 15, 16): Absent. Prothoracic appendage (Pt, Fig. 15) appearing as enlarged sensillum ampullaceum bordered posteromedially by single sensillum trichodeum and medially by smaller sensillum ampullaceum (Fig. 16); meso- and metathoracic appendages minute elevations with centrally located sensillum ampullaceum (Fig. 15).



Figs. 15, 16. *Zabrotes subfasciatus*, left thoracosternal area, ventral view. 15, legs absent, only small lobes present; prothoracic lobe (pl). 16, enlarged view of prothoracic lobe (pl) with pit-like sensilla ampullacea and spine-like sensilla trichodea.





Figs. 17, 18. *Zabrotes subfasciatus*. 17, thoracic spiracle; numerous atrial rings evident, as well as a reduction in number of sclerotized projections on rings. 18, transverse anal cleft (A).

Spiracle (Fig. 17): Atrium deep, walls fortified with numerous concentric folds supporting few superficial, sclerotized projections, projections becoming more numerous in depths of atrium.

Anus (A, Fig. 18): Transverse.

SPECIMENS EXAMINED. Twenty-five larvae, recovered from stock culture in 1973.

DISCUSSION. The value of the scanning electron microscope is evident when comparisons are made between published descriptions using light microscopy and the SEM's presented herein. The minute dimensions of certain structures either exceed the powers of resolution of most light microscopes or are impossible to recover for illustrative purposes. Published descriptions of larger structures appear to be quite accurate (fig. 34.774, Pfaffenberger, in press), whereas those descriptions of smaller anatomical structures contain many discrepancies when compared with those using SEM's.

Pfaffenberger (fig. 34.783, in press) illustrated the clypeolabrum, wherein he showed the presence of three rows of labral setae instead of an arc of six. He indicated the presence of two setae in the first row and five in the second. Moreover, he illustrated the setae among a mat of fine, short microtrichia instead of among the dense mat of elongate microtrichia (Figs. 8, 9). In an earlier description, Zacher (1930) illustrated the labrum with five elongate, truncated spines flanked by two sensilla trichodea. The minute microtrichia were confined to the bases of the five truncated spines. Finally, Arora (1964–69, 1978) published several illustrations which lack sufficient detail to make species diagnoses. In his figure 159 (Arora 1978), the six truncated spines alluded to above are shown to be seven epipharyngeal instead of labral setae (fig. 158, Arora 1978).

Although the arc of decurved, labral spines (Figs. 8, 9) has previously been described (*Z. subfasciatus,* Zacher 1930; *Caryedon palaestinicus* Southgate, Pfaffenberger 1984) nothing has been written about its probable function. The stoutness of their appearance strongly suggests that they are used to roughen, by gouging or "raking action," the surface of the endosperm making it possible for the mandibles to successfully carve provisions from the hardened and otherwise smooth surface. Moreover, their presence might enhance the effectiveness of the labral microtrichia in removing adhering detritus, frass, exuviae, etc., from the body surface and keeping the latter packed to one side of the chamber which they occupy during larval and pupal developmental stages. A third suggestion might be that the spines are used to gain purchase since thoracic appendages are absent. By engaging the ends of the spines in the endosperm the body could be drawn forward and positioned so that feeding could again commence.

The absence of legs (Figs. 15, 16) reflects a particular life style. Ova of *Z. subfasciatus* are oviposited directly on the surface of mature, host seeds (Center and Johnson 1974). The legless first instar (Pfaffenberger and Johnson 1976) bores directly into a seed devoid of resins, gums, etc., which are so often present in unripened seeds (Janzen 1969). Legs, which are used to navigate these substances, are therefore no longer needed to reach the seed endosperm. The double sensilla ampullacea (Figs. 15, 16) and neighboring sensilla trichodea indicate that at least some of the legs may have given way to organs of olfaction.

Pfaffenberger (1985) presented a discussion of probable sensillar function, as associated with sensillar structure, for *Acanthoscelides obtectus.* The functional morphology of all sensillar structures described in this paper are discussed in the latter work.

ACKNOWLEDGMENTS

I thank the Llano Estacado Center for Advanced Professional Studies and Research for financial support.

LITERATURE CITED

ARORA, G. L. 1964–69. A study of the taxonomy of adult and larval Bruchidae. Final Res. Prog. Rep. Dept. Zool. Panjab Univ., Chandigarh–14 (India), pp. 51–82.

———. 1978. Taxonomy of the Bruchidae (Coleoptera) of northwest India Part II. larvae. Oriental Insects. Supp. No. 8, pp. 1–48.

CENTER, T. D., AND C. D. JOHNSON. 1974. Coevolution of some seed beetles (Coleoptera: Bruchidae) and their hosts. Ecology 55:1096–1103.

DECELLE, J. 1975. Les Coléoptères Bruchides d'Angola. Companhia de Diamantes de Angola. Publ. Culturais. No. 89:15–32.

JANZEN, D. H. 1969. Seed-eaters versus seed size, number, toxicity and dispersal. Evolution 23:1–27.

JARRY, M., AND A. BONET. 1981. Premieres observations sur la contamination par *Zabrotes subfasciatus* Boh. (Coleoptera: Bruchidae) de gousses de *Phaseolus vulgaris* L. et *P. lunatus* L. au Mexique. Acta Oecol./Oecol. Applicata 2:311–315.

JOHNSON, C. D. 1985. Potential useful tropical legumes and their relationships with bruchid beetles [pp. 206–210]. *In:* Misra, K. C. (editor). Ecology and resource management in tropics. Vol. 1. Presented papers, Silver Jubilee Symposium of International Society for Tropical Ecology. Bhargava Book Depot, Varanasi, India, xi + 324 pp.

MCFARLANE, J. A., AND A. J. S. WEARING. 1967. A means of differentiating between *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boh.) (Coleoptera, Bruchidae) in white haricot beans at the pupal stage. J. Stored Prod. Res. 3:261–262.

PFAFFENBERGER, G. S. 1984. Description of first instar larva of *Caryedon palaestinicus* Southgate, new status (Coleoptera: Bruchidae). Coleopts Bull. 38:220–226.

———. 1985. Description, differentiation, and biology of the four larval instars of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Coleopts Bull. 39:239–256.

———. (In press). Family Bruchidae. *In:* Stehr, F. (editor). An introduction to immature insects of North America. Kendal/Hunt Publ., Dubuque.

———, G. S., AND C. D. JOHNSON. 1976. Biosystematics of the first-stage larvae of some North American Bruchidae (Coleoptera). U.S.D.A. Tech. Bull. No. 1525, 75 pp.

SHARMA, S. P., AND G. SHARMA. 1979*a*. Age-related protein changes in bruchids *Zabrotes subfasciatus* & *Callosobruchus maculatus*. Ind. J. Exp. Biol. 17:1197–1200.

———, AND ———. 1979*b*. Age-related lipid studies in bruchids. Curr. Sci. 48:955–956.

———, S. RATTAN, AND G. SHARMA. 1979. Temperature dependent longevity of *Zabrotes subfasciatus* Boh. (Coleoptera, Bruchidae). Comp. Physiol. Ecol. 4:229–231.

SINGH, T. 1978. The male accessory glands in Bruchidae (Coleoptera) and their taxonomic significance. Ent. Scand. 9:198–203.

———, I. KAUR, AND M. S. SAINI. 1979. Biology of *Zabrotes subfasciatus* (Boh.) (Bruchidae: Coleoptera). Entomon. 4:201–203.

VATS, L. K. 1972. Tracheal system in the larvae of the Bruchidae (Coleoptera: Bruchidae). Jour. New York Entomol. Soc. 80:12–17.

ZACHER, F. 1930. Untersuchungen zur Morphologie und Biologie der Samenkäfer (Bruchidae-Lariidae). Arb. Biol. Reichsant. Land- u. Forst. 18:233–384.

(Received 2 February 1989; accepted 7 September 1989)