THE ULTRASTRUCTURE OF MALE NUPTIAL PADS IN SOME AUSTRALOPAPUAN FROGS

by MICHAEL J. TYLER & KERSTIN LUNGERSHAUSEN*

Summary

TYLER, M. J. & LUNGERSHAUSEN, K. (1986) The ultrastructure of male nuptial pads in some Australopapuan frogs. *Trans. R. Soc. S. Aust.* **110**(1), 37-41, 30 May, 1986.

Amongst Australopapuan hylid and leptodactylid frogs the male nuptial pad has an elaborate surface pattern of multicellular elevations bearing intricate structures. The disposition of these structures in representatives of five genera is documented and examples are figured. The study constitutes the most extensive undertaken of nuptial pads in the Anura.

KEY WORDS: Anura, ultrastructure, nuptial pad, Australopapuan.

Introduction

Male frogs are unique amongst vertebrates in the nature of their secondary sexual characteristics. These structures comprise inflatable vocal sacs located beneath the floor of the buccal cavity and, in most species, pigmented nuptial pads on the first digit of the hand. The structure of vocal sacs and associated submandibular musculature has been demonstrated to be of considerable significance in determining phylogenetic relationships (Liu 1935; Tyler 1971, 1972; Drewes 1984).

Nuptial pads enable the male to obtain a secure grasp of the female during amplexus. But even at a macroscopic level it is evident that the shape and appearance of the nuptial pad varies between species. Amongst Middle American hylid frogs possessing nuptial pads Duellman (1970) observed three major structural forms: 1. an enlarged and "generalised" nuptial excrescence; 2. a cluster of enlarged spines and 3. a single enlarged pre-pollical spine. Tyler (1968) noted that the shape and surface area of nuptial pads (that fit Duellman's 1970 "generalised" category) varied amongst Papuan species now referred to *Litoria* Tschudi.

The male nuptial pad consists of a superficial, keratinous layer and a deeper layer containing large mucous cells. Hypertrophy of the mucous cells during periods preceding the breeding season is accompanied by an enlargement and elaboration of the pad (Iwasawa & Asai 1959; Prakkal & Ellis 1963). In testectomised subadult *Rana nigromaculata* it has been demonstrated that nuptial pads developed following the administration of testosterone 15 μg/g body weight for 30 days (Iwasawa & Kobayashi 1985).

There appear to be few studies of the ultrastructure of the surface architecture of nuptial pads. There is an SEM illustration of the nuptial pad in the pipid *Xenopus laevis* (Karabuchi & Inoue 1981), whilst Zweifel (1983) has documented differences between two Papuan species of the hylid genus *Nyctimystes*.

As a part of a continuing interest in the phylogenetic relationships of Australian hylid and leptodactylid frogs we examined a diverse selection of species to document the surface architecture of nuptial pads, and to explore the potential application of any variability as a taxonomic character.

Material and Methods

Representatives were examined of adult males with pigmented nuptial pads of the following five genera and 36 species: Family Hylidae: *Cyclorana australis, C. longipes, C. novaehollandiae, C. vagitus, Litoria adelaidensis, L. angiana, L. bicolor, L. booroolongensis, L. caerulea, L. chloris, L. coplandi, L. fallax, L. freycineti, L. gracilenta, L. inermis, L. infrafrenata, L. latopalmata, L. lesueuri, L. meiriana, L. microbelos, L. nannotis, L. nasuta, L. nyakalensis, L. pallida, L. peroni, L. rubella, L. spenceri, L. splendida, L. tornieri, L. wotjulumensis;* Family Leptodactylidae: *Lechriodus fletcheri, L. melanopyga, Limnodynastes convexiusculus, L. ornatus, L. spenceri, Neobatrachus pictus.*

All of the specimens used in this study had been killed in a 3% solution of chloral hydrate, fixed in 3% formalin and stored in 65% alcohol. Following removal from the thumb the nuptial pads were transferred to absolute alcohol for a minimum period of 12 hr, air dried, mounted and coated with 15 nm carbon and 20 nm gold/palladium (80:20), and viewed in an ETEC scanning electron microscope. In all of the specimens selected the nuptial pad was black.

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Estimates of the frequency of the components upon each pad were obtained by counting within a transparent acetate grid (usually with a surface area equivalent to 100 μ^2), placed in random positions upon photographs of the pad structure.

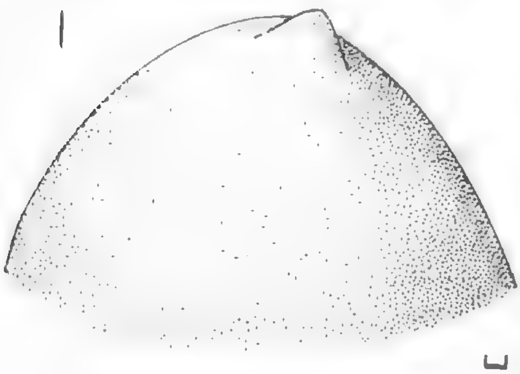
Results

In each of the species examined the surface of the nuptial pad was found to be composed of numerous, similar, multicellular structures. Interspecific variation involved the number of cells incorporated in each elevated structure, the nature of the surface, and the density of the elevations.

Amongst the species examined we observed the following seven structural forms:

(1) *Large spines* (Fig. 1)—very broad spines, each measuring approximately 200 μ across the base; each terminating in a distinct papilla.

1



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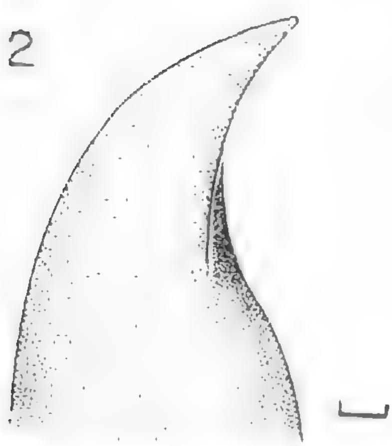


Figs 1-2. Nuptial pad architecture: 1, large spines in *Litoria nannotis* (scale bar = 100 μ); 2, large thorns in *Lechriodus melanopyga* (scale bar = 10 μ).

(2) *Large thorns* (Fig. 2)—curved, thorn-like structures 50 μ in diameter at the base and 75 μ high, and terminating in a sharp cone.

(3) *Conical elevations* (Fig. 3)—conical elevations 25 μ broad at the base and 30 μ high, and bearing sparse, short projections.

(4) *Radial processes* (Fig. 4)—a series of separate, projecting processes arranged radially around a central spine. Some of the processes are slightly dilated at their extremities. Each process is approximately 20 μ in length.

(5) *Alary processes* (Fig. 5)—large, curved, wing-like projections.

(6) *Papillae* (Fig. 6)—dense papillae borne on short processes, each of which is 20-30 μ diameter.

(7) *Rosettes* (Fig. 7)—regularly or irregularly arranged projections covering the entire surface of the elevation.

In addition to each of these symmetrical structures we also observed in various species occasional preparations in which the structures were irregular (Fig. 8), compared with the normal symmetrical condition (Fig. 9).

Systematic Account

Family: Hylidae

Cyclorana Steindachner

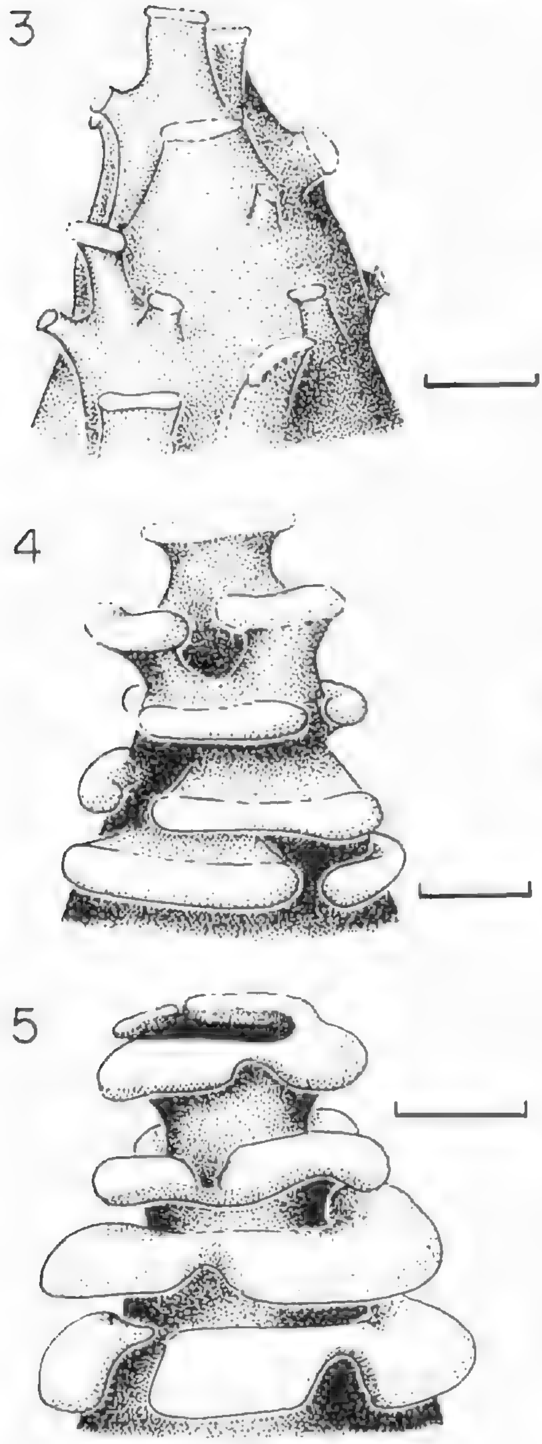
All four species exhibited nuptial elevations in the form of rosettes as depicted in Fig. 7. The density of elevations ranged from 106/mm^2 in *C. australis* to 235/mm^2 in *C. longipes*.

Litoria Tschudi

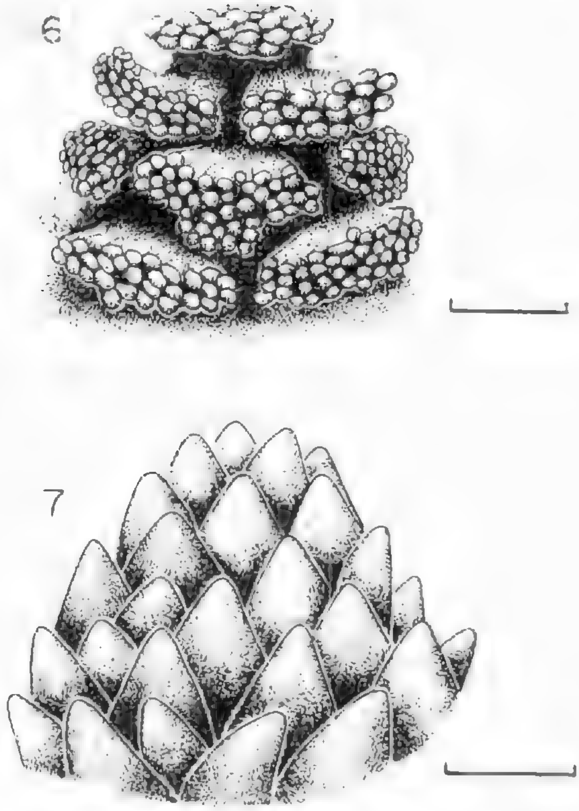
Three distinct forms of elevation are exhibited in this series. Firstly, large spines in *L. nannotis* (15/mm^2) (Fig. 10) and *L. nyakalensis* (in which resolution is inadequate to calculate density). The second form is that of rosettes in *L. booroolongensis* (107/mm^2), *L. coplandi* (218/mm^2) and *L. lesueuri* (167/mm^2).

The remaining species of *Litoria* examined all exhibited papillae: *L. adelaidensis* (191/mm^2), *L. angiana* (120/mm^2), *L. bicolor* (200/mm^2), *L. caerulea* (92/mm^2), *L. chloris* (164/mm^2), *L. fallax* (110/mm^2), *L. freycineti* (188/mm^2), *L. gracilenta* (225/mm^2), *L. latopalmata* (195/mm^2), *L. infrafrenata* (65/mm^2), *L. inermis* (280/mm^2), *L. microbelos* (222/mm^2), *L. meiriana* (278/mm^2), *L. nasuta* (104/mm^2), *L. pallida* (232/mm^2), *L. peroni* (172/mm^2), *L. rubella* (237/mm^2), *L. spenceri*[1] (61/mm^2), *L. tornieri* (153/mm^2), *L. watjulumensis* (158/mm^2) (Fig. 11).

[1] *Litoria spenceri* Dubois (1984) is a replacement name for *L. burrowsi* (Spencer) preoccupied.



Figs 3-5. Nuptial pad architecture: 3, conical elevations in *Lechriodus fletcheri* (scale bar = 10 μ; 4, radial processes in *Limnodynastes spenceri* (scale bar = 10 μ); 5, alary processes in *Limnodynastes ornatus* (scale bar = 10 μ).



Figs 6-7. Nuptial pad architecture: 6, papillae in *Litoria booroolongensis* (scale bar = 10 μ); 7, rosettes in *L. nasuta* (scale bar = 10 μ).

Family: Leptodactylidae

Lechriodus Boulenger

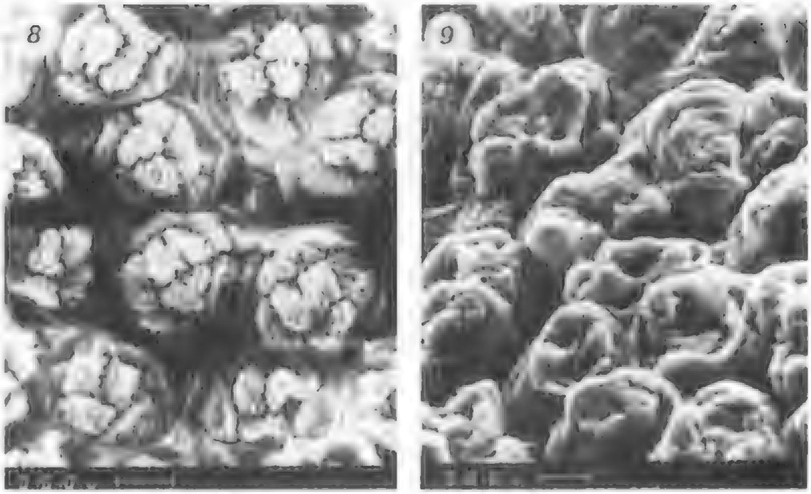
The two species examined have different forms of nuptial elevations: *L. fletcheri* conical elevations (96/mm^2) (Fig. 12) and *L. melanopyga* large thorns (33/mm^2) (Fig. 2).

Limnodynastes Fitzinger

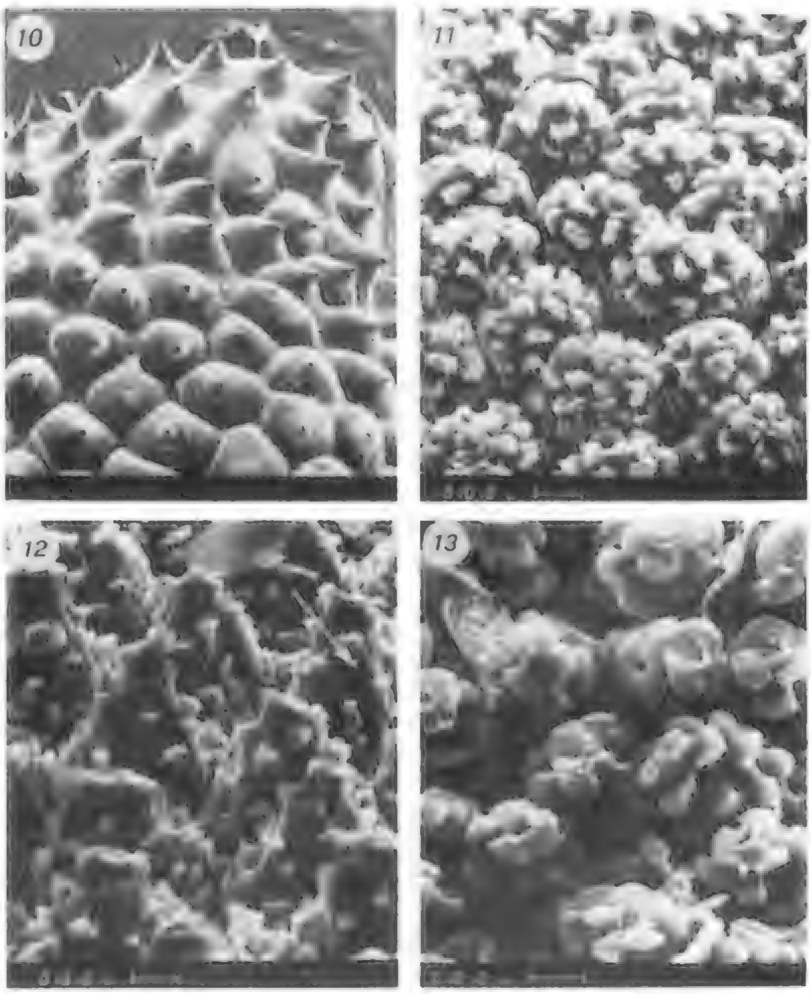
The three species appear to possess distinct types of nuptial elevations: *L. spenceri* radial processes (221/mm^2), *L. ornatus* alary processes (212/mm^2) (Fig. 13) and, although the resolution is poor, *L. convexiusculus* appear to exhibit papillae.

Neobatrachus Peters

The single species examined *(N. pictus)* exhibits nuptial elevations in the form of rosettes (134/mm^2).



Figs 8-9. Nuptial pad architecture in *Litoria caerulea*: 8, symmetrical form (scale bar = 10 μ); 9, irregular structures presumed to be associated with degeneration of the nuptial pad (scale bar = 10 μ).



Figs 10-13. Nuptial pad architecture: 10, *Litoria nannotis* (scale bar = 100 μ); 11, *L. wotjulumensis* (scale bar = 10 μ); 12, *Lechriodus fletcheri*. The arrow points to an individual squamous epithelial cell (scale bar = 10 μ); 13, *Limnodynastes ornatus* (scale bar = 10 μ).

Discussion

We have demonstrated that in the species of Australopapuan hylid and leptodactylid frogs examined the elaboration of the surface of the pad may be highly complex. We cannot exclude the possibility that some of the forms of elaboration that we have observed such as that of *Limnodynastes ornatus* and *L. spenceri*, represent intermediate stages in the development of other forms, but we suggest that the irregular form of the elevations upon the pad observed in some preparations (e.g. Fig. 8 and Zweifel 1983, Figs 7 and 13) represent regression of the pad prior to sloughing and not the perfectly formed pads.

Zweifel (1983) was the first contributor to note interspecific variation in the frequency of the elevated structures upon nuptial pads, citing counts of 700/mm^2 for *Nyctimystes trachydermis* and *N. disrupta*. Our data range from 15/mm^2 for *Litoria nannotis* to 278/mm^2 for *L. meiriana*. At the extremes of the range there is little doubt that the differences between the figures are significant between species, but the lower end of the scale is no more than a quantification of the differences in structure obvious to the naked eye.

We experienced great difficulty in obtaining good quality images and were unable with a dissecting microscope to eliminate preparations disintegrating prior to sloughing. We did not find any features distinguishing hylid from leptodactylid frogs, and there seems little likelihood of SEM appearance of nuptial pads providing a simple tool for studies of phylogenetic relationships in these families.

Acknowledgment

We are indebted to the staff of the Electron Optical Unit of the University of Adelaide for technical advice. Figures 1–7 are the work of Ruth Evans and the manuscript was typed by Sandra Lawson. The work was undertaken with the support of an Australian Research Grants Scheme award to M. J. Tyler and M. Davies.

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COLEOPTERA, SCORPIONIDA AND REPTILIA COLLECTED IN PITFALL TRAPS IN ENGELBROOK NATIONAL TRUST RESERVE, SOUTH AUSTRALIA

BY G. H. BAKER

Summary

Engelbrook National Trust Reserve is an open forest of Eucalyptus obliqua and E. baxteri in the Mt Lofty Ranges, South Australia. Pitfall trapping there over a one year cycle yielded 21 species of beetles active on the soil surface. Celibe (=Saragus) simplex (Tenebrionidae) was especially common. Few predatory beetles (Carabidae, Staphylinidae) were trapped. One species of scorpion (Urodacus manicatus), five species of lizard (Lampropholis guichenoti, Leiolopisma trilineata, Lerista bougainvillii, Hemiergis decresiensis, Aprasia striolata) and one frog (Pseudophryne) were also trapped.