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Short Note
Endoscopic Sexing of Juvenile Softshell Turtles, *Amyda cartilaginea*

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Whilst the sex of offspring in most vertebrates depends on a genotypic sex determination (GSD, sex determined by maternal and paternal genes and/or sex chromosomes), temperature dependent sex determination (TSD) seems to be the most common form of sex determination in turtles. Most turtles have a prolonged juvenile phase and take many years or even decades before they reach maturity and can be sexed externally. However, in contrast the non-lethal sexing of juveniles is increasingly important in ecological studies and in conservation and recovery projects of threatened turtle species. Endoscopy is currently the only 100% accurate non-lethal method available to sex juvenile turtles that do not show external sexual dimorphism[1,2,3]. Until now juvenile softshell turtles (family Trionychidae) have never been sexed with this method, probably because the currently accepted wisdom is that softshell turtles have GSD and not TSD.

Recently, we found a male-biased sex ratio in the captive breeding program for the critically endangered Siamese Narrow-headed Softshell Turtle, *Chitra chitra* Nutphand, 1986, at Kanchanaburi Inland Fisheries Development Center (KIFDC), Thailand[4]. Obviously, this is not an ideal situation or a basis for a recovery program of a critically endangered species, where a balanced or even a female biased sex ratio would be preferable[5]. For that reason it is important to evaluate endoscopy as a non-lethal sexing technique for juvenile softshell turtles, but preferably not in a critically endangered species. To this aim, we sexed four captive-bred juvenile *Amyda cartilaginea*, a common softshell turtle species in SE-Asia, at KIFDC using endoscopic imaging.

Body mass and standard morphological parameters, including carapace length (CL, to the nearest mm), was measured for all four turtles (Table 1). The turtles were anesthetized by intravenous injection (carpal sinus) of ketamine hydrochloride (20 mg/kg body mass). Optimum anaesthetic depth was achieved after about 15 minutes. Both hind legs were pulled backwards and tied together. The left inguinal pocket and neighbouring skin, shell, and leg were scrubbed with antiseptic soap and povidone-iodine. A 2.7

TABLE 1. Size and sex of juvenile *Amyda cartilaginea*.

ID	Body mass (g)	Carapace length (mm)	Sex
1	704	181	M
2	462	162	M
3	460	162	M
4	349	147	F

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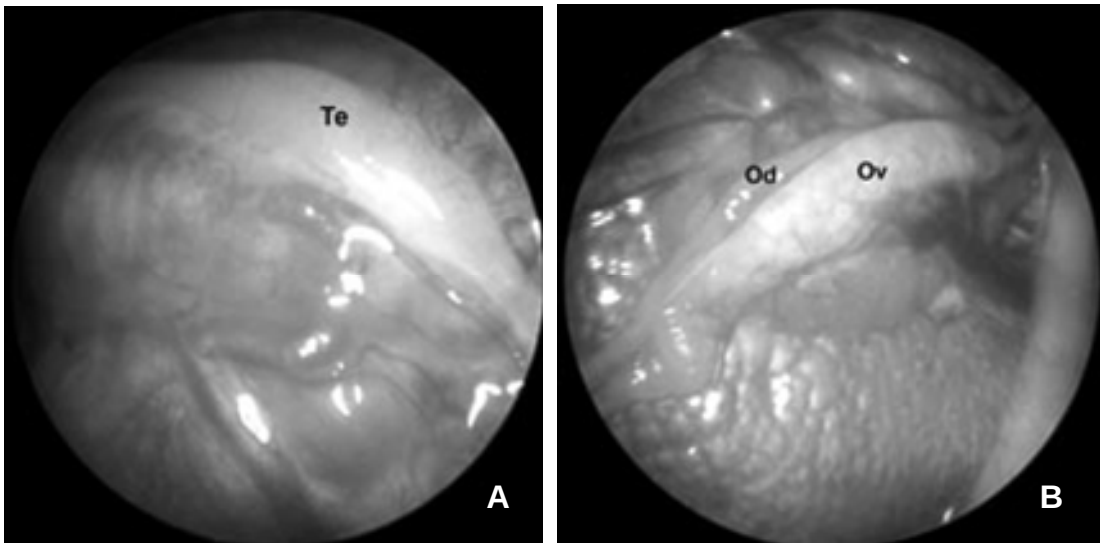

FIGURE 1. The (A) Testis (Te) of juvenile *A. cartilaginea* of 162 mm carapace length (#3 in Table 1), and (B) the ovary (Ov) and oviduct (Od) of juvenile *A. cartilaginea* of 147 mm carapace length (#4 in Table 1).

mm diameter rigid Storz Hopkins endoscope was inserted into the abdominal cavity through a stab incision in the lower anterior part of the inguinal pocket. Note that the abdominal cavity was not insufflated in order to minimize post-operative buoyancy problems. A Storz cold-light fountain 482B was used as light source. A digital camera with macro function (Nikon Coolpix 995) was used for photo documentation. The surgical procedure took about 10 minutes. After completion of endoscopy the skin wound was sutured using two stitches of 4/0 vicryl. The turtles recovered from anaesthesia 1-2 hours after the surgical procedure. The turtles were kept overnight individually in dry buckets after the endoscopic examination and the following day their diving behaviour was observed at release into their tanks.

The gonads of juvenile *A. cartilaginea* are thin and elongate and fixed to the dorsal part of the body cavity, very close to the kidneys, adrenal glands and lungs. Despite being attached to the dorsal coelomic wall by various membranes, gonads and reproductive tracts move and change their position easily relative to the kidneys, adrenals and lungs. The gonads also move with the breathing movements of the lung and can be unambiguously identified as testes or ovaries due to their external appearance:

The testes (Fig. 1A) are bound to the kidneys by the mesorchium, are elongate, roundish in cross section and look like elongate sausages with pointed ends. They are whitish-yellow and have a smooth surface (teca) containing a net of blood vessels. At a close distance it is possible to see the small, yellowish tubuli seminiferi. The epididymes and the vas deferens are transparent and barely visible.

The ovaries (Fig. 1B) are attached to the dorsal wall of the coelomic cavity or to a membrane that separates them from the lungs (which are adherent to the carapace). They are elongate, transparent, slightly convoluted caudally and contain many yellow and whitish follicles that appear like flat discs. Follicles are particularly located in the latero-cranial parts of the ovaries, giving the surface of the ovaries a bumpy appearance. No blood vessels are apparent on the surface. The

oviduct is a straight, flat, transparent-whitish band positioned lateral and is parallel to the ovary and extends further cranially than the ovary.

Of the four examined captive raised juveniles, three were males and one was a female (Table 1). All of the four juvenile *A. cartilaginea* continued to show perfect health and growth rates over the five months following the endoscopic examination, and indeed no negative impact of the procedure could be detected. Although any surgical procedure poses a certain risk, with an experienced operator risks are minimal and the endoscopic sexing of juvenile turtles is accurate and straight forward.

Endoscopic sexing of head started juveniles has already been performed in captive breeding and conservation programs of a number of critically endangered turtles, including *Geochelone yniphora*[6], *Erymnochelys madagascariensis*[2], *Batagur baska* and *Callagur borneoensis*[7], which are all listed under "the world's top 25 most endangered turtles" (www.conservation.org /xp/news/press_releases/2003/turtle_kit/25TurtProfiles0503.pdf) together with *Chitra chitra*. *Chitra chitra* has nearly disappeared in the wild[8] and captive breeding is essential for its long-term conservation[9]. In conservation terms, the advantage of knowing offspring sex ratios in a captive breeding and species recovery program of a critically endangered species, such as *C. chitra,* far outweighs any residual risk to individuals posed by endoscopy.

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