



## Short Communication

## The curious case of the endemic freshwater crocodile *Crocodylus mindorensis* as incidental host of marine fish acanthocephalan

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## ABSTRACT

We performed the first host-parasite survey of the Philippine crocodile, *Crocodylus mindorensis*, a critically endangered species for which ecological information is lacking. We collected by gastric lavage samples of the stomach contents of crocodiles ( $n = 10$ ) residing at the Palawan Wildlife Rescue and Conservation Center in Puerto Princesa, Palawan, Philippines. The only parasite detected was an acanthocephalan, which was identified as *Neorhadiorhynchus nudus* ( $n = 68$ ), a parasite typically found in the marine fish species consumed by three crocodile individuals. Given the known hosts of *N. nudus*, its parasitism of *C. mindorensis* in captivity is likely established by consumption of marine fish. Our findings have implications for the conservation management of *C. mindorensis*, particularly in terms of preventing introduction of parasites that could lead to development of infectious disease or alter the fitness of captive animals.

The Philippine crocodile *Crocodylus mindorensis* Schmidt, 1935, is a small crocodile endemic to the Philippines that typically inhabits freshwater lakes, rivers, creeks, ponds, and marshes [1]. It is classified as the most threatened or critically endangered species in the world by the International Union for Conservation of Nature and is protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Appendix I) and by Philippines law (Wildlife Act of 2004) [1]. There are no records of natural parasitic infections of *Crocodylus mindorensis*, likely due to a lack of research [2].

Crocodiles and parasites have a long co-evolutionary history, which has resulted in high host-parasite specificity and adaptation [2]. The relationships between crocodiles and their parasites can be disturbed by human activities, such as pollution and ecosystem disruption, and may influence host health and population regulation [3].

We performed the first parasitic survey in *C. mindorensis* in captivity to determine the prevalence of gastric parasite infection in captive crocodiles, assess the morphology of the parasites obtained, and

evaluate the contribution of conservation management to the prevalence of parasite infection.

We examined a total of 10 individuals from Palawan Wildlife Rescue and Conservation Center (PWRCC) (formerly known as the Crocodile Farm Institute) in Puerto Princesa, Palawan, in June 2016 with the permission of the Biodiversity Management Bureau of the Department of Environment and Natural Resources and Palawan Council for Sustainable Development. Both farm-bred and wild-caught crocodiles were sampled; however, we were unable to discriminate between the two cohorts. The diet of the crocodiles in the PWRCC includes wild-caught marine fishes of the family Scombridae (Tulingan), such as *Auxis thazard*, *Euthynnus affinis*, *Rastrilliger kanagurta*, and *Thunnus albacares*.

We performed gastric lavage following a standard protocol [4] to obtain stomach contents, because dissection for parasitological examination was not allowed as *C. mindorensis* is a critically endangered species. The stomach contents were examined for parasites under a dissecting microscope. Only acanthocephalans were isolated from the

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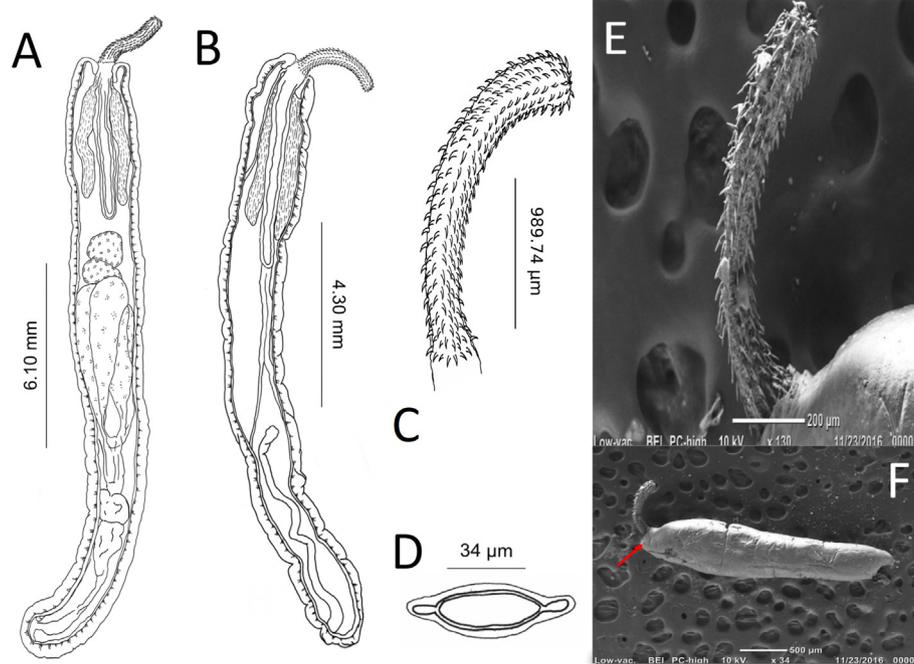
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**Fig. 1.** *Neorhadiorhynchus nudus* isolated from *Crocodylus mindorensis*. A: adult male B: adult female, C: female proboscis, D: egg, E: SEM of fully everted proboscis. F: entire trunk showing the cone (posterior end broken).

samples. The parasites were stained using Heidenhain's Iron Hematoxylin and mounted on a glass slide [5]. Unmounted parasites were viewed under a scanning electron microscope (JEOL JCM-6000PLUS NeoScope Benchtop) using hexamethyldisilazane and a standard procedure. Illustrations of the parasite were created using a drawing tube attached to a light microscope (Olympus CX21). Standard morphometric measurements of acanthocephalans were performed and recorded in millimeters (mm). The acanthocephalans were identified using the taxonomic keys of Amin [6]. Mounted specimens were deposited as voucher specimens in the Parasite Section of the Zoological Reference Collection of the University of Santo Tomas, Manila, Philippines (UST-ZRC-P Accession No. CMP001). We computed the prevalence, mean abundance, and mean intensity of acanthocephalans using the formulae of Bush et al. [7] and Rózsa et al. [8].

Of the 10 individual crocodiles examined, 3 (2 females and 1 male) harbored one species of acanthocephalan parasite. The infected crocodiles had 14 to 34 parasites (mean abundance 6.8; mean intensity, 22.67). In total, we collected 68 acanthocephalans (28 males and 40 females), all of which were identified as *Neorhadiorhynchus nudus* (Harada, 1938) Yamaguti, 1939 (Fig. 1) based on the presence of paired testes in contact with each other and distant from the posterior extremity, a cylindrical proboscis [9], and a total of 27 hooks per row (compared to 15–16, 14–15, 17, or 9–10 hooks per row in *N. macrospinus*, *N. atlanticus*, *N. myctophumi*, and *N. basrahiensis*, respectively) [10–12]. The most closely related species, *N. atypicalis*, has 27 hooks per row, which overlaps with range found in *N. nudus* (10–27) [13,14]. However, the acanthocephalans identified in this study had dorsal hooks that were longer and slender than their ventral hooks ([in  $\mu\text{m}$ ] anterior hooks, dorsal  $35 \times 7$  and ventral  $28 \times 10$ ; middle largest hooks, dorsal  $50 \times 11$  and ventral  $36 \times 15$ ; posterior hooks, dorsal  $36 \times 9$  and ventral  $32 \times 14$ ), similar to *N. nudus* but not to *N. atypicalis* [14,15]. *Neorhadiorhynchus nudus* also differs from the other species in terms of the absence of spines and sclerotized plaques on the trunk [11,16,17]. The morphology of the *N. nudus* identified is largely in agreement with that of Hassanine [11] and Li et al. [18] (Table 1), but the dimensions of the parasites isolated in this study were markedly smaller. Although the parasites identified had a shorter proboscis and hook length than reported previously, this species displays far greater

intraspecific variation in proboscis length and hook number than do other acanthocephalan species [13].

This is to the best of our knowledge the first attempt to determine the parasite fauna of the gastrointestinal tract of *C. mindorensis* and the first report of *N. nudus* in the Philippines [19]. All species of *Neorhadiorhynchus* have to date been recorded only in squid and marine fish (Perciformes: Carangidae and Scombridae), which are widely distributed throughout the South China Sea [12,16]. Therefore, we infer that captive *C. mindorensis* likely act as an accidental host of *N. nudus*, which they acquire by consuming wild-caught marine tuna.

Cases of crocodylians becoming accidental hosts of parasites are uncommon but not unknown. For example, *Gorgorhynchoides cystacanth* was isolated from gastric lavage samples from *Crocodylus acutus* from Mexico which had been fed infected fish [20]. Because the stomach is an atypical site of acanthocephalan infection, it must be careful to decide if they infect crocodiles by diving their proboscis into the stomach wall or not. In addition, a necropsy for parasitological examination of *C. mindorensis* that died from natural causes is needed to confirm parasitism by *N. nudus*.

In this study, we speculated that *Neorhadiorhynchus nudus* is a possible or probable parasite of *C. mindorensis* even though marine tuna is the final host of this acanthocephalan and is the preferred food for captive crocodiles (PWRCC, pers. comm.) to prevent infection with other common pathogens (e.g., *Trichinella*) from farm animals. However, feeding *C. mindorensis* with marine fish also comes with a risk of infection of virulent parasite of crocodylians, such as *Sebekia* spp.. We report here a rare case of introduction of a parasite to animals in captivity, which was caused by conservation-related activities. Further study is needed to determine the evidence of parasitism.

#### Declaration of interest

None.

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**Table 1**  
Comparison of measurements between *Neorhadiorhynchus nudus* in this present study to descriptions of the species *Diplosentis nudus* of Hassanine (2006) and to *Neorhadiorhynchus nudus* of Li et al. (2018). Measurements in mm.

Sex	This study			Hassanine (2006)			Li et al. (2018)		
	Male (n = 10)	Female (n = 16)	Male (n = 30)	Male (n = 30)	Female (n = 30)	Males (n = 23)	Females (n = 15)		
Trunk	3.68–6.25 × 0.16–0.75	2.25–6.5 × 0.5–0.98	7.42–11.13 × 1.10–1.64	7.28–10.91 × 1.29–1.93	5.60–8.40 × 0.83–1.25	5.60–8.40 × 0.83–1.25	5.70–9.10 × 0.99–1.58		
Proboscis	0.64–0.97 × 0.10–0.15	0.60–0.98 × 0.11–0.16	1.15–1.71 × 0.16–0.24	1.36–1.78 × 0.17–0.21	0.60–1.52 × 0.14–0.17	0.60–1.52 × 0.14–0.17	0.64–1.71 × 0.15–0.23		
NHR × NHRP	14 × 27	14 × 27	14 × 24–25	14 × 24–25	14 × 24–25	13–14 × 10–27	13–14 × 10–27		
Hook	0.026–0.047 × 0.008–0.012	0.028–0.050 × 0.008–0.012	0.03–0.07;	0.03–0.07;	0.03–0.07;	0.026–0.060 × 0.008–0.045	0.028–0.060 × 0.008–0.020		
Neck	0.10–0.15 × 0.10–0.20	0.09 × 0.08	0.18–0.27	0.18–0.27	0.22–0.32	0.11–0.35	0.12–0.30		
Proboscis Receptacle	1.19–2.04 × 0.13–0.17	1.15–2.25 × 0.12–0.20	1.98–2.93 × 0.23–0.34	1.98–2.93 × 0.23–0.34	1.99–2.99 × 0.23–0.31	1.75–2.78 × 0.20–0.43	2.10–3.15 × 0.24–0.38		
Lemnisci	1.35–2.22 × 0.08–0.14	1.48–2.51 × 0.06–0.15	2.10–3.06 × 0.08–0.12	2.10–3.06 × 0.08–0.12	2.51–3.61 × 0.25–0.36	1.06–2.78	1.18–3.00		
Cement glands	0.70–1.90 × 0.27–0.53	—	0.98–1.45 × 0.25–0.39;	0.98–1.45 × 0.25–0.39;	—	0.74–2.13	—		
Anterior Testis	0.21–0.60 × 0.20–0.58	—	0.99–1.46 × 0.56–0.84	0.99–1.46 × 0.56–0.84	—	—	—		
Posterior Testis	0.20–0.68 × 0.20–0.39	—	0.92–1.35 × 0.58–0.87	0.92–1.35 × 0.58–0.87	—	—	—		
Uterus	—	1.38–3.75 × 0.08–0.20	—	—	2.96–4.18	—	2.20–3.76		

Abbreviations: NHR, number of longitudinal rows of proboscis hooks; NHRP, number of hooks per row.

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**References**

- [1] M. Van Weerd, J. Van der Ploeg, A new future for the Philippine crocodile, *Crocodylus mindorensis*, Sylvatrop, Tech. J. Philippine Ecosyst. Nat. Res. 13 (2003) 31–50.
- [2] M. Tellez, A Checklist of Host-Parasite Interactions of the Order Crocodylia, vol. 136, University of California Press, Los Angeles, California, U.S.A, 2013.
- [3] D.J. Marcogliese, Parasites of the superorganism: are they indicators of ecosystem health? Int. J. Parasitol. 35 (2005) 705–716, <https://doi.org/10.1016/j.ijpara.2005.01.015>.
- [4] L.A. Fitzgerald, An evaluation of stomach flushing techniques for crocodylians, J. Herpetol. 23 (1989) 170–172, <https://doi.org/10.2307/1564024>.
- [5] J.C. Briones, R.D.S. Papa, G.A. Cauyan, M. Urabe, The first report of three acanthocephalan parasite species isolated from Philippine fishes, Helminthologia 52 (2015) 384–389, <https://doi.org/10.1515/helmin-2015-0061>.
- [6] O.M. Amin, Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida), J. Parasitol. 73 (1987) 1216–1219, <https://doi.org/10.2307/3282307>.
- [7] A.O. Bush, K.D. Lafferty, J.M. Lotz, A.W. Shostak, Parasitology meets ecology on its own terms: Margolis et al. revisited, J. Parasitol. 83 (1997) 575–583, <https://doi.org/10.2307/3284227>.
- [8] L. Rózsa, J. Reiczig, G. Majoros, Quantifying parasites in samples of hosts, J. Parasitol. 86 (2000) 228–232 [https://doi.org/10.1645/0022-3395\(2000\)086\[0228:QPISOH\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2).
- [9] C.R. Bursey, D. Vrcibradic, F.H. Hatano, C.F.D. Rocha, New genus, new species of Acanthocephala (Echinorhynchidae) from the Brazilian frog *Hyloides phyllodes* (Anura: Leptodactylidae), J. Parasitol. 92 (2006) 353–356, <https://doi.org/10.1645/GE-3518.1>.
- [10] O.M. Amin, F.M. Nahhas, Acanthocephala of marine fishes off Fiji Islands, with descriptions of *Filisoma longementglandatus* n. sp., *Neorhadiorhynchus macrospinus* n. sp. (Cavisomidae), and gravid females of *Rhadiorhynchus johnstoni* (Rhadiorhynchidae); and keys to species of the genera *Filisoma* and *Neorhadiorhynchus*, J. Parasitol. 80 (1994) 768–774, <https://doi.org/10.2307/3283256>.
- [11] R.M.E. Hassanine, Acanthocephalans from Red Sea fishes. Family Cavisomidae Meyer, 1932: the seasonal cycle of *Diplosentis nudus* (Harada, 1938) Pichelin et Cribb, 2001 in a definitive fish host, and a comment on *Sclerocollum* Schmidt et Paperna, Acta Parasitol. 51 (2006) (1978) 123–129, <https://doi.org/10.2478/s11686-006-0019-3>.
- [12] L.R. Smales, H.A.H. Al-Hasson, K.S. Al-Niaeeem, S.A. Al-Azizz, A new species of *Neorhadiorhynchus* (Acanthocephala: Cavisomidae) from *Platax teira* (Ephippidae) from Iraqi marine waters, Trans. R. Soc. S. Aust. 140 (2016) 90–95, <https://doi.org/10.1080/03721426.2015.1115458>.
- [13] L. Li, M.T. Wayland, H.X. Chen, Y. Yang, Remarkable morphological variation in the proboscis of *Neorhadiorhynchus nudus* (Harada, 1938) (Acanthocephala: Echinorhynchida), Parasitology (2018) 1–8, <https://doi.org/10.1017/S003118201800166X>.
- [14] O.M. Amin, R.A. Heckmann, N.V. Ha, Descriptions of *Neorhadiorhynchus nudus* (Cavisomidae) and *Heterosentis paraholospinus* n.sp. (Arhythmacanthidae) (Acanthocephala) from fish along the pacific coast of Vietnam, with notes on biogeography, J. Parasitol. 104 (5) (2018) 486–495 <https://doi.org/10.1645/17-176>.
- [15] O.M. Amin, N.V. Ha, On four species of echinorhynchid acanthocephalans from marine fish in Halong Bay, Vietnam, including the description of three new species and a key to the species of Gorgorhynchus, Parasitol. Res. 109 (2011) 841–847, <https://doi.org/10.1007/s00436-011-2310-y>.
- [16] G.D. Schmidt, I. Paperna, *Sclerocollum rubrimaris* gen. Et sp. n. (Rhadiorhynchidae: Gorgorhynchinae), and other Acanthocephala of marine fishes from Israel, J. Parasitol. 64 (1978) 846–850, <https://doi.org/10.2307/3279515>.
- [17] S. Pichelin, L.R. Smales, T.H. Cribb, A review of the genus *Sclerocollum* Schmidt & Paperna, 1978 (Acanthocephala: Cavisomidae) from rabbitfishes (Siganidae) in the Indian and Pacific oceans, Syst. Parasitol. 93 (2016) 101–114, <https://doi.org/10.1007/s11201-016-9101-1>.

1007/s11230-015-9609-5.

- [18] L. Li, H.X. Chen, Y. Yang, Morphological and molecular study of *Neorhadiorhynchus nudus* (Harada, 1938) (Acanthocephala: Cavisomidae) from *Auxis thazard* Lacepede (Perciformes: Scombridae) in the South China Sea, *Acta Parasitol.* 63 (2018) 479–485, <https://doi.org/10.1515/ap-2018-0057>.
- [19] C.C. Velasquez, S.L. Eduardo, Index catalogue of parasites of Philippine vertebrates, National Academy of Science and Technology, Philippines, 1994 391pp.
- [20] A. Villegas, D. Gonzales-Solis, Gastrointestinal helminth parasites of the American crocodile (*Crocodylus acutus*) in southern Quintana Roo, Mexico, *Herpetol. Conserv. Biol.* 4 (2009) 346–351.