

# The histology of dermal glands of mating *Breviceps* with comments on their possible functional value in microhylids (Amphibia: Anura)

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During mating male and female *Breviceps* become 'glued' together. The distribution of multicellular dermal glands varies between the sexes. The male has a large number of holocrine glands on the ventrum, while the female has similar glands on the dorsum of the back. It thus appears that both sexes contribute to the 'glueing' mechanism. New hypotheses to explain the adhesion between the sexes are proposed, viz., that the shift to terrestrial habits and subterranean laying in *Breviceps* have secondarily resulted in male size diminution. In other microhylids which adhere during aquatic oviposition, yet demonstrate a terrestrial morphology and habits, buoyancy is suggested to be of primary functional value.

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Tydens paring raak die manlike en vroulike diere aan mekaar 'vasgeplak'. Die verspreiding van multisellulêre velkliere verskil tussen die geslagte. Die mannetjie het 'n groot aantal holokriene kliere op die ventrum, terwyl die wyfie weer soortgelyke kliere op die dorsum van die rug het. Dit wil dus voorkom asof altwee geslagte bydra tot die 'vasplak' meganisme. Nuwe hipoteses word voorgestel om die vashegting tussen die geslagte te verklaar, naamlik dat die verskuiwing na 'n terrestriële leefwyse en die ondergrondse lê van eiers in *Breviceps* aanleiding gegee het tot 'n sekondêre vermindering in grootte van die mannetjie. In ander mikrohieliede paddas wat gedurende akwatiese eierlegging aan mekaar heg maar tog 'n terrestriële morfologie en gedrag het, word voorgestel dat die vermoë om in water te kan dryf die primêre funksionele waarde is.

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During mating the male and female *Breviceps* become 'glued' together before, during and for an unknown period after oviposition. The animals cannot be separated without tearing the adhering skin. This preliminary study reports on the histological sex differences of the skin of the mating animals and discusses the functional significance of mating adhesion to both terrestrial and aquatic breeding microhylids.

## Materials and Methods

Several *Breviceps gibbosus*, collected at Rondebosch, were sent by air from Cape Town to Mendoza in October 1971. On arrival in Argentina, some pairs were found 'glued' together. Skin from dorsal and sternal regions of manually separated adhering pairs was first fixed with 5% Glutaraldehyde/S-Collidine Buffer (pH 7,9) and later with 1% Osmium Tetroxide (OsO<sub>4</sub>), and embedded in epoxy resin. Sections of 1 µm were stained with Toluidine Blue-Borax and Basic Fuchsin.

## Results

Sex differences in dermal structures and dermal glands were found to be significant. In the sternal region of the males, 3–4 layers of epidermal cells can be observed. The total thickness of the epidermis is about 75–100 µm with a very thin stratum corneum. Scanty intra-epithelial glands, or single-celled glands are present (Figure 1). Several types of multicellular glands are recognizable,

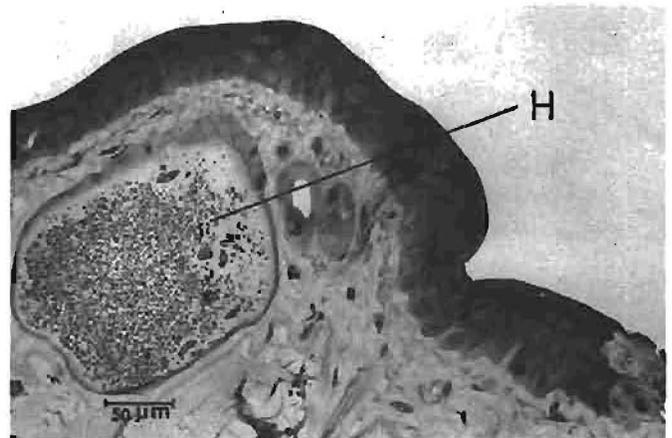


Figure 1 Sternal region of male. H holocrine granular glands. Scanty single-celled epidermal glands are also recognizable.

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namely mucous glands, holocrine granular glands and glands comparable to the apocrine 'breeding glands' of Conaway & Metter (1967). These apocrine glands are less common and active in the sternal region of males than in the dorsal region of females where a remarkable secretory activity is seen (Figure 2). Holocrine granular glands, however, are well represented in the sternal region of the males (Figure 1,H). In the sternal region of females the scattered single-celled epidermal glands increase but only spent apocrine glands, comparable to the 'breeding glands' of *Microhyla*, were observed (Figure 3). No secretory holocrine granular glands were recognizable in the sections. The thickness of the epidermal layers is 45–50  $\mu\text{m}$ , and the stratum corneum is very thin. The thickness of the dermis is about 100–120  $\mu\text{m}$ .

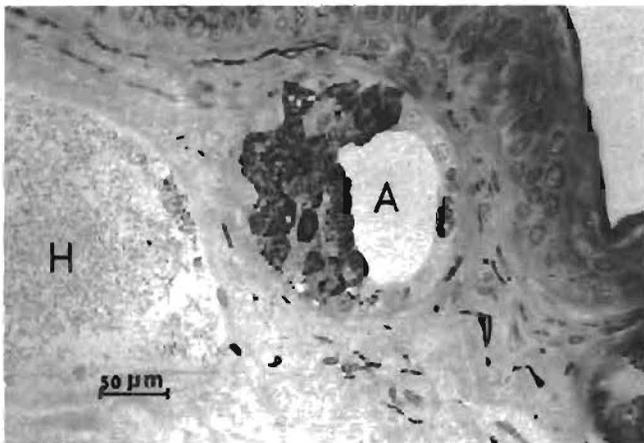


Figure 2 Dorsal region of female. H holocrine granular glands. A apocrine glands ('breeding glands' of *Microhyla*).

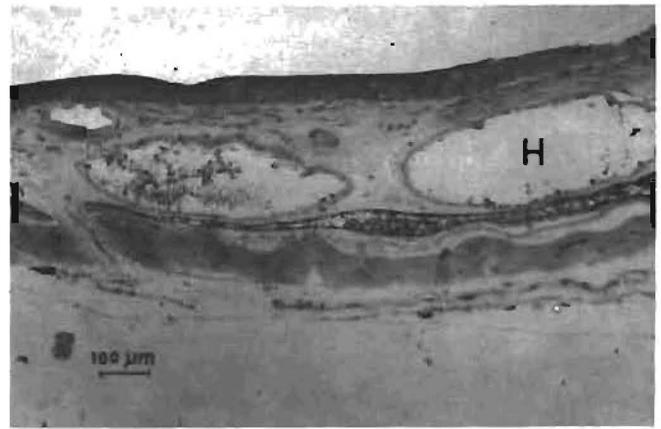


Figure 4 Dorsal region of male. H holocrine granular gland.

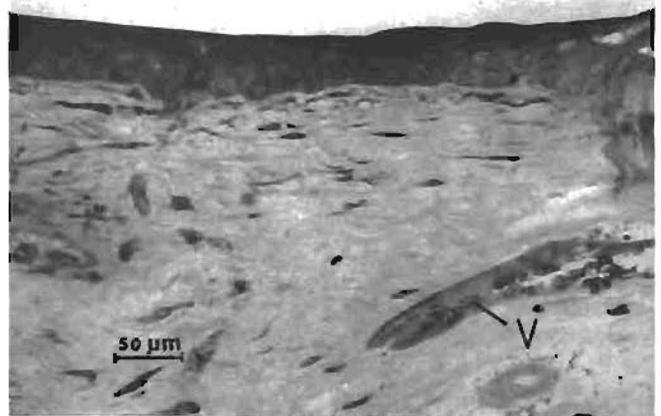


Figure 5 Dorsal region of male. V vestigial apocrine gland. Single-celled epidermal glands are illustrated.

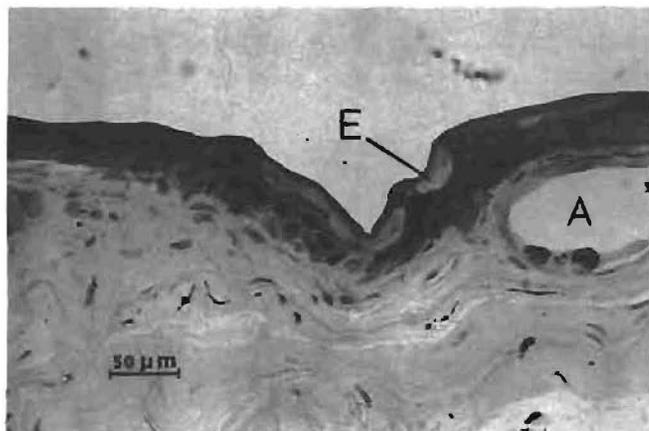


Figure 3 Sternal region of female. A spent apocrine gland. E single-celled epidermal gland.

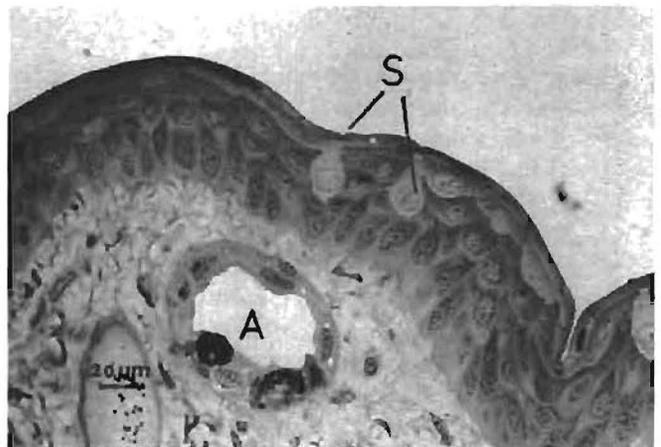


Figure 6 Dorsal region of female. A active apocrine gland. S single-celled epidermal glands.

The backs of the males apparently have only moderate secretory activity, possessing few scattered holocrine granular glands (Figure 4) and only scattered and almost spent or vestigial apocrine glands (Figure 5,V). Only two epidermal cell-layers were observed with a relatively thick stratum corneum (Figures 4 & 5). Very few single-celled epidermal cells could be seen. The thickness of the epidermis is about 225  $\mu\text{m}$ . In contrast,

the back of the female is characterized by a thick stratum corneum, 4–5 layers of epidermal cells and several bottle-shaped, pale, single-celled glands (*cf.* 'flask cells' described by Whitear 1975). The thickness of the dermis is about 450–500  $\mu\text{m}$ . Secretions of the apocrine glands, considered to be 'breeding glands' by Conaway & Metter (1967) are very obvious (Figures 2 & 6) and are released by the top portions of the cells breaking off. Cuboidal cells and spent 'apocrine glands' are thus uncommon. Large, tightly packed, holocrine granular glands are very

abundant, also in the main apocrine gland secretory region (Figure 2,H).

### Discussion

The apocrine glands of *Breviceps* are comparable to the 'breeding glands' of *Microhyla* and it is probably the holocrine granular glands which produce the secretions which cause the adhesion of mating *Breviceps*. An attractive hypothesis to explain the bonding effect is that the adhesion occurs by means of the mixing of complementary chemical substances, in a manner similar to the hardening mechanism in some commercial 'epoxy' glues. The irreversible and permanent effect of the 'epoxy' chemistry cannot, however, be hypothesized for adhering *Breviceps*. The apparent lack of a permanent bond could, nevertheless, be due to the frequent periodical loss of outer layers of the amphibian epidermis. The observations on heterospecific pairs reported by Jurgens (1978) for *Breviceps adspersus* (male) and *Tomopterna delalandii* (female) do not add significantly to the detailed explanation of the intrinsic chemical or structural mechanisms of adhesion.

Regions involved in adhesion during mating are the gular and/or sternal regions of the male and the dorsal sacral area of the female back. Both these regions exhibit the above-mentioned glandular types, the apocrine and granular glands being especially well developed in the female. The gular and/or sternal regions of the female apparently lack holocrine glands.

Both glandular types — those previously referred to as 'breeding glands' and granular glands (Conaway & Metter 1967) — are vestigial or absent in the back of the female. Further histochemical and biochemical assays are required to explain the composition and nature of the hypothesized complementary secretions or other 'glueing' agents which may be associated with the glands during activity.

Within the Amphibia, dermal adhesion during mating is presently known for only three microhylid genera, one each in North America, South Africa and the Philippines. It has been suggested that adhesion functions to prevent pair separation during mating in the disturbance-prone *Microhyla olivacea* and, alternatively, to prevent displacement of a clasping male by a rival (Fitch 1956). Conaway & Metter (1967) give the disturbance factor little credence and believe that in *M. carolinensis* the rotund body and short arms of the male made clasping impossible and that without being 'glued' to the female's back the male would not be able to easily maintain a mating posture. Both *M. olivacea* and *carolinensis* are pond breeders and oviposit while floating. The arms do not adhere to the female's body.

Wager (1965) also asserts that the males' arm span and size are inadequate for normal clasping in *Breviceps*, and that without 'glueing' they would be dislodged during burrowing. The size differential between the sexes of *Breviceps* is the greatest of all the microhylids (Figure 7). The male *Breviceps* does not clasp the female during mating (Figure 8) and his arms in fact adhere to her body (Poynton 1964). *Breviceps* come together above ground and burrow backwards to make a nest chamber underground (Wager 1965). According to this author, the male assists in the burrowing.



Figure 7 *Breviceps fuscus* 'glued' together during mating. Males of the genus are commonly a third to half the length of females and very much smaller in size.



Figure 8 The hands of the male frog do not clasp the female and, as shown, face outwards. The ventral surfaces of the arms are 'glued' to the female's back.

In the Philippinian *Kaloula*, some forms of the *conjuncta-picta-rigida* group practise adhesion (Inger 1954). A 'belly gland' is a common secondary sex character to the males of this group. Mating pairs adhere

to each other during floating oviposition in quiet ponds with the males in axillary clasp. The arms do not adhere during this activity. Taylor (1920) believed that it was the males that adhered to the females but Inger (1954) commented that it might be 'a mistake to overlook the part played by the female in this copulatory adjustment, for both sexes have well-developed mucous glands on the back'.

The functional value of adhesion is clearly independent of breeding mode within the Microhylidae for those genera for which it has been recorded, as the one African genus mates and lays underground while the American and Philippinian genera mate and lay in water. There is also a very marked difference in size between the male and female brevicepsids while very little size difference between the sexes is apparent in the other genera, the differential in the latter being similar to that shown by most frogs.

Common to the three genera which practise mating adhesion is their familial relationship (all are microhylids), rotund build and terrestrial burrowing habits. Many well-studied Australian and New Guinean microhylids (Tyler 1973; Zweifel 1972) that lay in subterranean nests in the manner of *Breviceps*, as also frogs of other families with a similar breeding mode, share a rotund morphology and terrestrial habits. None are known to become 'glued' during mating and the supposed physical handicap to mating posed by male or female body shape and/or burrowing oviposition are unconvincing as an explanation for the adhesive habit in those forms that do practise it. Too little is known of the pre-mating behaviour of the essentially terrestrial burrowing frogs that oviposit in water, to make suggestions for the functional value of the habit. It is evident, however, that the burrowing morphology is hardly compatible with either swimming ability or the pyrotechnics of the usually prolonged aquatic laying process which is common to most, if not all, frogs that lay in this medium. As an interim hypothesis we therefore suggest that adhesion plays a major role in the maintenance of bouyancy in *Microhyla* and *Kaloula*.

The widely acknowledged primitiveness of the aquatic oviposition mode within the Amphibia as also the belief

that the brevicepsids are phylogenetically antecedent to the microhylines (Lynch 1973), favour the view that the 'glueing' habit is a derived condition. Adhesion, once established as part of the burrowing laying behaviour, would appear to have considerable functional value to frogs which burrow backwards to lay in a nest chamber, quite regardless of the respective sizes of the mating frogs. The reduction of male size, as seen in *Breviceps*, would, we believe, multiply both survival and functional values by obviating the need for synchronized burrowing effort, reducing the surface time while compromised and aiding underground progression, as well as permitting the construction of a smaller nesting chamber. It is suggested therefore that male diminution in *Breviceps* is a secondary development which arose as a benefit of the adhesive habit. An earlier suggestion that the 'glueing' habit arose *de novo* to compensate for the small size of this sex is rejected and we suspect that detailed observation of mating *Breviceps* will show his role in burrowing to be insignificant.

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