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## Squamous Cell Carcinoma in Two Wild Bell's Turtles (*Myuchelys bellii*)

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**ABSTRACT:** Squamous cell carcinomas in Testudines are rarely reported. Most cases in the literature describe these tumors in captive animals. We report spontaneously arising squamous cell carcinomas affecting the plastron of two wild Bell's turtles (*Myuchelys bellii*) from the Macdonald River in New South Wales, Australia.

**Key words:** Bell's Turtle, *Myuchelys bellii*, neoplasia, squamous cell carcinoma.

Squamous cell carcinomas (SCC) are rarely reported in Testudines. Isolated cases in the captive yellow-bellied slider (*Trachemys scripta scripta*; Lanza et al. 2015), captive British Indian turtle (*Melanochelys trijuga*), captive spiny soft shell turtle (*Apalone spinifer spinifer*), captive common snake-necked turtle (*Chelodina longicollis*; Sykes and Trupkiewicz 2006), European pond turtle (*Emys orbicularis*) of unknown origin, and wild loggerhead sea turtle (*Caretta caretta*; Oros and Torrent 2000) have been described, comprising masses in the skin or mucocutaneous junctions, with and without metastases to the coelomic parenchyma. A single report describes a carapace-associated SCC in a captive Hermann's tortoise (*Testudo hermanni*; Von Deetzen et al. 2012).

We report nonmetastatic SCC arising within the plastron of two wild adult Bell's turtles (western sawshelled turtle, *Myuchelys bellii*) from the Namoi River catchment in New South Wales (NSW), Australia. The Bell's turtle is a medium-sized, saw-shelled, freshwater turtle from disjunct populations in the Namoi and Gwydir Rivers of NSW, and the Border Rivers systems of NSW and southeastern Queensland (Fielder et al. 2015). The species is listed as Endangered by the International Union for Conservation of

Nature's Red List of Threatened Species, and as Vulnerable under the Environment Protection and Biodiversity Conservation Act of 1999. The Bell's turtle is a high-profile conservation species in both NSW and Queensland due to ongoing population and landscape-management threats such as habitat loss and degradation, and disturbance of both turtles and nests by feral predators (New South Wales Office of Environment and Heritage 2019).

Case 1 was an adult female (weight 3 kg; straight carapace length=260 mm) caught in the Macdonald River at Bendemeer, NSW (30°52'37"S, 151°9'29"E), in a cathedral trap during routine population surveillance operations in December 2015. On clinical examination, the left humeral-pectoral scutes were disrupted by a locally extensive area of ulceration covered with a friable, tan colored, malodorous plaque (Fig. 1a). The turtle was otherwise in good body condition. A swab of the lesion tested negative for the Bellerophon River virus (Zhang et al. 2018) by PCR, and the animal was medically managed for 6 wk with no improvement. A diagnosis of SCC was made following incisional biopsy of the mass and routine histopathology. The turtle was palliated for a further 3 mo; however, euthanasia was elected due to tumor progression and the poor prognosis for recovery, rehabilitation, and release.

The turtle was euthanized in April 2016 using IV Lethobarb Euthanasia Injection (Virbac Pty. Ltd., Sydney, Australia). On postmortem examination, the plastron was ulcerated and a poorly demarcated, 14×18 cm mass with irregular margins extended into the subjacent tissue, with locally extensive

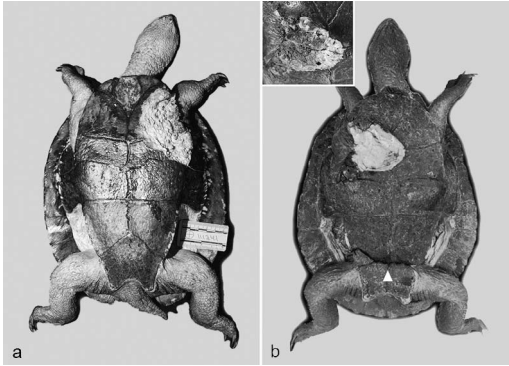


FIGURE 1. (a) Case 1: Adult female Bell's turtle (*Myuchelys bellii*) captured from the Macdonald River in New South Wales, Australia, with a 14×18 cm ulcerated mass covered by a tan colored, friable plaque on the left humeral-pectoral scutes of the plastron. (b) Case 2: Adult female *Myuchelys bellii* with a debried, 5.5×4 cm irregularly shaped, ulcerated mass with infiltrative margins on the right humeral-pectoral scutes of the plastron. A smooth, linear depression of the caudal plastron (arrowhead) is interpreted as a healed fracture. Inset: Prior to debridement, the mass was soft, exophytic, and pale yellow.

bone loss. The coelomic wall was spared; however, the hilar pulmonary parenchyma was bilaterally firm and consolidated. A 1-cm-diameter mature granuloma adhered to the liver capsule and stomach wall was interpreted as an incidental finding.

Tumor histopathology was characteristic of SCC, comprising neoplastic polygonal cells arranged into infiltrative nests and anastomosing trabeculae with scattered areas of dyskeratosis and necrosis, and frequent keratin pearls (Fig. 2). Neoplastic cells were characterized by distinct cell margins bridged by desmosomes, abundant brightly eosinophilic, slightly glassy cytoplasm, and round to oval-shaped nuclei with marginated chromatin and prominent nucleoli. Pleomorphism was moderate with occasional karyomegaly and frequent mitotic figures.

Case 2, also an adult female (weight=1.5 kg; straight carapace length=232 mm) caught in a cathedral trap during routine population surveillance operations in December 2018, was from Muluerindie, NSW (31°4'42"S, 151°27'53"E) in the mid-reaches of the

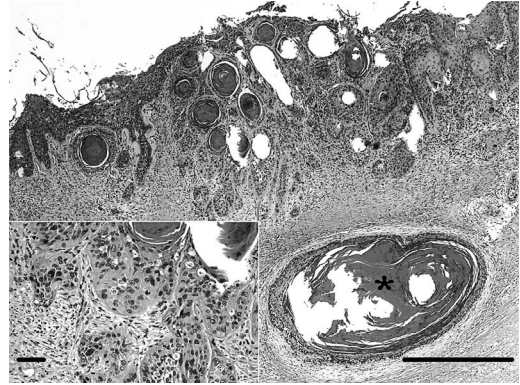


FIGURE 2. Adult female Bell's turtle (*Myuchelys bellii*) captured from the Macdonald River in New South Wales, Australia. Histologic section of the skin with a squamous cell carcinoma arising from the epidermis. Keratin pearls (asterisk), smaller nests, and trabeculae infiltrate the dermis, accompanied by a chronic inflammatory infiltrate. Bar=500  $\mu$ m. H&E. Inset: High magnification of infiltrating neoplastic squamous cells, characterized by marked variation in cell and nucleus size. Bar=50  $\mu$ m. H&E.

Macdonald River. The turtle was in good body condition. Arising from the right humeral-pectoral scutes of the plastron was an irregularly shaped 5.5×4 cm, soft, depressed, pale yellow mass (Fig. 1b). Biopsy findings were consistent with squamous cell carcinoma.

The turtle remained in care for 2 wk before being euthanized using IV Lethobarb Euthanasia Injection due to a poor prognosis for recovery. On necropsy, tumor margins were poorly defined with infiltration of the surrounding bone and axillary subcutaneous soft tissue. A smooth, linear depression in the caudal plastron, at the level of the femoral-anal scute junction, was interpreted as a mature scar indicative of prior trauma (Fig. 1b). The liver contained multiple 9–12-mm diameter, multifocal, nodular yellow lesions.

Histologic findings were similar to those present in case 1. The mass extended from the ulcerated epidermal surface into the underlying dermis, panniculus, and skeletal muscle. The mass was comprised of pleomorphic epithelial cells with brisk mitotic activity arranged into infiltrative nests and cords interspersed with prominent keratin pearls.

The mass was locally aggressive; however, there was no evidence of vascular invasion or metastasis. Sections of the liver were characterized by well-demarcated areas of nodular hyperplasia, consistent with necropsy changes and considered an incidental finding.

In both cases, the tumors were large and locally aggressive, replacing a significant portion of the plastron and effacing surrounding bony and soft tissue structures. Neither turtle was a candidate for surgical treatment, and ongoing tumor progression was considered likely, with implications for shell stability, mobility, and systemic health.

Reports of neoplasia in free-ranging turtles are rare. We report SCC of the plastron in two free-ranging adult, female turtles from the same river system. An additional mature female Bell's turtle from the same river system has been recorded with similar scute lesions; however, tissues were not available for analysis. Over 2,000 individual Bell's turtles have been captured and handled as part of population monitoring efforts over a period of 15 yr (Chessman 2015; Fielder et al. 2015). The first report of such lesions was from case 1 in 2015 and might represent an emerging disease in this population.

The tumors described herein might simply reflect spontaneous carcinogenesis in ageing wild turtles. Nevertheless, a common etiologic pathway warrants consideration. Tumorigenesis of SCC might occur spontaneously or be induced by ultraviolet light exposure, environmental carcinogens, genetic factors, oncogenic viruses, or chronic tissue injury (von Deetzen et al. 2012; Hill et al. 2016).

Little is known about viruses of freshwater turtles; however, recent population crashes of the congeneric Bellinger River snapping turtle (*Myuchelys georgesi*) highlights the role of infectious disease in wildlife mortalities (Zhang et al. 2018). There was no evidence of a viral dermatopathy in either turtle, and there are no reports of freshwater turtle papillomas, which might implicate neoplastic transformation to SCC. Ancillary testing to exclude the possibility of an oncogenic virus could be warranted if additional cases are identified.

Solar damage is considered an unlikely predisposing factor, given the ventral location of the masses. Chronic tissue injury is likewise considered unlikely, based on necropsy findings. There was an unusual defect to the plastron of the second turtle which might indicate previous trauma; however, the plastron of the first animal appeared unscarred. Shell damage, wounds, limb amputations, and undescribed eye lesions have been recorded previously in this species without associated neoplasia (Chessman 2015; Fielder et al. 2015).

The river from which these two turtles were captured is generally in poor condition (Murray-Darling Basin Authority 2012), and it flows through agricultural lands that can expose turtles to harmful runoff. The threatened status of this species warrants ongoing monitoring with particular attention to neoplastic lesions, which could implicate predisposing infectious, toxic, or genetic factors.

Turtle capture and handling procedures were authorized by the New South Wales Department of Primary Industry Scientific Collection permit P17\_0062, New South Wales Department of Planning, Industry, and Environment Scientific license SL101876, and University of New England Animal Ethics Committee approval AEC17-110. Turtles were captured as part of a monitoring program that has been assisted by the New South Wales Government through its Environmental Trust.

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