



SHORT COMMUNICATION

First record of cannibalism in the giant mottled eel *Anguilla marmorata* (Actinopterygii: Anguilliformes) as revealed by DNA barcoding

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ABSTRACT. Although the giant mottled eel, *Anguilla marmorata* Quoy & Gaimard, 1824, is widely distributed in the Indo-Pacific region, few ecological studies have been conducted on the species. We investigated the stomach contents of *A. marmorata* visually and used the DNA-barcode region of the mitochondrial cytochrome c oxidase subunit 1 (CO1) to confirm the species' identification. The stomach content analysis revealed that teleosts and crustaceans are the major prey items of *A. marmorata*. Interestingly, the stomach content of one of the specimens, which was 1029 mm in total length (TL), contained an eel-like fish identified as *A. marmorata* measuring 510 mm in TL. This study is the first to record cannibalism in the diet of *A. marmorata*. Although the diet of anguillid eels is generally selective for a single prey species, larger eels are more likely to adopt a diverse feeding habit that includes cannibalism in the tropical river ecosystems.

KEY WORDS. Aquatic ecosystem, Borneo Island, diet, molecular, piscivory, tropical anguillid eels.

The anguillid eels, *Anguilla* Schrank, 1798, comprise 19 species and subspecies that are distributed throughout the world from tropical to temperate regions (Ege 1939, Tesch 2003, Arai 2016). These catadromous fishes migrate between their offshore spawning grounds and the water (coastal and freshwater), where they spend most of their life cycle (Arai 2020). Anguillid eels have commercial, cultural, and ecological importance especially in European and Asian countries (Arai 2014, 2021, Jacoby et al. 2015).

The giant mottled eel, *A. marmorata*, is broadly distributed and is found in the Indo-Pacific region (Ege 1939, Tesch 2003, Arai 2016, 2020, Arai and Taha 2021). Although *A. marmorata* is considered an important substitute for the temperate eel species in aquaculture and fisheries in East Asia (Gollock et al. 2018), there is limited information available on the life history of almost all tropical anguillids – including *A. marmorata*.

Previous studies on the feeding ecology of *A. marmorata* are scarce. Investigations on the feeding ecology of *A. marmorata* may contribute to the understanding of the life history of the species and its role in the trophic ecology of aquatic ecosystems. Information on the diet of a fish contributes to the understanding of various aspects of its life history such as habitat preference, prey selection, predation, resource partitioning, competition, energy transfer and evolution in aquatic ecosystems (Arai et al.

2015a, 2015b, Amalina et al. 2016) and is fundamental to the development of conservation measures for the species (Arai et al. 2015a, 2015b, Amalina et al. 2016).

After examining the stomach contents of *A. marmorata* specimens collected in Brunei Darussalam, and Malaysia in the western part of Borneo Island we document the first record of cannibalism for the species. The identification of the prey species was confirmed by DNA barcoding. Additionally, we provide further insights into the diverse feeding habits of *A. marmorata* in the tropical river ecosystem.

A total of four specimens of *A. marmorata* were caught by the local people at the Wariu River, Kota Belud, Sabah, Borneo Island, East Malaysia (6°20'23"N; 116°27'51"E; 3 specimens), from 4 to 10 November 2019, and at a stream at Lamunin Village, which is connected to the Tutong River, Tutong District, Brunei Darussalam, Borneo Island (64°39'54"N; 114°43'18"E; 1 specimen), on 17 February 2021. Collecting was conducted with the help of an eel trap. Anguillid eels, including *A. marmorata*, are rare in Brunei Darussalam and Malaysia on the western part of Borneo Island (Wong et al. 2017, Zan et al. 2020).

All samples were frozen at -20 °C immediately after sampling. The external morphometric characteristics were measured for each sample according to the morphological descriptions of Ege (1939), Watanabe et al. (2004) and Arai (2016). Based on the

morphological identification keys for anguillid eels developed by Ege (1939), the fin difference index (FDI) provides the highest resolution with the least ambiguity when distinguishing eels at the species level. The FDI was calculated as follows: $FDI = 100 Z L_T^{-1}$, whereby Z is the distance from the beginning of the dorsal fin (Z) to the anus (ano-dorsal length) and L_T is the total length (Ege 1939).

The stomach contents of the eels were examined. Prior to the examination, the outer surface of each stomach was cleaned to avoid cross-contamination (e.g. blood, tissue attached to stomach from predator). Subsequently, each stomach was dissected with clean scissors and tweezers, and the contents were flushed with distilled water and 95% ethanol. For DNA barcoding using mitochondrial cytochrome c oxidase subunit 1 (CO1), the dorsal fins were clipped from the four specimens, and a muscle tissue was dissected from an eel-like specimen found in the stomach. The CO1 gene was analysed according to our previous studies (Arai et al. 2020, Zan et al. 2020), in which genomic DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Germany), and CO1 gene was amplified using Taq PCR Master Mix (QIAGEN, Germany). However, we used the primers AngF (5'TCA CCC GTT GAT TCT TTT CT3') and AngR (5'CCG ATA GCC ATT ATT GCT CAG3') (Norarfan et al. 2021). PCR amplicon was purified using QIAquick Gel Extraction Kit (QIAGEN, Germany) and sequenced bi-directionally with the same primers. Generated sequence trace files were analysed and assembled using MEGA version X (Kumar et al. 2018). The contig sequences were compared for percentage similarity with the reference sequences in the GenBank database using BLAST search.

The four eel samples (593–1029 mm in total length (TL); 450–3105 g in body weight) presented skin with variegated markings (Table 1). All of the eels had narrow maxillary bands of teeth and FDI of 14.3–18.7%. Based on the key morphological characteristics used for identification (Ege 1939, Watanabe et al. 2004) and the geographic distribution of anguillid eels, all of the eel samples were considered as *A. marmorata*.

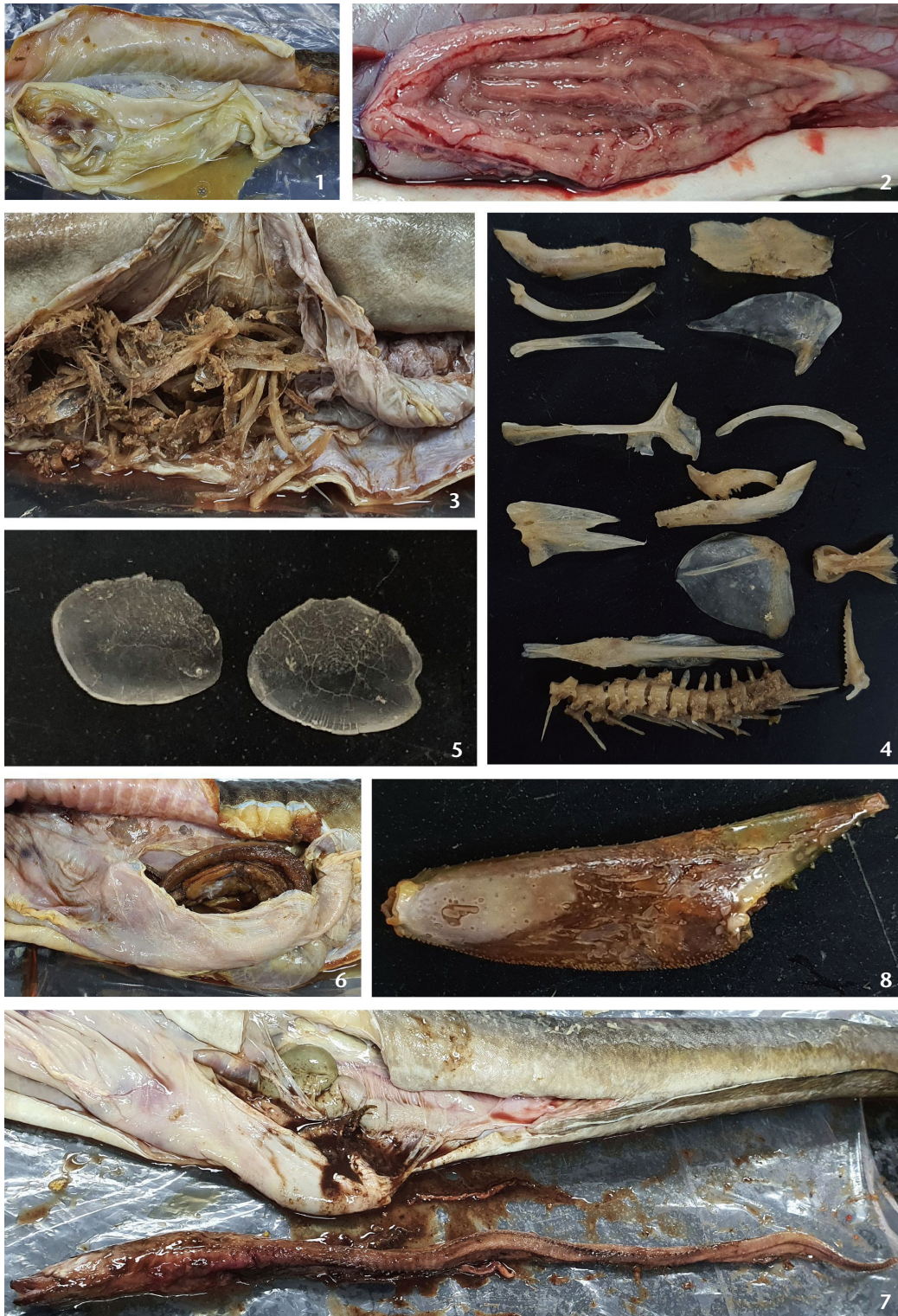
The ~700 bp mitochondrial CO1 genes from the four eel samples were successfully amplified and sequenced. Using BLAST, all of the DNA sequences showed definitive matches of 99–100% to the GenBank reference sequence of *A. marmorata*. Thus, the four eel samples were confirmed as *A. marmorata* (Table 1). These four DNA sequences were deposited in the GenBank database with the accession numbers MW283181 to MW283183 and MZ435985.

Two of the four *A. marmorata* specimens had stomach contents, while the stomachs of two specimens were empty (Table 1, Figs 1–8). The stomach of one *A. marmorata* specimen had fish bones and otoliths that belong to a teleost fish (Figs 3–5). It was not possible to identify the fish further because its muscles (soft) tissues had already been digested. Another *A. marmorata* specimen had eaten at least an eel-like fish and a crustacean (crayfish) based on the presence of the fish and a claw in the stomach (Figs 6–8). The TL of the eel-like fish was 510 mm, with some intact muscle tissues present, which allowed for DNA barcoding to be carried out. The mitochondrial CO1 sequence analysis revealed a definitive identity match of 99.9% with the GenBank reference sequence of *A. marmorata*, confirming that it was an eel of the same species. The DNA sequence was deposited in the GenBank database with the accession number MZ435986.

The main prey items of *A. marmorata* in this study were fish and crustaceans. Only a limited number of specimens could be studied in the western Borneo Island, which lies outside the species' main distribution range (Wong et al. 2017, Zan et al. 2020). Nevertheless, their prey items were similar to the ones observed for the other anguillid species such as the European eel *A. anguilla* Linnaeus, 1758 (Lammens et al. 1985, Lammens and Visser 1989, Dörner et al. 2009), the New Zealand eels *A. australis schmidtii* Phillips, 1925 and *A. dieffenbachii* Gray, 1842 (Jellyman 1989) and the Japanese eel *A. japonica* Temminck & Schlegel, 1846 (Kaifu et al. 2013). Although Anguillid eels consume a wide range of prey, they generally select a single prey species from what is available (Jellyman 1989). For example, the European eel *A. anguilla* will shift its diet according to the availability of specific prey species (Lammens et al. 1985) but will for the most part consume a single species at a time (Lammens and Visser 1989, Dörner et al. 2009). The Japanese eel also basically consumes a single prey species, based on the previous study of its stomach contents (Kaifu et al. 2013). The diet of anguillid eels can also change depending on their growth stages. In the case of the New Zealand eels, the prey of the smaller eels are mainly amphipods and insect larvae. Snails are the dominant prey of slightly larger *A. australis* and fish become an important prey when the eel is longer than 700 mm in TL (Jellyman 1989). In the present study, the stomach contents of two specimens had fish. The TLs of the eels in this study were more than 700 mm (980 and 1029 mm), a suitable size for chasing and capturing fish. Although Anguillid eels are optionally piscivorous in aquatic

Table 1. Specimens and stomach contents used in this study.

Adult specimens	Location	Total length (mm)	Body weight (g)	Species by morphology	Species by DNA barcoding	Stomach contents
1	Malaysia	784	935	<i>A. marmorata</i>	<i>A. marmorata</i>	Empty
2	Malaysia	980	2890	<i>A. marmorata</i>	<i>A. marmorata</i>	Teleost fish
3	Malaysia	1029	3105	<i>A. marmorata</i>	<i>A. marmorata</i>	Teleost fish (eel-like fish)* crayfish
4	Brunei Darussalam	593	450	<i>A. marmorata</i>	<i>A. marmorata</i>	Empty
*Teleost fish in specimen 3		510	Not determined	Eel-like fish	<i>A. marmorata</i>	<i>A. marmorata</i>



Figures 1–8. Stomach contents of the giant mottled eel *Anguilla marmorata*: (1) 784 mm, (2) 593 mm in TL with empty stomachs; (3) 980 mm in TL with (4) bones and (5) otoliths of teleost fish(es); (6) 1029 mm in TL with (7) *A. marmorata* and (8) a claw of crayfish.

ecosystems, they are generally not considered major predators of fish when compared to other piscivorous fishes (Dörner et al. 2009). Anguillid eels are night-active hunters (Tesch 2003), and seek their prey close to the bottom of the water column (Barak and Mason 1992).

The stomachs of two specimens were empty. Empty stomachs were observed in various other anguillid eels (e.g. Jellyman 1989, Dörner et al. 2009, Kaifu et al. 2013). In studies conducted in the wild, 27% of *A. australis*, 34% of *A. dieffenbachii* (Jellyman 1989), 49–54% of *A. anguilla* (Dörner et al. 2009) and 30–40% of *A. japonica* (Kaifu et al. 2013) had empty stomachs. The absence of prey in the stomach might not be the result of overnight digestion, since the digestion rates of wild eels range from 24 to 36 hours (Cairns 1942, Burnet 1952). Furthermore, fish bones and otoliths with the soft tissues digested were observed in this study in the stomach of one specimen. A high incidence of empty stomachs has been recorded in anguillid eels such as *A. australis* and *A. dieffenbachii* (Cairns 1942, Burnet 1952, Jellyman 1989), *A. reinhardtii* Steindachner, 1867 (Beumer 1979, Sloane 1984), *A. rostrata* Lesueur, 1817 (Godfrey 1957), *A. anguilla* (Sinha and Jones 1967) and *A. japonica* (Kaifu et al. 2013). Additional studies using stable isotope and DNA barcoding are needed to understand the diets and trophic position of anguillid eels.

Based on the DNA barcoding, one prey in the stomach of *A. marmorata* was identified as an eel of the same species. The TL of the prey was surprisingly large, with more than half the TL of the predator (Table 1, Figs 6–8). Although previous research has not documented that cannibalism is common in anguillid eels, piscivory has been well documented in *A. australis* and *A. dieffenbachii* (Cairns 1942, Burnet 1952, Ryan 1986, Jellyman 1989), *A. anguilla* (Hartley 1948, Moriarty 1972, Ezzat and El-Seraffy 1977, Dörner et al. 2009), *A. rostrata* (Godfrey 1957, Ogden 1970), *A. bengalensis bengalensis* (Gray, 1831) (Pantulu 1957), *A. reinhardtii* (Beumer 1979, Sloane 1984) and *A. japonica* (Kaifu et al. 2013). It has been suggested that *A. anguilla* eels tend to consume juveniles (elvers) and small eels of the same species because they are an easy source of food (Sinha and Jones 1967). The stomach contents of *A. australis* and *A. dieffenbachii* indicated that these eels are prey for both species, although their prey sizes are unknown (Jellyman 1989). In the present study, however, the prey of *A. marmorata* was longer than 500 mm which indicates the adult stage. In the tropical ecosystem of Malaysia, various size groups of *A. bicolor* McClelland, 1844 (400 mm to 800 mm) and *A. bengalensis bengalensis* (300 mm to 900 mm) were found in the same habitat (Arai and Abdul Kadir 2017). It is possible that in this case the smaller eels are targeted and become prey of larger eels, and that their feeding behavior and diet are opportunistic in their habitat and ecosystem. *Anguilla marmorata* is considered an important species in aquaculture (Gollock et al. 2018) and for this reason the results of the present study can be used to improve eel farming.

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Author Contributions

TA designed the experiment; NDZ, HT and TA conducted the experiments; NDZ, HT and TA contributed to analyzing data and writing the paper.

Competing Interests

The authors have declared that no competing interests exist.

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