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Skin structure in the snout of the Australian lungfish, *Neoceratodus forsteri* (Osteichthyes: Dipnoi)

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ABSTRACT

Many fossil lungfish have a system of mineralised tubules in the dermis of the snout, branching extensively and radiating towards the epidermis. The tubules anastomose in the superficial layer of the dermis, forming a plexus consisting of two layers of vessels, with branches that expand into pore canals and flask organs, flanked by cosmine nodules where these are present. Traces of this system are found in the Australian lungfish, *Neoceratodus forsteri*, consisting of branching tubules in the dermis, a double plexus below the epidermis and dermal papillae entering the epidermis without reaching the surface. In *N. forsteri*, the tubules, the plexus and the dermal papillae consist of thick, unmineralised connective tissue, enclosing fine blood vessels packed with lymphocytes. Tissues in the epidermis and the dermis of *N. forsteri* are not associated with deposits of calcium, which is below detectable limits in the skin of the snout at all stages of the life cycle. Canals of the sensory line system, with mechanoreceptors, are separate from the tubules, the plexus and the dermal papillae, as are the electroreceptors in the epidermis. The system of tubules, plexus, dermal papillae and lymphatic capillaries may function to protect the tissues of the snout from infection.

1. Introduction

Fossil lungfish have a loose branching system of mineralised tubules in the dermal tissues of the snout, below an ossified "exoskeleton" described as consisting at least in part of a hard tissue called cosmine in some fossil genera. The tubules pass through the dermal tissues of the snout, form a plexus in the outer layers of the dermis and join the pore canals that surround the cosmine nodules in the calcified tissues of the snout (Miles, 1977; Schultze, 1987; Cheng, 1989; Pridmore and Barwick, 1993; Campbell and Wragg, 2014). The system of mineralised tubules is present in the dermis of every Devonian dipnoan with suitable preservation, and in a number of crossopterygians such as *Porolepis posaniensis* (Schultze, 1987). It occurs in basal sarcopterygians like *Meemannia eos* (Zhu et al., 2010) and in the enigmatic *Diabolepis speratus* (Campbell and Barwick, 2001; Chang and Yu, 1997), but the system is not found in tetrapods (Chang and Yu, 1997; Schultze, 1994). Mineralised tubules have even been found in *Ferganoceratodus martini*, a Mesozoic lungfish from Thailand (Cavin et al., 2007). Similar branching tubules, which are not mineralised, are present in one living dipnoan, the Australian lungfish, *Neoceratodus forsteri*. The tubules terminate in a double plexus below the epidermis, and dermal papillae extend into the epidermal tissue (Bemis and Northcutt, 1992). In the Australian lungfish, the tubules appear to be linked with the efferent branchial artery of arch 1, and fill with latex when this vessel is injected (Bemis and Northcutt, 1992). However, at least in *N. forsteri*, the snout and lips, although often scratched or cut during feeding activities, do not bleed (Kemp, 2012a).

The skin of the Australian lungfish contains several important systems, associated in position but not in function. This contribution examines the structure of the tubule system and its termination within the skin of the snout of the Australian lungfish, as well as the sense organ systems associated with the snout and lips. Although all are found within the tissues of the snout and lips, they are not actually linked, and their functions are separate.

2. Materials and methods

Samples of snout, scale and lip tissue from two adult lungfish collected from the Brisbane River in southeast Queensland, and ten laboratory reared juveniles and two subadults from the same source were used for this project. Hatchling and juvenile lungfish were reared from eggs collected in the Brisbane River at Lowood

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Table 1
Lengths of individual animals used. Subadult and adult specimens have acquisition numbers.

Stage	Length
45	Hatchling, 13.8 mm
48	Hatchling, 18.0 mm
50	Hatchling, 22.5 mm
52	Juvenile, 28.1 mm
53 (i)	Juvenile, 34.2 mm
53 (ii)	Juvenile, 36.7 mm
54	Juvenile, 40.3 mm
57	Juvenile, 76.5 mm
58	Juvenile, 86.4 mm
60	Juvenile, 190 mm
#AN96-34	Subadult, 34 cm
#AN98-45	Subadult, 45 cm
#AN99-102	Adult, 102 cm
#AN97-114	Adult, 102 cm

in southeast Queensland according to the methods described in Kemp (1981, 1999). Stages follow Kemp (1982, 1999). The hatchlings and juveniles ranged from stage 45 to stage 60 (Table 1). The subadult specimens, AN96-34 and AN98-45, were reared in the laboratory. The adult fish, AN99-102 and AN97-114, were caught in the river and heavy wear on the tooth plates confirmed that they were old.

Blocks of tissue, including epidermis and dermis, from adult and subadult lungfish were fixed in 10% neutral buffered formalin, rinsed in water, and dehydrated in a graded series of ethanols. Specimens from the lips and snout were sectioned with a razor blade to reveal structures in the dermis and in the epidermis. Two planes of section were employed, transverse and sagittal to the long axis of the head. For scanning electron microscopy, processed tissues were dehydrated in a critical point dryer before being mounted on stubs and coated with platinum.

Sections of subadult and adult skin, prepared as described above for scanning electron microscopy, were not coated and were examined in a JEOL 6460 scanning electron microscope fitted with a detector for energy dispersive spectroscopy (EDS analysis).

Blocks of subadult and adult tissue for histology were washed and dehydrated in a graded series of ethanols, and then embedded in Technovit resin (Kemp, 1999) or in Epon Araldite resin, sectioned at 2 μm and stained with Toluidine blue in buffer. Heads of hatchling and juvenile lungfish, fixed in neutral buffered formalin, rinsed in water and dehydrated in ethanol, were embedded whole in Technovit resin, then sectioned and stained with 1% Toluidine blue in phosphate buffered saline. Preparation of dental structures followed the methods outlined in Kemp (2003) and Kemp and Barry (2006).

3. Results

3.1. Definitions

Structures present in the lungfish snout are illustrated in Fig. 1, to show the relative positions and dimensions of the different structures.

Tubules consist of thick, branching strands of connective tissue radiating through the dermal tissues of the snout and mandible of adult and subadult *N. forsteri* (Fig. 1A). The tubules have minute foramina along the length, and each one encloses a fine *lymphatic vessel* packed with lymphocytes. The foramina are 1–5 μm in size. Tubules terminate in a double dermal *plexus*, lying below the epithelium and consisting of connective tissue. The dermal plexus also has minute foramina, 1–5 μm in size, and also encloses lymphatic vessels (Fig. 1B). The inner layer of the plexus is intermittent, and the outer layer, closer to the epidermis, is uninterrupted.

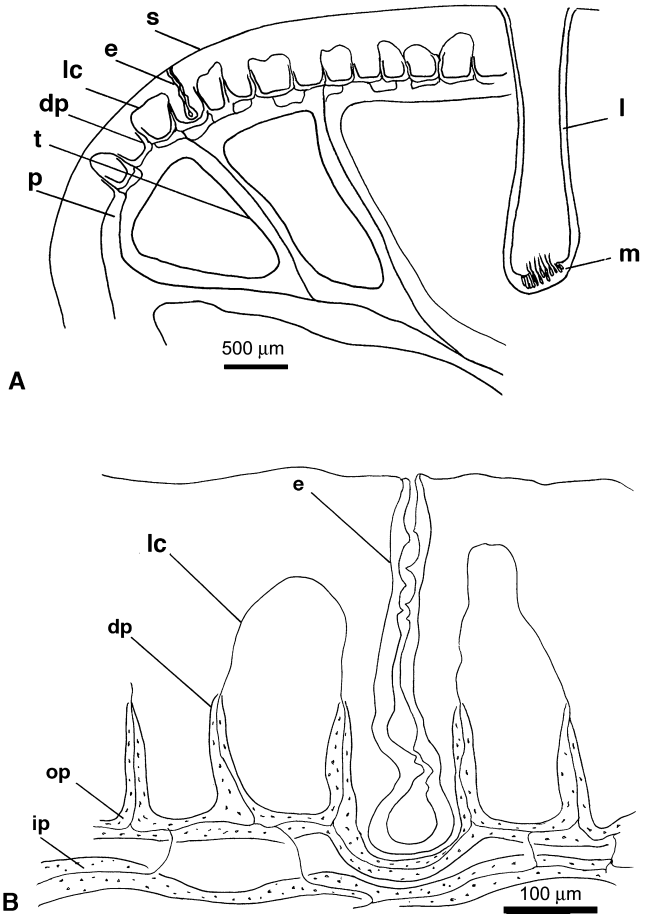

Fig. 1. Drawings to define the structures present in the epidermis and dermis of a lungfish, and their relative dimensions. (A) Low power. (B) High power of epidermis and plexus. dp, dermal papilla; e, electroreceptor; l, lateral line canal; lc, lymphatic capillary; m, mechanoreceptor; ip, inner plexus; op, outer plexus; p, plexus; t, tubule.

Dermal papillae arise from the outer layer and enter the epidermis. They are conical in shape and made up of connective tissue with minute foramina, 1–5 μm in size. They surround a *lymphatic capillary*. The capillaries emerge from the dermal papillae and form loops among the epithelial tissue, without reaching the surface of the skin (Bemis and Northcutt, 1992: Fig. 12). This system, in a less organised form, is present in young hatchlings and juveniles, and consists of *lymphatic capillaries*, with no covering of connective tissue, among the other structures present in the dermis. These do not enter the epidermis in young stages.

Sensory (lateral) lines of the head are wide canals deep within the dermis, made up of a single layer of epithelial cells supported by connective tissue (Fig. 1A). At intervals along the length of the canal are *mechanoreceptors*. The sensory lines and the tubules, the plexus and the dermal papillae in the living lungfish may be found in the same anatomical region of the head, but there are no functional or anatomical connections between them.

Electroreceptors (ampullary pits) are small sensory organs, confined to the epidermis or to superficial layers of the dermis (Fig. 1B). On the snout they are single, and on the dorsal, lateral and ventral surfaces of the head they are arranged in *pit lines* (Kemp, 1999). Rows of electroreceptors run above and below the lateral line of the trunk (Kemp, 2012b). Despite a close topographic association with the dermal papillae and the sensory lines