RESEARCH NOTE

Assessing the species composition of tropical eels (Anguillidae) in Aceh Waters, Indonesia, with DNA barcoding gene *cox1*. [version 1; referees: 1 approved, 2 approved with reservations]

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Abstract

The objective of the present study was to evaluate the species diversity of eels native to Aceh waters based on genetic data. Sampling was conducted in western coast waters of Aceh Province, Indonesia, from July to August 2016. Genomic DNA was extracted from the samples, a genomic region from the 5' region of the *cox1* gene was amplified and sequenced, and this was then used to analyse genetic variation. The genetic sequences were blasted into the NCBI database. Based on this analysis there were three valid species of eels that occurred in Aceh waters, namely *Anguilla marmorata*, *A. bicolor bicolor*, and *A. bengalensis bengalensis.*

Open Peer Review

Referee Status: ✔ ? ?

	Invited Referees 1	2	3
version 1 published 13 Mar 2017	✔ report	? report	? report

1 **Salman Abdo Al-Shami**, University of Tabuk, Saudi Arabia

2 **Mudjekeewis D. Santos**, National Fisheries Research and Development Institute, Philippines

3 **Murugaiyan Kalaiselvam**, Annamalai University, India

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Comments (0)

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Competing interests: No competing interests were disclosed.

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Introduction

There are 114 species of freshwater and brackish water fish distributed around 17 sampling locations across Aceh waters[1]. Several of these have the potential for aquaculture, e.g. the *Anguilla* spp. of tropical eels, locally known as sidat or illeah in Acehnese language[2–3]. Based on morphological characteristics, only two species of eels have been recorded in Aceh waters, *Anguilla bicolor* and *Anguilla marmorata*[1], but it is believed that the true number of species is greater because some parts of the inland waters in Aceh province have not been explored yet. According to Miller and Tsukamoto[4], there are 19 species of eels that have been identified worldwide, 7 of which are found in Indonesian waters[5]. It is therefore very likely that new species will be found in Aceh waters.

For fisheries management it is crucial to identify these species in order to plan a better conservation strategy, since each one has unique behavioral patterns, and should be independently managed. Eels are very similar morphologically, so it is very difficult to distinguish one species from the other based on morphological characteristics only. Analysing genetic data through DNA barcoding can solve this problem[6], so that the true number of eel species living in the waters of Aceh can be evaluated. The objective of the present study was to verify the taxonomic status of eels in Aceh waters by amplifying the *cox1* gene and analysing the genetic data.

Methods

The study was conducted on the western coast of Aceh Province, Indonesia, from July to November 2016. The samples were processed and analyzed in the School of Biological Sciences, Universiti Sains Malaysia. Sampling was done at night from 18.00 to 06.00 hours. Adult eels were caught using line fishing, while traps were used to catch glass eels. Eel larvae are called glass eels; they have translucent white bodies and measure about 5–10 cm. The length of adult eels is species dependent but most measure between 40–120 cm.

Approximately 1 cm^2 of caudal fin tissue was taken from each specimen using a sterile procedure to avoid contamination of specimens. The tissue was placed into 2.0 ml tubes containing 96% alcohol. Genomic DNA was isolated using Aqua Genomic DNA solution following the manufacturer's protocol[7–8]. DNA electrophoresis was carried out on a 0.8% agarose gel at 100V. The quality and quantity of extracted DNA was assessed using a spectrophotometer. A genomic region approximately 655 bp in size was amplified from the 5' region of the Mitochondrial Cytochrome Oxidase Subunit I (*cox1*) gene following the protocol from Ward *et al.*[9] with these primer pairs:

FishF1: 5'TCAACCAACCACAAAGACATTGGCAC3'

FishR1: 5'TAGACTTCTGGGTGGCCAAAGAATCA3'

After amplification, PCR products were run on 1.2% agarose gels at 100V. The clearest band was selected and purified using purification kits (PCR Clean-Up System, Promega), following the manufacturer's protocol. The purified products were run on 1.2% agarose gels at 100V to check for bands and only clear products were sent for sequencing to First BASE Laboratory Sdn Bhd in Kuala Lumpur, Malaysia. All obtained sequences were edited and aligned using MEGA 6.0 program[10]. Multiple sequence alignments were performed on the edited sequences with Cluster *W*, which is integrated into the MEGA 6.0 program. The sequences were then blasted into the NCBI database to compare and identify species. Nucleotide divergence among sequences was estimated for their genetic distance by Neighbour-Joining (NJ) based on Kimura 2 parameter. NJ was also used to construct phylogenetic trees to determine genetic relationships among haplotypes.

Statement on animal ethics

All procedures involving animals were conducted in compliance with The Syiah Kuala University Research and Ethics Guidelines, Section of Animal Care and Use in Research (Ethic Code No: 958/2015). Please refer to Supplementary File 1 for the completed ARRIVE guidelines checklist.

Results

Genomic DNA from the 5' region of the *cox1* gene from a total of 13 glass eel samples and 31 adult eel samples were successfully amplified (Table 1). The results from NCBI BLAST identified two species of eel from adult eel samples, shortfin eel *A. bicolor bicolor* and giant mottled eel *A. marmorata*. In addition, there were three species of eels that were recognized among the glass eel samples, namely *A. bicolor bicolor*, *A. marmorata* and Indian mottled eel *A. bengalensis bengalensis.* A total of 20 haplotypes, consisting of 3 haplotypes of the *A. bengalensis bengalensis*, 1 haplotype of the *A. marmorata*, 15 haplotypes of the *A. bicolor bicolor* and 1 haplotype of the *Uroconger lepturus* (out-group) were produced from 44 samples (Table 2), out of 132 variable sites. and a haplotype diversity (Hd) of 0.8742. The haplotype number four

Table 1. Total sample and code of the tropical eels collected from Aceh waters.

District	Sampling site	Total sample	Stage
Aceh Besar	Beureunut River	13	Glass eels
	Kajhu swamp	5	Adult
	Tibang reservoir	5	Adult
	Aceh River	1	Adult
	Pulo Aceh Island	1	Adult
Nagan Raya	Geutah River	2	Adult
Gayo Lues	Alas River (Blangkejeren)	1	Adult
Aceh Barat Daya	Kuala Batee River	1	Adult
Aceh Singkil	Singkil swamp	5	Adult
Aceh Jaya	Lamno River	1	Adult
Aceh Tenggara	Alas River	4	Adult
Aceh Barat	Meurebo River	3	Adult
Pidie Jaya	Ulim River	1	Adult
Aceh Selatan	Terbangan	1	Adult
Total		44	

belongs to *A. marmorata* and was shared by 9 samples from 4 different locations. The haplotype number 5 belongs to *A. bicolor bicolor* and was shared by 13 samples from 6 locations. All of the haplotype sequences have been deposited in the NCBI GenBank with accession numbers KY618767 to KY618795.

Therefore, the study revealed that there are three valid species of tropical eels found in Aceh waters: *A. bicolor*, *A. marmorata,* and *A. bengalensis;* the last species being a newly recorded species in Aceh waters. The study indicates that multiple species of glass eels migrate from the sea into freshwater. One interesting finding was that one sample of conger eels (*Uroconger lepturus*) was detected among the Tropical glass eel samples. This is indicatory of DNA barcoding being successful in identifying species of eels in Aceh waters which cannot be identified by biometric data. Genetic data has become an important tool in assessing gene flow between marine populations[11], species identification[12] and monitoring the resources of marine fisheries[13].

The genetic divergence between *A. bicolor* and *A. marmorata* was 5.0%, between *A. bicolor* and *A. bengalensis* it was 6.7% and between *A. marmorata* and *A. bengalensis* genetic divergence was 4.0% (Table 3). The phylogenetic tree showed a close relationship between *A. marmorata* and *A. bengalensis* (Figure 1). Based on

Table 2. Haplotypes according to species and sampling location.

Haplotype	Species	Total sample	Sampling location
1	*Anguilla bengalensis bengalensis*	2	Beureunut River (Glass eels)
2	*Anguilla bengalensis bengalensis*	1	Beureunut River
3	*Anguilla bengalensis bengalensis*	2	Beureunut River
4	*Anguilla marmorata*	9	Beureunut River, Blangkeujeren, Geutah River, Alas River,
5	*Anguilla bicolor bicolor*	13	Beureunut River, Kajhu swamp, Singkil swamp, Meurebo River, Ulim River, Tibang reservoir
6	*Anguilla bicolor bicolor*	1	Beureunut River
7	*Anguilla bicolor bicolor*	1	Beureunut River
8	*Anguilla bicolor bicolor*	1	Beureunut River
9	*Anguilla bicolor bicolor*	3	Aceh River, Pulo Aceh River, Terbangan
10	*Anguilla bicolor bicolor*	1	Kuala Batee River
11	*Anguilla bicolor bicolor*	1	Kajhu swamp
12	*Anguilla bicolor bicolor*	1	Kajhu swamp
13	*Anguilla bicolor bicolor*	1	Meurebo River
14	*Anguilla bicolor bicolor*	1	Meurebo River
15	*Anguilla bicolor bicolor*	1	Lamno River
16	*Anguilla bicolor bicolor*	1	Singkil swamp
17	*Anguilla bicolor bicolor*	1	Tibang reservoir
18	*Anguilla bicolor bicolor*	1	Tibang reservoir
19	*Anguilla bicolor bicolor*	1	Tibang reservoir
20	*Uroconger lepturus* (out-group)	1	Beureunut River
Total	3 species eels (minus out-group)	44	14 locations

Table 3. The genetic distance between three species of Anguilla.

No	Species	1	2	3
1	*Anguilla bicolor bicolor*	-	-	-
2	*Anguilla marmorata*	5.0		-
3	*Anguilla bengalensis bengalensis*	6.7	4.0	-

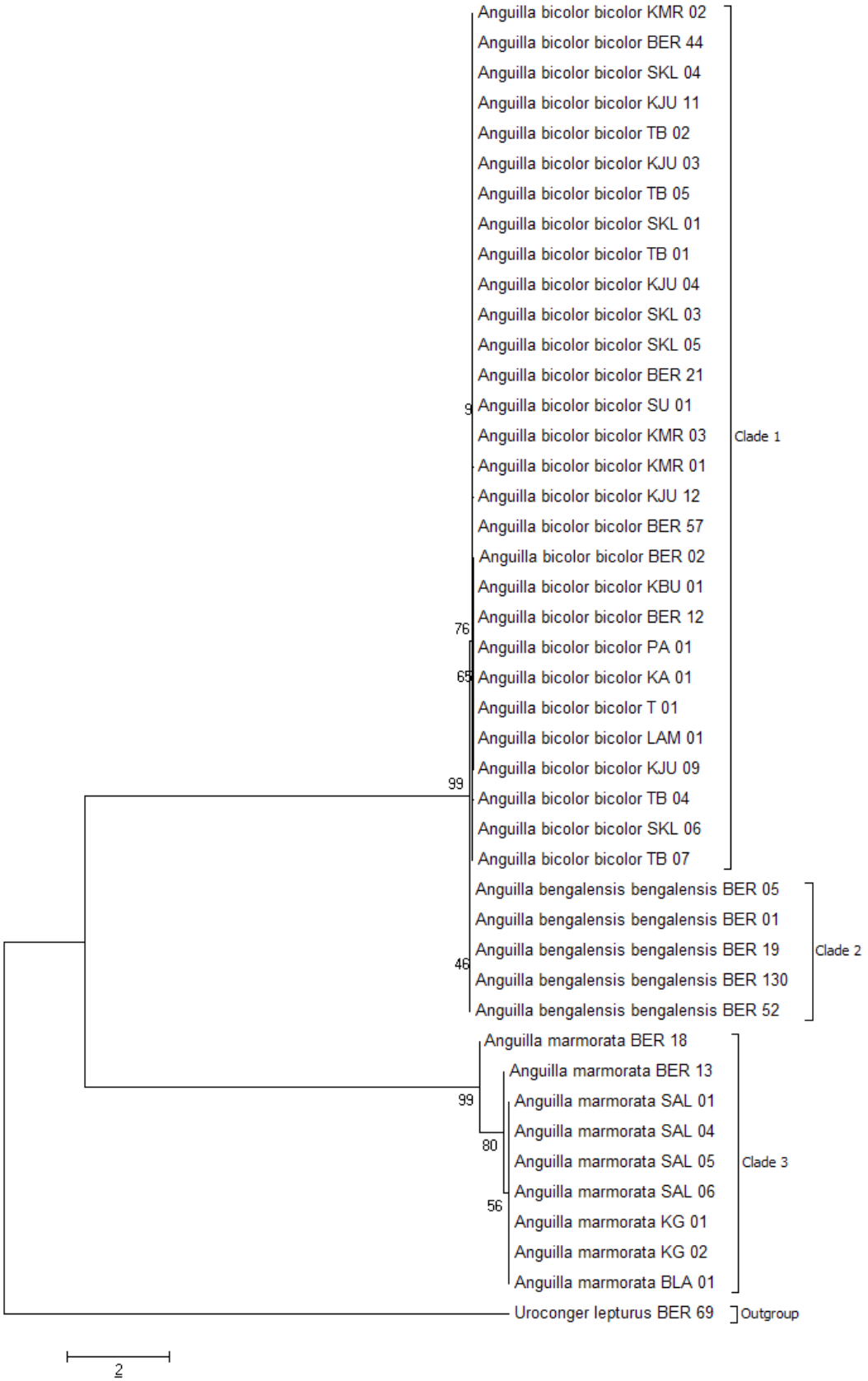

Figure 1. The phylogenetic tree of individual of *Anguilla* samples using the Neighbour-Joining (NJ) method.

IUCN[14] data, *A. bengalensis bengalensis* and *A. bicolor bicolor* are categorized as near threatened, while the status of *A. marmorata* is on least concern. However, based on direct sampling in Aceh waters the shortfin eels are still abundant and most frequently caught, and are distributed over a wide range of areas including small streams, marshes, peat swamp, estuaries and irrigation channel in paddy fields[1,15]. Indian mottled and giant mottled eels on the other hand have been very rarely caught and are generally only found in large rivers directly connected to the sea.

Conclusion

It is concluded that three species of tropical eels are found in Aceh waters, namely, *A. marmorata, A. bicolor bicolor,* and *A. bengalensis bengalensis* where *A. bengalensis bengalensis* is the newly recorded species.

Data availability

Sequenced DNA of Tropical eels from Aceh waters can be found in the NCBI GenBank repository (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers KY618767 to KY618795.

Author contributions

ZAM is responsible for developing research proposal and study design and approved the final draft of the paper. ASB, NF, AAM, NF and AIU are responsible for sample collection, sample processing, and data analysis. MNS is responsible for manuscript sequence alignment and proofreading of the draft.

Competing interests

No competing interests were disclosed.

Grant information

This study was supported by Syiah Kuala University through the 2016 H index scheme (Contract number: 230/UN11.2/2016).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Current Referee Status: ✔ ? ?

Version 1

Referee Report 11 May 2017

doi:10.5256/f1000research.11554.r22410

? **Murugaiyan Kalaiselvam**
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Tamil Nadu, India

1. In Introduction, add few more points regarding the importance of Genetic identification, and demerits of conventional identification strategies (Only one point had been given in introduction for name sake, add few more)

2. In addition, author will add the possible outcome after identification of eels, in what way this work serves to the research community?

3. Prior to molecular identification, the author done the sample identification by morphometric characters? Though it's a old procedure, it is of much importance and the results of morphometric analysis acts as an base step for identification.

4. Data on Taxonomic characters will serve as a guide for identification of the same, whereas having sequences on hands will not be useful for further reference.

5. Authors stated that only two species of eels have been recorded in Aceh waters and in results they recorded 3 species with molecular results? Thus the morphometric identification of eels should be included so that what are the distinct features of 3 eels can be clarified to the readers.

6. Generally for DNA barcoding analysis lateral tissue from the left side of fish will be taken in to consideration, but the authors had chosen caudal fin tissue, is there any justification for taking the caudal fin tissue, if so justify that and add proper reference for that methodology.

7. Totally 13 glass eel and 31 adult eels were, so totally out of the 44 samples, the results inferred belongs to only 3 species, so care should be taken prior to analyzing the samples for molecular identification as it is cost effective process and wastage of chemicals.

8. How did author arrived the genetic divergence?

9. Materials needs clear cut procedures and reference alone doesn't enough:
   - Genomic DNA was isolated using Aqua Genomic DNA solution following the manufacturer's

protocol[7-8]
- Mitochondrial Cytochrome Oxidase Subunit I (*cox1*) gene following the protocol from Ward *et al.*[9]

10. Discussion part need to be written with the comparative studies made by author authors regarding the availability of eel in study area, identification problems, and results of the present study with respect to molecular identification and highlights the importance of the obtained results.

11. Conclusion seems to be the result and what is the inference made from the study should be written precisely and accurately.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 18 April 2017

doi:10.5256/f1000research.11554.r21916

Mudjekeewis D. Santos
Genetic Fingerprinting Laboratory, National Fisheries Research and Development Institute, Quezon City, Philippines

This paper has some scientific merit in providing new information about species composition of eel in Aceh waters and in using COX1 gene as a marker. As such I find it suitable for indexing after major revisions:

1. Authors need to highlight in the Introduction the existing information/status about eels in Aceh waters.

2. In addition, they need to relate the study on existing eel trade (domestic or export) in the area if any since this is the main threat for the said species.

3. The paper of Asis et al. (2014)[1] would help enrich the objective of this paper.

4. The reference DNA sequences used in the paper/trees are not clear. Did the authors established their own reference sequences for cox1? This should be indicated or made clear.

References

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PubMed Abstract | Publisher Full Text

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 22 March 2017

doi:10.5256/f1000research.11554.r20933

Salman Abdo Al-Shami
Department of Biology, University College of Taymma, University of Tabuk, Tabuk, Saudi Arabia

The manuscript in hands present interesting information about species diversity of tropical eels (Anguilla spp.) in Aceh, Indonesia. Although the manuscript is nicely presented, few justifications and clarification are still required.

Title

- For general readers, using the word "waters" may make the readers confused about what type of water bodies in which the samples collected from. For example, how about using "coastal line" instead of "waters" or just delete the word "waters".
- Please replace the family name of eels with "Anguilla spp." to be more precise.

Abstract

I believe adding an introductory sentence will make the research summary more meaningful. This introductory sentence will highlight the importance of the study and make a sound justification of the study objectives.

- Should be read "...the present study is to evaluate..."
- Should be read "the western"
- "coastal waters" change into "coastal line" or "marine environment".
- Add semicolon after "namely;"
- Add comma after "Based on this analysis"
- The word "genomic" makes me confused. Is it mitochondrial or genomic gene? Please correct me if I am wrong.

Introduction

- I would suggest extending the introduction in a way that gives the readers a comprehensive background about the research based on the available literature.
- It would be nice to start the introduction with an introductory paragraph to give the readers the brief understanding about the research context.

Methods

The procedures and tools used to collect the eel samples should be described elaborately. For example, it was stated that traps were used to catch the glass eels. It was not mentioned what type of traps? How did the researchers set the trap? For how long did they leave the traps?

- It will be excellent if the authors provide a geographical map showing the approximate locations of sampling sites.
- It would be nice to add a reference to the method of sampling the eels' tissue.
- Please add a reference to "Kimura 2 parameters"

Results

The presentation of the results is adequate and no further corrections or additions are required.

- Figure 1: please add "Anguilla spp." to the figure caption.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.