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Confirmation of Host Plant of Cave-Dwelling Cixiid Planthoppers (Hemiptera: Cixiidae) by Histological Sectioning of Fig Roots

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ABSTRACT The food sources of cave-dwelling insects are difficult to document, and those of root-feeding cixiid nymphs (Hemiptera: Cixiidae) particularly so because of their tendency to jump from their substrates upon the slightest disturbance. By histologically staining the stylet sheath left by *Oliarus* sp. feeding on *Ficus* roots, we establish the host association beyond doubt. Histological sections also demonstrate that *Oliarus* feeds in the cells of the endoderm and within the xylem vessels. The technique we describe can be used to determine host relationships for cave-dwelling Cixiidae in general, providing an additional angle for the study of the evolutionary history of cave adaptation in this group.

Introduction

A major problem in describing the natural history of new or little-known cave insects lies in determining their principal food source(s). The cave ecosystem usually lacks primary producers (because there is no light to sustain photosynthesis), and feeding substrates for cave insects are generally surmised by observing what few energy inputs may be available (e.g. guano, washed in leaf litter, carcasses; Howarth 1983). Cixiid nymphs in caves present a special problem, despite belonging to a family in which nymphs are primarily root feeders (Carver *et al.* 1991). Although they can safely be assumed to feed on roots which penetrate into the cave, it can be difficult to determine which plant species they feed on. For example, the roots of *Eucalyptus*, *Melaleuca*, *Ficus* and *Brachychiton* often penetrate caves in the dry tropics of North Queensland (Hock and Howarth 1989a,b; Howarth and Stone 1990; pers. obs.), sometimes following adjacent paths to moisture below. Once disturbed, cixiid nymphs are commonly found on all root systems in such areas, as well as on rock faces and on the ground (pers. obs.). They jump readily and powerfully (30-40 cm) in response to disturbance, making their undisturbed distribution difficult to ascertain.

Recent work on cave dwelling Cixiidae in both Queensland and Hawaii has demonstrated their usefulness in interpreting the evolutionary history of cave adaptation (Hoch and Howarth 1989a,b, 1993). Such interpretation has nevertheless been hampered by an incomplete knowledge of the biology of the animals, and Hoch and Howarth (1989b) point out the importance of a knowledge of cixiid host plants to "provide significant clues to understanding the evolution of cave faunas". In this paper we describe a method by which host plant data for cave dwelling Cixiidae can be reliably obtained, and then apply it to confirm that *Ficus* sp. is the host plant of at least one species of *Oliarus* in northern Queensland caves.

Materials and methods

Collection of specimens. Three nymphs and one dead adult female cixiid were collected on and near roots under rocks in the transitional zone (*sensu* Howarth 1988) of Stop Press Cave (CH 30, Piano Karst), Chillagoe, northern Queensland (22.vi.1993, G. Taylor and P. Weinstein). The specimens represent an undetermined species of *Oliarus* Stål (Hemiptera: Cixiidae) (Fennah 1956; H. Hoch, pers. comm.). Root material was taken in the same location from terminal roots with actively growing meristems. The roots were returned to the laboratory for processing in vials with dampened tissue paper. The exudation of latex from cuts made in more proximal portions of this root mass confirmed the roots as being those of *Ficus* sp. Furthermore, the roots were morphologically identical to those of aerial *Ficus* roots collected from Townsville, upon which the nymphs also readily fed.

Histological preparation. Transverse sections of *Ficus* roots, several complete cells in thickness (about 0.5 mm), were cut using a clean scalpel, and fixed and stained in lactophenol (20 g phenol, 20 mL lactic acid, 40 mL glycerol, 20 mL water) to which several drops of 2% aniline blue were added to colour the lactophenol medium blue. Sections were washed in 100% ethanol, 50% xylol in ethanol and 100% xylol, and mounted in Gurr's thin Canada balsam in xylene. Stylet sheaths stained selectively dark blue.

Alternatively, sections were placed in Carnoy's fixative, rinsed in 100% ethanol, transferred to 95% ethanol, stained for several seconds in 1% light green in 95% ethanol, washed in ethanol with several changes, placed in 50% xylol in ethanol followed by 100% xylol and mounted in Canada balsam. This method was adapted from one previously developed to demonstrate the stylet sheaths left by feeding aphids (G. S. Taylor, unpublished data).

Results

Stylet sheaths of *Oliarus* sp. were demonstrated

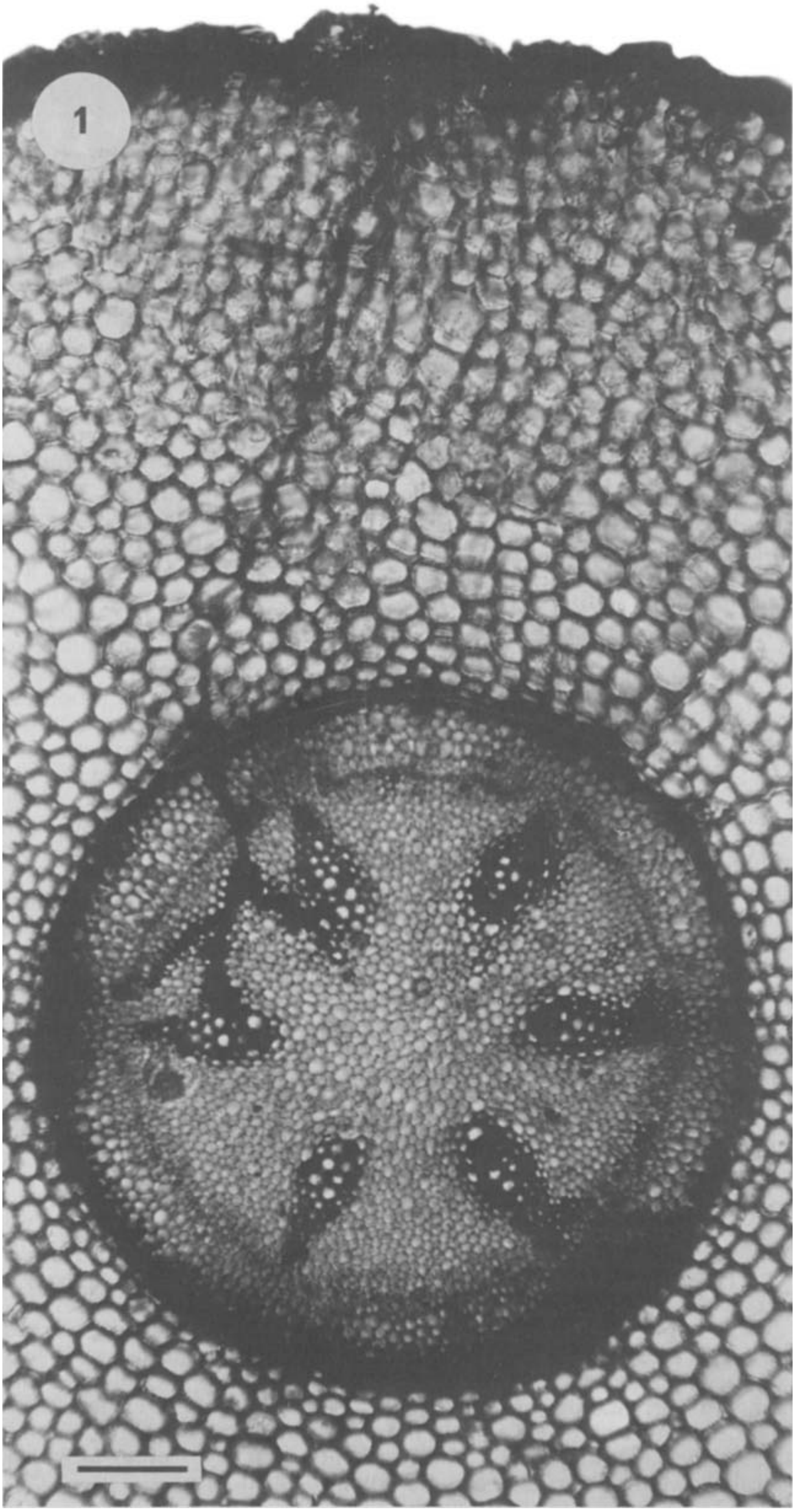

Fig. 1. Transverse section of aerial *Ficus* sp. root with feeding track of nymph of *Oliarus* sp. Note that vascular bundles are selectively targeted. Scale = 0.1 mm.

in the *Ficus* roots (Figs 1, 2), confirming host association to, at least, generic level. Aniline blue proved an excellent stain for the stylet sheath. Feeding punctures were indicated externally by the presence of a stylet sheath flange. Stylet tracks passed intra- and intercellularly through the cortex of parenchyma (Figs 1, 2). As stylet tracks passed through the cell wall its diameter became somewhat constricted (Fig. 2). Intercellularly its diameter was rather uniform, except for the occasional flow into cellular cavities during secretion (Fig. 2). Beyond the endodermis stylet tracks often branch to target xylem bundles (Fig. 1). Depth of insect feeding was typically 0.75-0.95 mm.

Discussion

The function of the stylet sheath produced during the feeding process by Homoptera and Heteroptera: Pentatomorpha remain largely speculative. Secreted during penetration of the host plant, it solidifies to form a tubular structure surrounding the insects' stylets (Miles 1972). It has been postulated that it serves to seal off plant cells that are often under turgor pressure, to provide a solid lining to prevent leakage of watery saliva into intercellular spaces, and to minimise the wound reaction of plants (Miles 1987). Following withdrawal of the stylets the stylet sheath persists in plant tissue (sometimes observable externally by the presence of a stylet sheath flange). Whatever

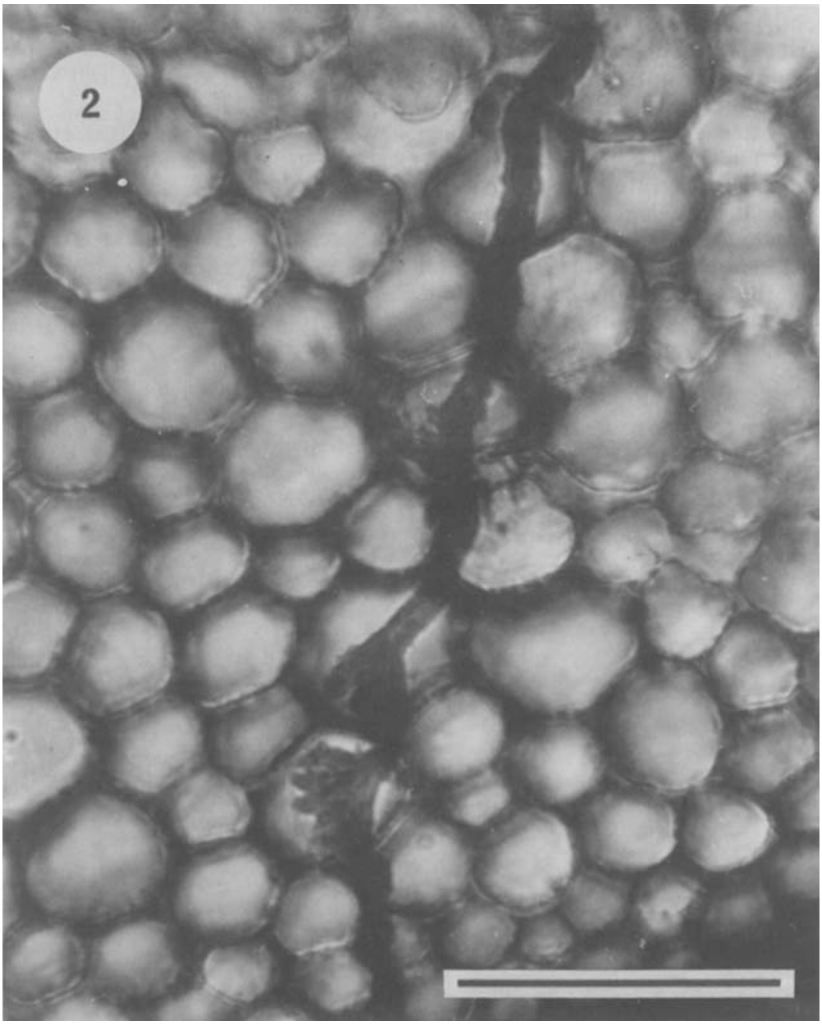

Fig. 2. Portion of stylet track of *Oliarus* sp. showing constriction during cell wall penetration and occasional outflow of stylet sheath material into cell cavities. Scale = 0.1 mm.

its function, the presence of a stylet sheath indicates previous bug feeding. Utilising this attribute we have demonstrated the usefulness of an histological technique to establish reliably which roots have been fed upon by cave-dwelling cixiid nymphs. The technique should enable the host plants of all cave-dwelling Cixiidae to be established beyond doubt, providing useful additional information on the evolutionary history of this group (Hoch and Howarth 1989b). As well as histological evidence confirming a host association, identification of the host species is clearly also required. We used the exudation of latex from a wound to identify the host species to generic level (*Ficus* sp.) and confirmed this by comparison of the structure of aerial *Ficus* roots collected elsewhere. Where no such obvious clues are available, we suggest that reliable identifications can be made by histological comparison of root sections from caves and from the roots of identified trees on the surface. Root segments for such comparisons can be fixed in the field in lactophenol (without aniline blue) and transported to the laboratory for subsequent staining of stylet tracks.

An incidental finding was the exceptional clarity of the stylet tracks demonstrated for *Oliarus* sp. in *Ficus* roots. The histological sections (Figs 1, 2) demonstrate that *Oliarus* sp. feeds on both cellular contents and on xylem; within the endodermis, stylet tracks penetrated cell walls, and beyond the endodermis vascular bundles were selectively targeted. Only in aphids have stylet sheaths been used to demonstrate feeding sites with similar clarity, and in these the stylet sheath follows a mainly intercellular route to the phloem vessels (Miles and Taylor 1994). The significance of these different feeding strategies remains speculative, but the approach of *Oliarus* sp. could perhaps be regarded as less specialised.

Acknowledgments

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References

Carver, M., Gross, G. F. and Woodward, T. E. (1991). Hemiptera. *In* CSIRO (eds), The Insects of Australia, 2nd ed. Melbourne University Press: Melbourne.

Fennah, R. G. (1956). Homoptera: Fulgoroidea. Insects Micronesia 6: 39-211.

Hoch, H. and Howarth, F. G. (1989a). Reductive evolutionary trends in two new cavernicolous species of a new Australian cixiid genus (Homoptera: Fulgoroidea). Systematic Entomology 14: 179-196.

Hoch, H. and Howarth, F. G. (1989b). Six new cavernicolous cixiid planthoppers in the genus *Solonaima* from Australia (Homoptera: Fulgoroidea). Systematic Entomology 14: 377-402.

Hoch, H. and Howarth, F. G. (1993). Evolutionary dynamics of behavioural divergence among populations of the Hawaiian cave dwelling planthopper *Oliarus polyphemus* (Homoptera: Fulgoroidea: Cixiidae). Pacific Science 47: 303-318.

Howarth, F. G. (1983). Ecology of cave arthropods. Annual Review of Entomology 28: 365-389.

Howarth, F. G. (1988). Environmental ecology of North Queensland caves: why there are so many troglobites in Australia. Australian Speleological Federation 17th Biennial Conference preprints, Lake Tinaroo, December 1988.

Howarth, F. G. and Stone, F. D. (1990). Elevated carbon dioxide levels in Bayliss Cave, Australia: implications for the evolution of obligate cave species. Pacific Science 44: 207-218.

Miles, P. W. (1972). The saliva of Hemiptera. Advances in Insect Physiology 9: 183-255.

Miles, P. W. (1987). Feeding processes of Aphidoidea in relation to effects on their food plants. *In* Minks, A. K. and Harrewijn, P. (eds) Aphids, their Biology, Natural Enemies and Control, Vol. A. Elsevier: Amsterdam.

Miles, P. W. and Taylor, G. S. (1994). 'Osmotic pump' feeding by coreids. Entomologia Experimentalis et Applicata 73: 163-173.

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