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VENTRAL MESOSOMAL CHANGES IN EMBRYOS FROM THREE SCORPION FAMILIES: IURIDAE, BUTHIDAE AND VAEJOVIDAE

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ABSTRACT. The scanning electron microscope was used to examine embryos at a stage when booklungs and spiracles are forming. Earlier studies with scorpion fossils suggest there was ventral mesosomal transition from gills or booklungs above ventral plates to sternites, booklungs and spiracles. In *Hadrurus arizonensis* (Iuridae), ventral plates and then sternites are formed on the ventral surface of mesosomal segments before spiracles appear. Bilateral invaginations in body segments XII–XV apparently give rise to the booklungs, with spiracles formed lateral to the site of invagination. Sternites with bilateral depressions were also present before spiracles in embryos of the buthid *Centruroides exilicauda*. In the developmental stages herein examined, spiracles were formed in embryos of *Paruroctonus mesaensis* (Vaejovidae); but there was no indication of ventral plates or sternites on the ventral mesosoma. Spiracles appear in the intersegmental area posterior to body segments XIII–XV. Booklungs may form later from primordia associated with bilateral depressions observed in a later stage in these segments.

The earliest scorpion fossils (Silurian) suggest these animals were aquatic, while all surviving species are terrestrial (Selden & Jeram 1989; Sissom 1990; Jeram 1994). A critical stage in scorpion evolution was the change (ventral mesosoma) from gills to booklungs, probably in the Permian and Carboniferous periods. Kjellesvig-Waering (1986) provided some evidence that aquatic scorpions had gills above ventral plates in the ventral mesosoma. He proposed that there was gradual reduction of these plates and formation of sternites, booklungs and spiracles. Selden & Jeram (1989) and Jeram (1990) described a fossil Carboniferous scorpion with booklungs rather than gills above ventral plates.

Scorpion embryos were examined with the possibility they might provide some information about the water-to-land transition (Farley 1999a, b). These initial observations showed some differences in the ventral mesosoma during spiracle and booklung formation in embryos of the vaejovid, *Paruroctonus mesaensis*, and the iurid, *Hadrurus arizonensis*. The present study is an extension of that work, including embryos of *Centruroides exilicauda*, a buthid. The latter was examined since buthids are considered most primitive among extant scorpion families (Stockwell 1989, 1992; Sissom 1990), and mesosomal changes may reflect the ancestral condition.

METHODS

The composition of physiological saline and the procedures for collection and maintenance of specimens were described in an earlier publication (Farley 1987). Specimens of *Paruroctonus mesaensis* Stahnke 1957 were collected in the Colorado Desert near Indio and Palm Springs, California. Specimens of *Hadrurus arizonensis* Ewing 1928 (Williams 1970; Francke & Soleglad 1981) and *Centruroides exilicauda* Wood 1863a (Wood 1863b; Ewing 1928; Williams 1980) were collected in Arizona. Specimens of all three species are in the California Academy of Science, San Francisco.

Tissues were flushed with saline to remove debris as animals were dissected with microscissors and forceps. The ovariuterine tubules were opened and embryos removed. Surrounding membranes (amnion, serosa) were pulled away with microprobe and forceps.

Tissues were fixed (6–10 h, 23–25 °C) with 4% glutaraldehyde in 0.1 M cacodylate buffer with one drop of calcium chloride for each 10 ml of solution (Lane et al. 1981). The tissues were washed in cacodylate buffer-NaCl solution and postfixed (2 h, 23–25 °C) in 1% osmium tetroxide in 0.2 M cacodylate buffer with NaCl. The concentrations of these solutions were adjusted to approximate the osmolality of scorpion blood (630 mOsm; Yokota 1984). Tissues were dehydrated in acetone, critically-point dried (Balzers, CDD 020) and sputter-coated (EMscope SC500) with 20 nm thickness of gold/palladium. Tissues were examined at 12–15 KV with a Philips 15 scanning electron microscope (SEM).

RESULTS

At a stage before spiracle and booklung formation, embryos of *H. arizonensis* have plates demarcated on the ventral surface of mesosomal segments. Initially, only a narrow ridge outlines the ventral plates, with the delineated region much smaller than the ventral surface of the segment. The outlined region becomes a flap-like structure (Fig. 1) fused to the body wall anteriorly but free at the lateral and posterior margins. The early ventral plates do not extend the full width of the mesosoma nor overlap antero-posteriorly. Embryos were not sectioned, but no indications of an opening or gill-like structures were observed at the posterior margin of the ventral plates. Paired indentations in body segments XII–XV (Hjelle 1990) are presumably booklung primordia.

In later stages, the invaginations in segments XII–XV become more prominent (Fig. 2), and the ventral cuticle increases in length and width, forming structures that resemble adult sternites with the perimeter joined to pleural or intersegmental integument. The sternites extend the full width of the mesosoma and overlap in the longitudinal axis. Intrasternal spiracles eventually form at the adult location (Farley 1990a, b), just lateral to the site of booklung invagination. Booklungs do not develop in segment XVI; the indentations (Fig. 2) eventually disappear, leaving no external trace. Mesosomal development in the buthid, *C. exilicauda*, appears to be similar to that of *H. arizonensis*. In Fig. 3, an embryo of the former species has sternites with bilateral depressions, presumably for booklungs.

In embryos of *P. mesaensis*, there is no demarcation of ventral plates or sternites at the time when spiracles first appear (Fig. 4). In the stages observed in this study, spiracles were seen near the mesosomal midline in the intersegmental tissue posterior to segments XIII–XV. In later stages, bilateral depressions were seen in segments XII–XVI, but there was still no indication of ventral plates or sternites. Advanced embryos of *P. mesaensis* were not available to determine if booklungs and new spiracles form at these depression sites, or if the initial spiracles (Fig. 4) move to the adult position, farther anterior and lateral in the segment (Farley 1990a, b). The early spiracles differed in shape among the embryos, but usually had a smooth, apparently cuticular margin and a slit-like opening (Fig. 4), in comparison with the oval shape in the adult.

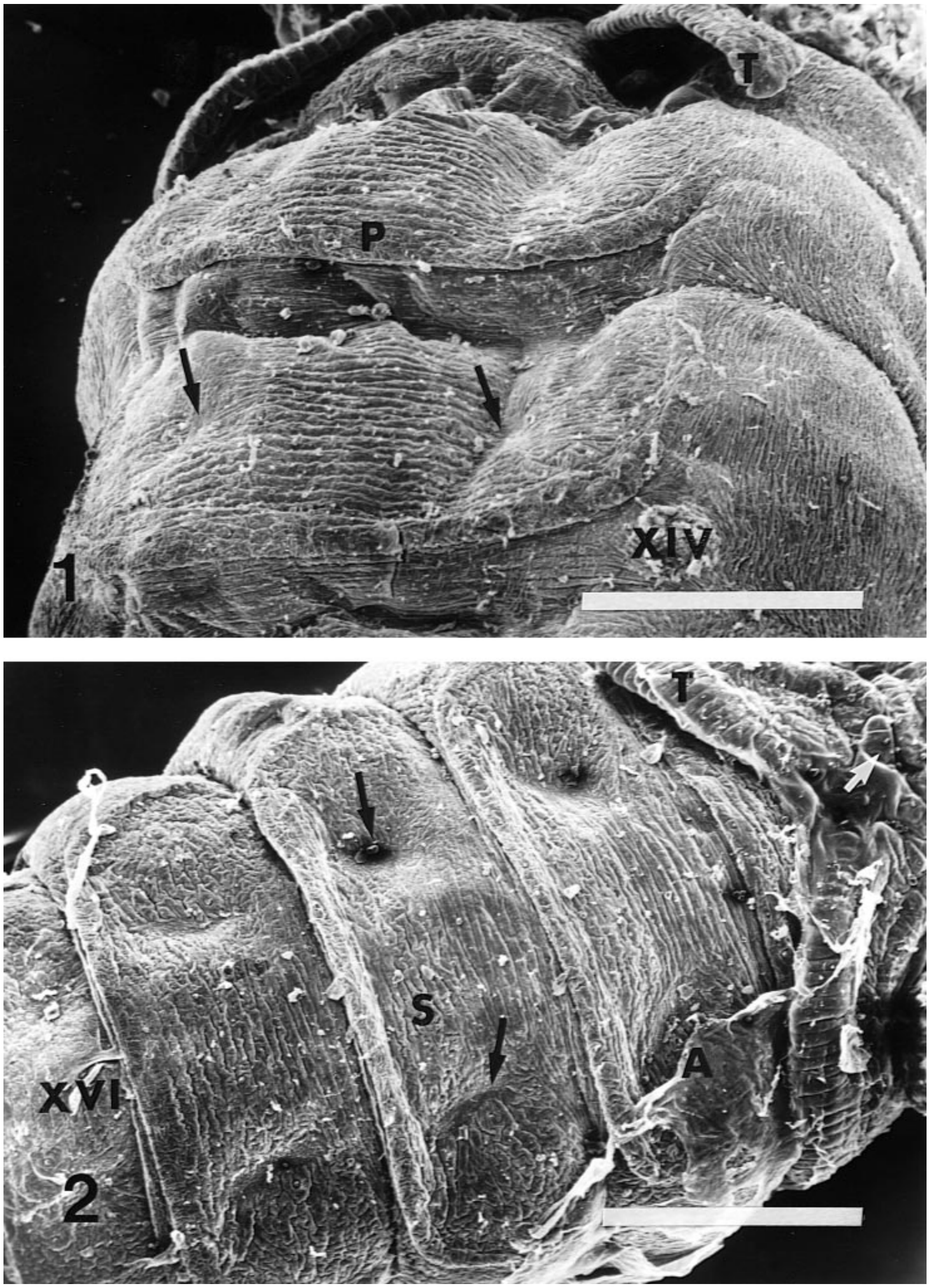
Although ventral plates or sternites are not evident in embryos of *P. mesaensis* when spiracles first appear (Fig. 4), the ventro- posterior margin of each mesosomal segment was examined for indications of invagination or gill-like structures. In some embryos, the lateral intersegmental area shows differentiation suggestive of infolding, with vertical striations (Fig. 4). The spiracles form in the medial aspect of this distinctive intersegmental area.

Embryos were not sectioned, but during dissection in transmitted light, some internal structures can be seen. In embryos of *P. mesaensis*, there was no indication of a thickening or density anterior to the spiracles, as would be expected if booklungs were forming. The spiracle site in the intersegmental area (Fig. 4) did not appear to be a region of invagination as occurs in the bilateral depressions seen in the mesosomal segments of the iurid and buthid embryos (Figs. 1–3).

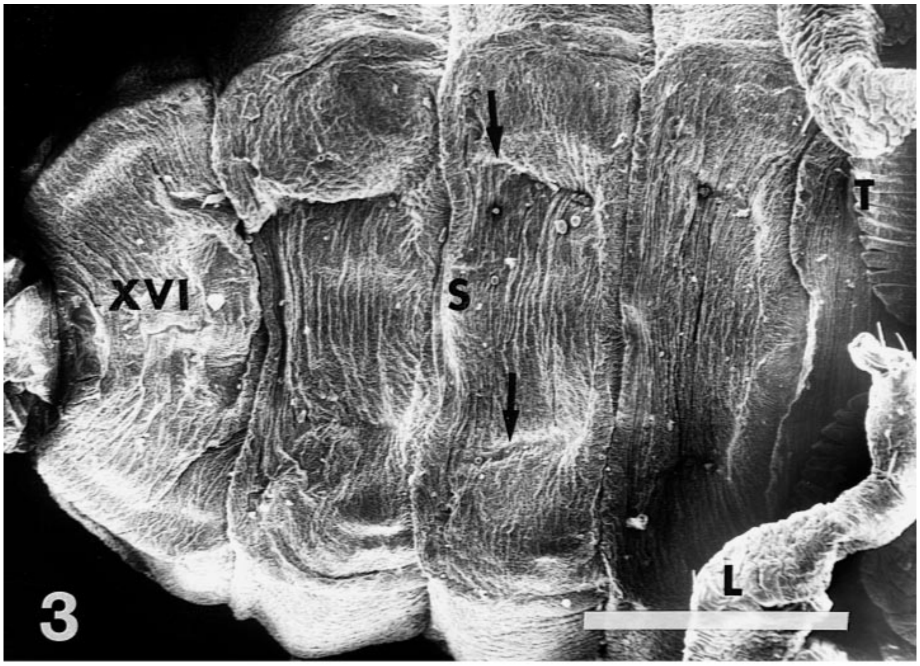
DISCUSSION

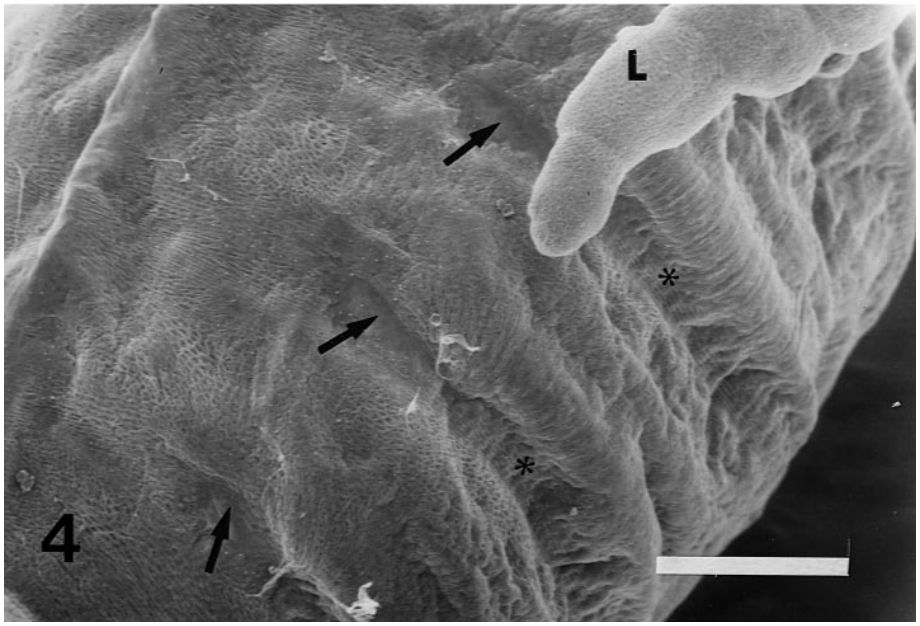
In *H. arizonensis* and *C. exilicauda*, booklung and spiracle formation appears to be like that described by earlier workers in species from the families Buthidae (Abd-el Wahab 1951), Chactidae (Laurie 1890; Brauer 1895) and Scorpionidae (Metschnikoff 1871; Laurie 1892). The bilateral depressions evident in mesosomal segments in Figs. 1–3 appear to be sites of invagination, and spiracles are later formed here at the location seen in adults (Farley 1990a, b). The early demarcation of flap-like structures (Fig. 1) supports the notion that ventral plates preceded (Kjellesvig-Waering 1986) or occurred with booklungs in ancient scorpions (Selden & Jeram 1989; Jeram 1990).

Differences were reported among scorpion species in the shape and texture of the cuticle of adult booklung lamellae (Lankester 1885; Berteaux 1889; Laurie 1896a, b). These were proposed as taxonomic criteria, but other features subsequently found acceptance (Stock-



Figures 1, 2.—SEMs of ventral surface of mesosoma of embryos of *Hadrurus arizonensis*. 1. Flap-like ventral plates (P) are fused to the body wall anteriorly and free at the posterior margin. Bilateral invaginations (arrows) are present where spiracles and booklungs will eventually form. Teeth (T) are evident at the posterior edge of the pectines. XIV, body segment; 2, Later stage. The ventral cuticle of each segment has broadened and is now a sternite (S) attached around the entire perimeter. Bilateral invaginations (black arrows) have deepened. Shallow depressions occur in body segment XVI, but booklungs do not develop in this segment. The white arrow indicates a pair of small, transitory appendages of unknown significance between gonopore and pectine. A, remnants of amnion not removed during preparation; T, pectinal teeth. Scales, 0.5 mm.





Figures 3, 4.—SEMs of ventral surface of mesosoma of embryos. 3, *Centruroides exilicauda*. Each segment has a sternite (S) like that of the irurid embryo of Figure 2. Bilateral invaginations (arrows) are presumably the site of booklung formation. Depressions are evident in body segment XVI although booklungs do not form in this segment. L, fourth walking leg. T, pectinal teeth. Scale, 0.5 mm; 4, *Paruroctonus mesaensis*, left side of ventral mesosoma. No ventral plates or sternites are evident, but spiracles (arrows) are present at the posterior margin of body segments XIII–XV. The spiracles are at the medial end of an invaginated intersegmental region (⋆) with vertical striations. L, fourth walking leg. Scale, 0.2 mm.

well, 1989, 1992; Sissom 1990). Developing booklungs were previously described as bilateral invaginations in the ventral mesosoma (Metschnikoff 1871; Laurie 1890, 1892; Brauer 1895; Abd-el Wahab 1951). Tissue sections showed that sac-like invaginations extend anteriorly in the segment from the initial site of ingress, which remains open to become the spiracle. A few lamellae are initially formed in the horizontal plane. These later rotate 90° to the dorso-ventral axis, along with development of many more lamellae (Laurie 1890, 1892).

There may be absence or delay of ventral plates, and sternites may form late in embryos of *P. mesaensis* in comparison with the iurid and buthid embryos. Among scorpion families, heterochrony occurs in embryogenesis in relation to the mode of maternal nourishment of the embryos (Matthew 1959; Farley 1999a, b). All extant scorpions have adaptations for terrestrialization (*i.e.*, oral tube, booklungs, latterly compressed podomeres), but may be polyphyletic with convergent evolution (Jeram 1994). The possibility of a different vaejovid derivation is raised in the present studies by the delay or absence of ventral plates (Fig. 4) and the development of spiracles at the medial end of lateral intersegmental specializations that may be indicative of ancestral respiratory structures. Fossils of British Triassic scorpions have slit-like spiracles in the intersegmental membrane of mesosomal segments or in the latero-posterior margin of the abdominal plates (Wills 1947).

Tissue sections are needed to determine if booklung formation is also distinctive in *P. mesensis*. The lack of tissue invagination at the place where spiracles first appear in the intersegmental area (Fig. 4) suggests this is not the site of booklung primordia. These spiracles may migrate from the intersegmental area to the adult position more anterior and lateral in the segment (Farley 1990a, b). Another possibility is that the early spiracles in Fig. 4 are transitory, and new spiracles form later with booklungs more anterior in the segments.

Kjellesvig-Waering (1986) proposed that ventral plates were abdominal flaps or appendages that overlay the body wall beneath, and sternites developed as the abdominal plates were reduced and eventually lost. From their review of fossil evidence, Selden & Jeram (1989) considered it more likely that ventral plates later became sternites by fusion with the body wall. The latter proposal is supported in the present study in embryos of *H. arizonensis*. Small regions, initially outlined by a ridge on the ventral surface of mesosomal segments, become flap-like plates (Fig. 1) and then the ventral cuticle is broadened to form sternites (Fig. 2). There was no indication of reduction or loss of the ventral plates, resulting in exposure of overlying sternites.

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LITERATURE CITED

Abd-el Wahab, A. 1951. Some notes on the segmentation of the scorpion *Buthus quinquestriatus* (H.E.). Proc. Egyptian Acad. Sci., 7:75–91.

Berteaux, L. 1889. Le poumon. La Cellule, 5:255–316.

Brauer, A. 1895. Beitrage zur kenntnis der entwicklungsgeschichte des skorpions. II. Zeitschrift fur wissenschaftliche Zoologie, 59:351–435.

Ewing, H.E. 1928. The scorpions of the Western part of the United States. U.S. Natl. Mus. Washington, 73:27–30.

Farley, R.D. 1987. Postsynaptic potentials and contraction pattern in the heart of the desert scorpion, *Paruroctonus mesaensis.* Comp. Biochem. Physiol., 86A:121–131.

Farley, R.D. 1990a. Functional organization of the respiratory and circulatory systems in the desert scorpion, *Paruroctonus mesaensis*. Acta Zool. Fennica, 190:139–145.

Farley, R.D. 1990b. Regulation of air and blood flow through the booklungs of the desert scorpion, *Paruroctonus mesaensis*. Tissue & Cell, 22:547–569.

Farley, R.D. 1999a. Scorpiones. Pp. 117–222. *In* Microscopic Anatomy of Invertebrates (F. W. Harrison, ed.), Vol. 8A. Chelicerate Arthropoda (F.W. Harrison & R.F. Foelix, eds). Wiley-Liss, New York.

Farley, R.D. 1999b. Structure, reproduction and development. Pp. 25–98. *In* Scorpion Biology and Research. (P.H. Brownell & G.A. Polis, eds.). Oxford University Press, Oxford/New York.

Francke, O.F. & M.E. Soleglad. 1981. The family Iuridae Thorell (Arachnida, Scorpiones). J. Arachnol., 9:233–258.

Hjelle, J.T. 1990. Anatomy and morphology. Pp. 9–63. *In* The Biology of Scorpions (G.A. Polis, ed.). University Press, Stanford, California.

Jeram, A.J. 1990. Book-lungs in a lower Carboniferous scorpion. Nature, 343:360–361.

Jeram, A.J. 1994. Scorpions from the Viséan of East Kirkton, West Lothian, Scotland, with a revision of the infraorder Mesoscorpionina. Trans. Roy. Soc. Edinburgh: Earth Sci., 84:283–288.

Kjellesvig-Waering, E.N. 1986. A restudy of the fossil scorpionida of the world. Palaeontographica Americana, 55:1–287.

Lane, N.J., J.B. Harrison & R.F. Bowerman. 1981. A vertebrate-like blood-brain barrier with intraganglionic blood channels and occluding junctions, in the scorpion. Tissue & Cell, 13:557–576.

Lankester, E.R. 1885. Notes on certain points in the anatomy and generic characteristics of scorpions. Trans. Zool. Soc. (London), 11:372–384.

Laurie, M. 1890. The embryology of a scorpion (*Euscorpius italicus*). Quart. J. Microsc. Sci., Ser. 2., 31:105–141.

Laurie, M. 1892. On the development of the lungbooks in *Scorpio fulvipes*. Zool. Anz., 15:102–105.

Laurie, M. 1896a. Notes on the anatomy of some scorpions, and its bearing on the classification of the order. Ann. Mag. Nat. Hist., ser. 6, 17:185–193.

Laurie, M. 1896b. Further notes on the anatomy and development of scorpions, and their bearing on the classification of the order. Ann. Mag. Nat. Hist., ser. 6, 18:121–133.

Mathew, A.P. 1959. Some aspects of the embryology of scorpions. J. Zool. Soc. India, 11:85–88.

Metschnikoff, E. 1871. Embryologie des scorpions. Zeits. für Wissenschaft. Zool., 21:204–232.

Selden, P.A. & A.J. Jeram. 1989. Palaeophysiology of terrestialization in the Chelicerata. Trans. Roy. Soc. Edinburgh: Earth Sci., 80:303–310.

Sissom, W.D. 1990. Systematics, biogeography and paleontology. Pp. 64–160. *In* The Biology of Scorpions (G.A. Polis, ed.). University Press, Stanford, California.

Stahnke, H.L. 1957. A new species of scorpion of the Vejovidae: *Paruroctonus mesaensis*. Entomol. News, 68:253–259.

Stockwell, S.A. 1989. Revision of the phylogeny and higher classification of scorpions (Chelicerata). Ph.D. dissertaion, Univ. California, Berkeley.

Stockwell, S.A. 1992. Systematic observations on North American scorpionida with a key and checklist of the families and genera. J. Med. Entomol., 29:407–422.

Williams, S.C. 1970. A systematic revision of the giant hairy-scorpion genus *Hadrurus* (Scorpionida: Vaejovidae). Occas. Pap. California Acad. Sci., 87:1–62.

Williams, S.C. 1980. Scorpions of Baja California, Mexico, and adjacent islands. Occas. Pap. California Acad. Sci., 135:1–127.

Wills, L.J. 1947. A monograph of British Triassic scorpions. Monogr. Palaeontol. Soc. (London), vol. 100/101. 137 pp.

Wood, H.C. 1863a. Descriptions of new species of North American pedipalpi. Proc. Acad. Nat. Sci. Philadelphia 1863, pp. 107–112.

Wood, H.D. 1863b. On the pedipalpi of North America. J. Acad. Nat. Sci. Philadelphia, 2nd ser., 5:357–376.

Yokota, S.D. 1984. Feeding and excretion in the scorpion *Paruroctonus mesaensis*: water and material balance. J. Exp. Biol., 110:253–265.

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