IUCN-SSC Crocodile Specialist Group
Fritz Huchzermeyer Veterinary Science Student Research Assistance Scheme
Project Result/Update

Project title: Conservation management must be wary of inadvertent parasite introductions: the curious case of the endemic freshwater crocodile *Crocodylus mindorensis* as incidental host of marine fish acanthocephalan

Researcher: Arvin Jet B. Marcaida (The Graduate School, University of Santo Tomas) **Adviser:** Arvin C. Diesmos (Herpetologist, National Museum of the Philippines) **Co-Adviser:** Jonathan Carlo A. Briones and Mae Lowe L. Diesmos (Research Center for the Natural and Applied Sciences, University of Santo Tomas)

Introduction

There are two species of crocodiles in the Philippines. The *Crocodylus mindorensis*, also known as the Philippine Crocodile, is a small crocodile endemic to the Philippines which lives in freshwater lakes, rivers, creeks, ponds and marshes. It is recognized as the most threatened species in the world and it is currently under protection (van Weerd & van der Ploeg, 2003). The other species is the Saltwater Crocodile *Crocodylus porosus*, which is widely distributed and is commonly bred in farms. The quality and size of its skin makes the price and demand high in the market. However there is limited number of individuals available for skin market globally compared to other crocodilian species. It can be found in brackish or estuarine water in the Philippines and other parts of Asia all the way to Australia. They are much larger in size and are more dangerous compared with *C. mindorensis* (Ross, 1998; Steel, 1989 Studies on crocodile parasites were widely scattered and published in inaccessible journals back in the early 1800s. Publication on reptilian parasites included crocodilians; however, not all species of crocodilians were included nor all parasites species were reported (Tellez, 2013). Most publications on crocodile-parasite in the Philippines were on the species of *Crocodylus porosus* by Tubangui and Masilungan (1936) and Machida, Araki, Regoniel, Pontillas, and Kurata (1992). According to Tellez (2013), there are no current records or documentation of natural parasitic infections in *Crocodylus mindorensis*. Junker and Boomker (2006) discovered a species of pentatosmid worm (*Alofia ginae*) in Philippine Crocodile but this may be highly unlikely. This is due to lack of research on this species rather than their lack of parasitism. This study was designed to study the prevalence of endohelminthes parasites infection in captive crocodiles, describe the morphology of parasites and assess the contribution of conservation management to infection prevalence.

Method

Parasite detection and isolation

A total of 10 individuals of the *Crocodylus mindorensis* from Palawan Wildlife Rescue and Conservation Center (PWRCC) in Puerto Princesa, Palawan, were surveyed in the months of June 2016. Being critically endangered, endorsement letter and permit were taken from the Biodiversity Management Bureau (BMB) of the Department of Environment and Natural Resources (DENR) and Palawan Council for Sustainable Development (PCSD) in order to conduct the study. Gastric lavage was done by the experts or collaborators to get samples of stomach contents and parasites according to protocol enumerated by Fitzgerald (1989) since it is the safest way to acquire parasites from live sample hosts. In addition to this, 14 individuals from another species of crocodile, the *Crocodylus porosus*, were surveyed from the carcass which was to undergo tanning for production of leather in Coral Agri-Venture Farm in Teresa, Rizal. These crocodiles sampled from this farm have a range of 181cm – 227cm Snout-Vent Length (SVL). Gastric lavage was not done to *Crocodylus porosus* since they are too big to handle and it will require more manpower. Therefore, the gastro-intestinal tract and other organs of the carcass of the Saltwater Crocodiles were examined and dissected. Organs examined include esophagus, trachea, lungs, liver, stomach, and intestines (Huchzermeyer, 2003; Tellez, 2013). They were placed inside a large container and were submerged in saline water. Vigorous shaking was done to release the parasites that may be attached on the linings of the organs. They were then left undisturbed for 30 minutes to allow the particles to settle at the bottom. After which, the organs were removed from the container and the particles were transferred in PETA bottles filled with mix 10 mL formaldehyde, 10 mL acetic acid, 200 mL 70 % ethanol (AFA) which serves as fixative. Parasites were thoroughly inspected with the use of dissecting microscope (Optika SZM-1) in the laboratory. However, this method resulted to negative results making this study focused only on *Crocodylus mindorensis*.

Processing of parasites

Collected parasites will then be preserved in 70% ethanol for long term storage. It will be viewed under disecting microscope for initial identification. Each parasite will be mounted on a glass slide. It will undergo a process

of staining using Heidenhain's Iron Hematoxylin (a mixture of 5mL of 90% EtOH and 5mL stock solution in 90mL distilled H2O) as staining agent and ammonium iron (III) sulfate dodecahydrate as mordant or destaining solution (2g of mordant lavender crystals mixed with 100mL distilled H2O). In between it will be wash in running water. After which it will be dehydrated in a series of gradient alcohol (95% and 100%) then will be bath in xylene solution. Lastly it will be mounted using Canada balsam. Specimens used for Scanning Electron Microscope (JEOL JCM-6000PLUS NeoScope Benchtop) were fixed in 70% ethanol, and were dehydrated in series of ethanol. Critical point drying was done using hexamethyldisilazane (HMDS) as standard procedure. Voucher specimens for DNA Analysis were stored in micro-centrifuge tube with 70% ethanol for future analysis.

Morphology identification of parasites

Parasite illustrations were done with the help of a drawing tube attached on a light microscope (Olympus CX21). Morphometric measurement of parasites was taken in millimeters (mm). Species Identification was accomplished using taxonomic keys of Amin, (1987) (acanthocephalan), Khalil et al., 1994 (cestode), Gibson et al., 2002 (trematode), Junker, 2002 (pentastomes), Anderson et al., 2009 (nematode). The checklist of Tellez (2013) served as baseline information for possible presence of parasite types found in specific species of crocodile. Sexual dimorphism for male and female was determined.

Parasitism and sexual dimorphism analysis

Parasites found in infected crocodiles were quantified. Then the prevalence, mean abundance, and mean intensity were computed with the following formula (Bush et al., 1997; Ro´zsa et al., 2000). It is recommended to use mean intensity and mean abundance to characterize samples of hosts. Statistical analysis includes determining the sexual dimorphism of the species of parasites. T-test (parametric) or Mann-Whitney (non-parametric) test were computed using PAST statistical tool software to compare the mean of the morphometric of both the male and female parasites to determine the sexual dimorphism.

Results

Parasitism in Philippine Crocodile

Of the 10 individuals of *Crocodylus mindorensis* examined, 3 or 30% in which 2 females and 1 male harbored at least one species of acanthocephalan parasite. A total of 68 adult specimens of acanthocephalan worms comprising of 41% males (n=28) and 58% females (n=40) were collected from the stomach of Philippine crocodile using gastric lavage. The infected hosts harbored 14 to 24 parasites (Mean: 23.33 SD: ± 10.06) with mean abundance value of 6.8 and mean intensity value of 22.67. This study also tried to sample from *Crocodylus porosus*, but unfortunately it gave negative result or there were no parasites found.

Taxonomy and identification of parasite

Parasites found was a species of acanthocephalan, *Neorhadinorhynchus nudus* (Harada, 1938) Yamaguti, 1939. Sexual dimorphism suggest that males with total length mean of 4.973 (± 1.013) are slightly larger than females with total length mean of 4.585 (± 1.064); however, this was confirmed using statistical tools such as t-Test and Mann Whitney test that females have wider trunk ($p=0.000126<0.05$) compared to males. This study provides the first record of parasite found in *Crocodylus mindorensis* and the first acanthocephalan belonging to the Family Cavisomidae found among crocodylians since only Family Polyacanthorhynchidae, Rhadinorhynchidae, and Polymorphidae were only recorded (Tellez, 2013). Only the genus of *Cavisoma* and *Flisoma* were recorded in the Philippines, making the genus *Neorhadinorhynchus* a new locality record to the Philippines (Velasquez, 1994; Arthur et al., 1995; Weave & Smales, 2013). Among these, *Neorhadinorhynchus* is the closest to resemble the species being described in this study because based on the key to genera of the Cavisomidae Meyer, 1932 provided by Bursey et al. (2006), the testes are away from posterior extremity and is in contact to each other while its proboscis is cylindrical rather than long and slender or claviform or spherical. In this present study, the species exhibits the characters of the *Neorhadinorhynchus nudus* according to Harada (1938) and Yamaguti (1939) and is distinguished from other 7 species considering the key provided by Amin and Nahhas (1994) by having 12-14 longitudinal hook rows with 24-25 hooks per row compared to 15-16, 14-15, 17, or 9-10 hooks per row of *N. macrospinosus*, *N. atlanticus*, *N. myctophumi* and *N. basrahiensis* respectively (Amin & Nahhas, 1994; Hassanine, 2006; Smales et al., 2016). Considerably the species being described is much shorter compared to the *N. nudus* described by Hassanine (2006) but it is almost short as the *N. nudus* collected by Amin and Nahhas (1994) which is identical to the described specimen of Harada (1938).

Geological distribution of parasite

The genus *Neorhadinorhychus* are distributed from Atlantic Ocean, down the eastern coast of Africa, and across the Indo-Pacific to Japan, Taiwan, and the Fiji Islands, Indian Ocean, as far as the Pacific Ocean and the most recently is from Iraqi marine waters (Golvan, 1969; Amin & Nahhas, 1994; Hassanine, 2006; Amin & Van Ha, 2011; Smales et al., 2015). Among the species, *N. nudus* have been recorded from Taiwan, Fiji Islands, Red Sea, and across the Indian Ocean. It is widely distributed mainly because it can utilize at least two families of fishes, Carangidae and Scombridae, as its host (Yamaguti, 1939; Amin & Nahhas, 1994; Hassanine, 2006; Smales et al., 2016).

Management of captive crocodiles

The presence of gastro intestinal helminth in captive animals in this study may be attributed by feeding or diet, housing or enclosure, and proximity or overcrowding. Acanthocephalan, having indirect life cycle, must have at least one intermediate (insects (Coleoptera and Orthoptera), isopods, centipede, millipedes, decapods, and crustaceans) host and definitive host (vertebrate) which might serve as food items for both wild and captive animals. Since *Neorhadinorhynchus nudus* is a parasite of fish, perhaps crocodiles acquired the infestation after feeding on infected fish caught from the wild. Considering this might suggest that crocodiles are probably accidental host. This is most likely to happen since the diet of the crocodiles in the Palawan Wildlife Rescue and Conservation Center (PWRCC) includes wild caught marine fishes (I.e. tuna and needlefish) where tuna is confirmed to be one of the definitive host of N. nudus.

The captivity or husbandry of both wild and farm bred animals in small enclosures and close proximity to each other or to other species of animals contribute to the prevalence and transmission of parasites through environmental contamination in water or soil (Mir et al., 2016). There is a chance of prevalence of parasites with the crocodile samples in the semi wild enclosure (with vegetation and pond waters with algal bloom) in the PWRCC compared to other type of enclosure in the same farm. This was supported by Mir et al. (2016) that well maintained and closed enclosures reduces the chance for any intermediate and definitive host to enter. With captivity, the change in environment and living condition increase the susceptibility of animals to parasitic infections (Goossensa et al., 2005; Singh et al., 2006). This means that keeping them in enclosure also makes them in close proximity along with other animals, where proximity increases the chance of transmission of diseases or parasites to uninfected animals (Moudgil & Singlia, 2013; Adeniyi et al., 2015). One of the conditions of stress in captivity or husbandry is overcrowding which can compromise the immune system and cause diseases or even mortality in hatchlings (Foggin, 1987; Huchzermeyer et al., 1994; Huchzermeyer, 2003). This is further explained by Morgan and Tromborg (2007) where they emphasize that small confinement or enclosure prevent captive animals to respond to other stress stimuli and reducing or eliminating control over exposure to other environmental variables such as light, sound, temperature, and food. This was observed with the samples obtained semi-wild enclosures in PWRCC than the well maintained and close enclosure where there was no presence of parasites. Tellez (2013) concluded that crocodiles and alligators seem to succumb to parasites in poor or stressful environments.

The parasitism of *Crocodylus mindorensis* by a marine parasite is an interesting and a rare situation in that this is a freshwater animal, natural host-parasite relationships develop evolutionarily selecting for more resistant hosts and less virulent parasites. If by any chance *C. mindorensis* is resistant or only moderately susceptible to infection, it is suggested that environmental stress or anthropogenic factors may have predisposed them to intense infection by this acanthocephalan. In the same case with the infection of a marine monogenean, *Neobenedenia melleni* to freshwater *Oreochromis mossambicus* in Hawaii where they conclude that environmental stressors can affect an animal's defenses and resistance to disease and parasites (Kaneko et al., 1988). It has been evident also that certain parasites can survive in marine and freshwater and they tend to develop differently, one study showed that the acanthocephalan, *Pomphorhynchus laevis*, grows but does not become sexually mature and poorly develop in freshwater host compared when it is in a marine host (Kennedy, 1984).

Conclusion

This study has observed the presence of parasite in the endemic species of crocodile, *Crocodylus mindorensis*. Through descriptions from other study and taxonomic keys of morphological characteristics along with measurements identified the acanthocephalan parasite infecting the Philippine Crocodile to be *Neorhadinorhynchus nudus*. However, further verification from experts and DNA analysis should confirm the status of this species. Sexual dimorphism of this parasite is not distinct however the female's width is significantly different. This is the first record of this parasite found in this species of crocodile and the species and genus of this acanthocephalan is of new locality record in the Philippines. This study suggests that *Crocodylus mindorensis* act as incidental host since acanthocephalan from this family are known parasite of fish. This will be proven if the life cycle and intermediate or definitive host of this

parasite will be traced in its environment and conduct parasite survey of fish from the sampling site. This study contributes to our understanding of the poorly known parasites of the Philippine Crocodile. The high prevalence at 30%, mean abundance at 6.8, and mean intensity at 22.67 of *Neorhadinorhynchus nudus* in *Crocodylus mindorensis* in Palawan Wildlife Rescue and Conservation Center (PWRCC) may be related to their diet, enclosure, and their proximity to each other in captivity. Further survey and investigation must be done for both species of crocodiles, especially for *Crocodylus porosus*, also in other areas including wild populations. Even though there may be a high prevalence of parasitism of crocodiles in this study, there was no reported mortality and clinical signs due to parasitic infection. Overall management of captive animals including nutrition or diet, sanitation, deworming, and reducing stress stimuli must be follow and should not be neglected. However, further studies should be conducted about the relationship of stress in captivity to the parasitic infection. Overall, farmers or breeders of crocodiles must be wary of inadvertent parasite introductions which will have an implication to the conservation management of the Philippine Crocodile and Saltwater Crocodile.

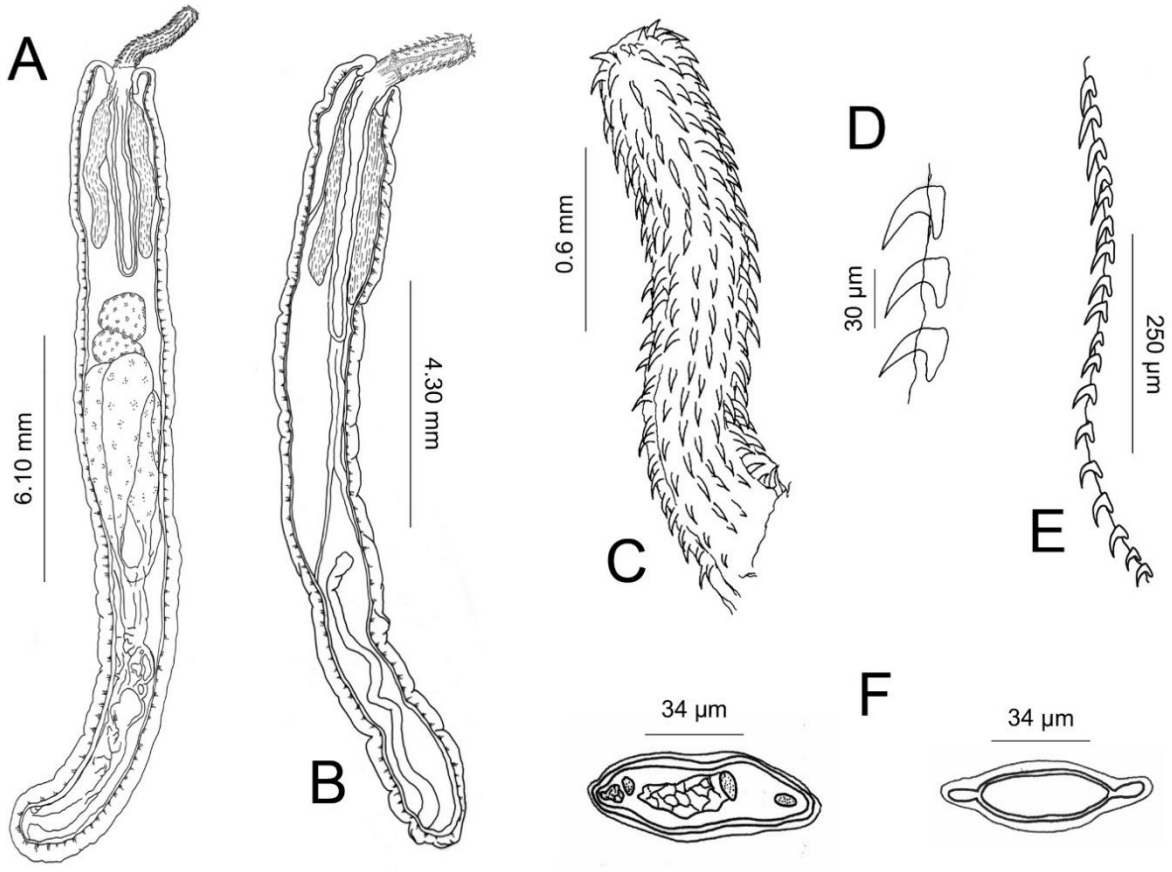


Figure 5. *Neorhadinorhynchus nudus* in *Crocodylus mindorensis.* **A.** Adult Male **B.** Adult Female **C.** Proboscis of male in A. **D.** Hook and Hook roots **E.** Hook roots in a single longitudinal row **F.** Mature and immature eggs showing hemiechinate characteristics.