We describe the pathology in a captive adult White lipped treefrog (Litoria infrafrenata) with a squamous cell carcinoma of the skin and a free-living adult Common green treefrog (L caerulea) with an adenocarcinoma of the skin. Although many amphibian neoplasms have been reported worldwide, none had been described in Australian species.

Many neoplasms of amphibians have been described but none has been reported from an Australian species. A large proportion of reported cases of anuran tumours are epithelial and many originate in the skin. Amphibian tumours include squamous cell carcinomas, adenomas, adenocarcinomas, papillomas and epitheliomas.

We describe the pathological findings in a captive adult Litoria infrafrenata (White lipped treefrog) with a squamous cell carcinoma of the skin and a free-living adult L caerulea (Common green treefrog) with an adenocarcinoma of the skin.

Methods
The L infrafrenata and L caerulea with obvious dermal lumps were submitted for diagnosis, the former preserved in 10% formalin and the latter unfixed. They were measured, photographed and necropsied. Samples of the proliferative lesions and a range of organ samples including liver, kidney, heart, spleen, lung, urinary bladder, brain, thigh muscle, stomach, intestines and skin were processed routinely for histological examination, sectioned at 6 µm and stained with haematoxylin and eosin. Measurements were made using a calibrated eyepiece graticule.

Results
Case 1- Squamous cell carcinoma in an adult L infrafrenata
On the skin of a captive female L infrafrenata (AAHL accession No. 95 203/1) with a snout-vent length of 93 mm, submitted from Adelaide in 1995, were five, well-demarcated, raised, grey/brown, rough, circular plaques between 8 and 14 mm in diameter and 2 to 5 mm in height (Figure 1). These occurred along the dorsal edge of the right tympanum; on the flexor surface of the right elbow; on the dorsolateral back 10 mm anterior to the right hindlimb; below the cloaca, extending to the right; and on the ventral surface of the right stifle. Plaques on the elbow and stifle were ulcerated. On the left side of the central abdomen was an ill-defined area of roughened skin, about 4 x 12 mm, with an elevated, grey surface.

Histologically, there were rounded pegs of epidermal cells proliferating downwards and invading the dermis, or thicker lesions comprised of multiple, solid or cystic balls of epidermal cells (Figures 2 and 3). These ranged between 56 and 400 µm in diameter and the cystic structures were lined by layers of about 5 to 8 cells. The cells were well differentiated, with frequent formation of keratin pearls. The moderately pleomorphic cell nuclei were oval or kidney-shaped with one or two very prominent nucleoli and there were about two mitoses per high power field. Intercellular bridges were seen. Cells had abundant eosinophilic...
mm in diameter) were present on dorsal skin caudal to the head, on the lateral edge of the right hind foot, dorsal skin of the right forearm and under the left eye, and three occurred in a cluster caudal to the left parotid gland. One large, smooth, encapsulated nodule 10 x 20 mm that was embedded under the superficial muscles behind the right tympanum was not connected to any epidermal growths and was easily shelled out.

Cytoplasm. Necrosis of individual cells was common with ballooning degeneration and formation of microvesicles. The surfaces of lesions were hyperkeratotic and focally ulcerated. Interstitial fibrosis and mixed inflammation occurred between epidermal structures in ulcerated areas and infiltrated the entire thickness of the dermis in the diffuse ventral lesion.

The frog had moderately sized fat bodies, fully developed ova with mature eggs, and the internal organs appeared normal. A greater than usual amount of melanin was present in the liver.

Case 2 - Adenocarcinoma in an adult L. caerulea

A 110 g female L. caerulea (AAHL accession No. 98 320/26) with a snout-vent length of 97 mm was found free-ranging in Lismore, New South Wales, in May 1998. The frog was lethargic on collection and died in transit to the laboratory. Thirteen nodules, 3 to 21 mm diameter, were present on the dorsal skin of the body and limbs (Figure 4). Most larger nodules were ulcerated, exposing a cream coloured, bleeding, friable surface of the tumour. Three large nodules (21 to 24 mm diameter) grew on the skin over the urostyle, together with two smaller ones about 5 mm wide. One of the larger nodules covered with intact skin had penetrated the dermis, and a few adhesions to underlying muscle fascia had formed. All other cutaneous nodules were superficial to the deeper connective tissue of the dermis. Smaller nodules (3 to 10 mm in diameter) were present on dorsal skin caudal to the head, on the lateral edge of the right hind foot, dorsal skin of the right forearm and under the left eye, and three occurred in a cluster caudal to the left parotid gland. One large, smooth, encapsulated nodule 10 x 20 mm that was embedded under the superficial muscles behind the right tympanum was not connected to any epidermal growths and was easily shelled out.
Histologically, the nodules in the dermis were composed of epithelial cells in various stages of differentiation. The complex tumour formed lobules ranging from 343 to 2525 µm wide surrounded by fibrous stroma (Figure 5). Most cells had dark, ovoid nuclei about 6.1 x 4.2 µm, and eosinophilic cytoplasm. The mitotic rate was low and there was little variation in cell and nuclear size. The bulk of the tumour was comprised of solid or glandular acini. There were some cystic structures filled with keratin pearls (400 to 2000 µm) or necrotic red blood cells. Scattered throughout were small ducts formed by a single layer of about eight cuboidal cells with pale, round nuclei (Figure 6). Larger, dilated ducts were also present and some contained necrotic cell debris. Occasional clusters of cells in solid acini had large, round, cytoplasmic vacuoles that did not stain with periodic acid-Schiff. Large areas of haemorrhage and necrosis occurred in the centre of lobules or were associated with surface ulceration. At necropsy, internal organs appeared to be normal. The frog had moderately-sized fat bodies and the ovaries contained mature eggs. Rhabdias sp occurred in the lungs. Histological examination of internal organs did not reveal any neoplastic lesions. A nonsuppurative cystitis was present with large colonies of bacterial rods and foci of inflammation. There was a greater than normal amount of melanin in the liver.

**Discussion**

The two epidermal neoplasms were considered to be malignant. Although they were mostly confined to the skin and had a low mitotic rate, features of malignancy included local invasion through tissue boundaries, ulceration, necrosis, haemorrhage and multiple sites of occurrence. It is possible these tumours metastasised along the dorsal cutaneous blood vessels or dorsal subcutaneous lymph sinuses which drain caudally along the back, but they may also have arisen spontaneously at multiple sites. Multicentric squamous cell carcinomas and adenocarcinomas have been reported in amphibians. The adenocarcinoma reported here also had features of a basal cell tumour, such as its differentiation into various epithelial cell types.

Compared with their reported occurrence in mammals, birds and fish, neoplasia in amphibians is uncommon. This may be due to neoplasms being over-looked and not reported, or alternatively, amphibians may have some resistance to their development. These two epidermal neoplasms, plus a possible intravascular lymphoma, represent 0.84% (3/356) of ill frogs in our survey of diseases in Australian frogs. This prevalence is similar to, or less than, that seen in amphibians in other countries. High prevalences have been reported in polluted areas and also with infectious tumours of amphibians (for example, Lucké renal tumour) where the same tumour occurs in many individuals of the same species, and incidence varies seasonally.

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**References**


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