





Reply

# Reply to Hendawitharana et al. Comment on “Arulananthan et al. The Status of the Coral Reefs of the Jaffna Peninsula (Northern Sri Lanka), with 36 Coral Species New to Sri Lanka Confirmed by DNA Bar-Coding. *Oceans* 2021, 2, 509–529”

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We appreciate the comments made by Hendawitharana et al. (2023) on our paper reporting on the corals of Northern Sri Lanka, including records of 38 coral species new to Sri Lanka, and genetic investigations of eight of these [1]. We agree that the identification of corals up to the species level is challenging, especially in some genera, notably *Acropora*, and understand the concerns raised on the use of different methods to identify coral species may lead to varying conclusions. In our study, species were primarily identified using morphological characteristics, and molecular methods only being used to check on some doubtful species. In any case, it was not completely unexpected to find species previously unreported in our study area in Northern Sri Lanka. The reefs of Northern Sri Lanka have been studied to a very small degree due to historical reasons and are located closer to the centers of coral biodiversity in the Western and Eastern Indian Oceans.

To discuss our methods further, we should emphasize that the intention was to use DNA methodology only on those species whose identity was otherwise in doubt. In regard to morphological identification, we relied principally on underwater photography to identify the species concerned. We believe that this method should be preferred where possible when working within marine protected areas or with species that appear to be scarce. However, in all cases, we took photographs from different angles and three different distances (macro, close-up, and wider view), in a way not possible before the latest generation of cameras became available. To enhance the accuracy of our identification, more than one author independently checked the identification of the sample corals by referring to both the most widely used coral reference books—The Corals of the World [2] and the World Register of Marine Species (WoRMS) [3]. In unclear cases, while one author read the description from the guide, a second simultaneously checked the macro morphological features of the coral. In some cases, the advice of other coral experts was sought.

In regard to the molecular work, we used the most common method of barcoding [4–8]. However, we agree that it would increase the accuracy and robustness of species identification if multiple markers, including mitochondrial *COI* gene, 16S rRNA, and nuclear ribosomal internal transcribed spacer (ITS) regions can be used together [8–17]. In practice, the results of our barcoding matched the conclusions reached using traditional morphology-based identification. We would emphasize that all DNA sequence chromatograms were assembled and edited using Unipro UGENE 1.29.0, and then, sequences were aligned using

ClustalW [18]. The aligned outputs were queried to identify the species, and the reference sequences of various coral species were retrieved from the National Center for Biotechnology Information (NCBI) [19–22], GenBank, and Barcode of Life Data System (BOLD) [23] by using the Basic Local Alignment Search Tool (BLAST). Those sequences corresponding to the genotype were analyzed for homologies with sequences in the NCBI database by performing BLAST and BOLD workbench. From the BLAST analysis, all samples provided a greater than 99.5% similarity. Nevertheless, we did discuss the limitations of this method in the Discussion Section of our paper [1].

In most cases, Veron's [1] "keys for genera and species" proved satisfactory for the robust identification of the corals observed. Identification of seven samples proved more challenging; these were *Acropora aspera* (COJP001), *Acropora digitifera* (COJP003), and two specimens from two different locations representing *Acropora gemmifera* (COJP002, COJP004), *Acropora hyacinthus* (COJP006), *Echinopora gemmacea* (COJP007), and *Montipora flabellate* (COJP009), which was present only in small colony form. Even though we accept that the COI is not definitive for *Acropora* species, the best fitting morphological descriptions from Corals of the World [2], and we have chosen the best fitting BLASTn output to conclude the identification. It should also be noted that even though, unsurprisingly, the COI sequences of COJP001, COJP002, and COJP006 did not discriminate between them, the indicated identity reinforced the identification previously made from the study of the sample's morphological parameters, including corallite structure (axial, radial, exserted radial), as well as colony form.

More generally, we are aware that the conventional classification of scleractinian corals based on their morphology alone has been called into question based on both molecular and small-scale morphological data, resulting in frequent revisions to their classification at all levels [8,13–15,24–26]. Advances in molecular analysis have revealed clearer inconsistencies between previously accepted coral taxonomy and their apparent evolutionary past. Further, the term "accepted" may also be used to gloss over significant differences in name games with corals. For example, *Acropora danai*, which is now considered a junior synonym of *Acropora abrotanoides*, as described in Wallace, 1999 [27], and Veron, 2000 [2]. *Acropora nobilis* is now considered a junior synonym of *A. intermedia*, as documented in Wallace, 1999, and Veron et al., 2022 [3,28].

Our study described the status of the reefs in the Jaffna Peninsula, from both a conservation and a bio-geographic point of view. The results of the biogeographic study were relevant because the database of already published work from the Indian Ocean and Red Sea regions indicated that most if not all the species that we recorded were present within these regions [29–36] (Table 1). While acknowledging the fact that Hendawitharana et al. (2023) found our title misleading, we would like to state the following. The phrase "Confirmed by DNA-Bar coding" referred only to the genetic confirmation of the eight newly recorded species studied in this way to check their identity. We do not believe that the title misleads the readership and trust that disagreement on this point can be disregarded. We accept the possibility that a few of the identifications based on confirmation by barcoding may turn out not to be as robust as we would hope, but we do stand by the validity of the identifications described in our paper.

**Table 1.** Details of scleractinian corals reported to be new to Sri Lanka, with notes on their taxon update details and known geographical distribution (individual closest locations near by Indian territory already clearly presented in our published article).

Sp No.	Family	Genus	Species	Updates on Taxon Details	Geographic Distribution [2,3,28–36]
1	Acroporidae Verrill, 1901	<i>Acropora</i> Oken, 1815	<i>Acropora aspera</i> Dana, 1846	3, 28	Red Sea, Indo Pacific, South Pacific Ocean
2			<i>Acropora digitifera</i> Dana, 1846	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, South Africa, South Pacific Ocean, Madagascar
3			<i>Acropora gemmifera</i> Brook, 1892	3, 28	Red Sea, Indo Pacific, South Pacific Ocean, Mozambique, Federal Republic of Somalia
4			<i>Acropora latistella</i> Brook, 1891	3, 28	Red Sea, Central Indo Pacific, South Africa, Mozambique
5			<i>Acropora pulchra</i> Brook, 1891	3, 28	Red Sea, Indo Pacific, Indian Ocean, Mozambique, South Pacific Ocean
6		<i>Alveopora</i> Blainville, 1830	<i>Alveopora allingi</i> Hoffmeister, 1925	3, 28	Red Sea, Indian Ocean, South Africa, Mozambique, South Pacific Ocean
7		<i>Astreopora</i> Blainville, 1830	<i>Astreopora myriophthalma</i> Lamarck, 1816	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, South Africa, South Pacific Ocean, Kenya
8			<i>Astreopora listeri</i> Bernard, 1896	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, South Pacific Ocean, Madagascar, Kenya
9			<i>Astreopora ocellata</i> Bernard, 1896	3, 28	Red Sea, Central Indo Pacific, Indian Ocean
10		<i>Montipora</i> Blainville, 1830	<i>Montipora flabellata</i> Studer, 1901	3, 28	South Pacific Ocean, Mozambique
11			<i>Montipora informis</i> Bernard, 1897	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, South Pacific Ocean, Madagascar
12	Agariciidae Gray, 1847	<i>Coeloseris</i> Vaughan, 1918	<i>Coeloseris mayeri</i> Vaughan, 1918	3, 28	Red Sea, Central Indo Pacific, South Africa, Mozambique
13	Dendrophylliidae Gray, 1847	<i>Turbinaria</i> Oken, 1815	<i>Turbinaria frondens</i> Dana, 1846	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, Kenya, New Zealand
14			<i>Turbinaria reniformis</i> Bernard, 1896	3, 28	Red Sea, Indian Ocean, Indo Pacific, Kenya, Federal Republic of Somalia
15			<i>Turbinaria stelluta</i> Lamarck, 1816	3, 28	Indo Pacific, Madagascar, South Pacific Ocean

Table 1. Cont.

Sp No.	Family	Genus	Species	Updates on Taxon Details	Geographic Distribution [2,3,28–36]
16	Oulastreidae Vaughan, 1919	<i>Oulastrea</i> Milne Edwards & Haime, 1848	<i>Oulastrea crispata</i> Lamarck, 1816	3, 28	Andaman Nicobar Islands, Western Basin
17	Scleractinia incertae sedis	<i>Pachyseris</i> Milne Edwards & Haime, 1849	<i>Pachyseris gemmae</i> Nemenzo, 1955	3, 28	Andaman Nicobar Islands, Indian Ocean
18	Merulinidae Verrill, 1865	<i>Coelastrea</i> Verrill, 1866	<i>Coelastrea palauensis</i> Yabe & Sugiyama, 1936	3, 28	Red Sea, Central Indo Pacific, Indian Ocean
19		<i>Cyphastrea</i> Milne Wdwards & Haime, 1848	<i>Cyphastrea japonica</i> Yabe & Sugiyama, 1932	3, 28	Philippines, Central Indian Ocean
20			<i>Cyphastrea microphthalma</i> Lamarck, 1816	3, 28	Red Sea, Indian Ocean, Indo West Pacific, Mozambique, Madagascar, South Africa, South Pacific Ocean, Kenya
21		<i>Dipsastraea</i> Blainville, 1830	<i>Dipsastraea amicorum</i> Milne Edwards & Haime, 1850	3, 28	Red Sea, Indian Ocean, Mozambique
22			<i>Dipsastraea rotumana</i> Gardiner, 1899	3, 28	Red Sea, Indian Ocean, Indo West Pacific, Mozambique, South Africa, South Pacific Ocean
23			<i>Dipsastraea lizardensis</i> Veron & Pichon, 1977	3, 28	Red Sea, Central Indo Pacific, Indian Ocean
24		<i>Echinopora</i> Lamarck, 1816	<i>Echinopora gemmacea</i> Lamarck, 1816	3, 28	Red Sea, Indian Ocean, Indo West Pacific, Mozambique, Madagascar, South Africa, South Pacific Ocean, Kenya, Federal Republic of Somalia
25		<i>Goniastrea</i> Milne Edwards & Haime, 1848	<i>Goniastrea minuta</i> Veron, 2000	3, 28	Seychelles, Philippines, South Pacific Ocean
26		<i>Platygyra</i> Ehrenberg, 1834	<i>Platygyra acuta</i> Veron, 2000	3, 28	Indo Pacific, Andaman and Nicobar Islands
27	Lobophylliidae Dai & Horng, 2009	<i>Acanthastrea</i> Milne Edwards & Haime, 1848	<i>Acanthastre ishigakiensis</i> Veron, 1990	3, 28	Andaman and Nicobar Islands, South Pacific Ocean
28		<i>Micromussa</i> Veron, 2000	<i>Micromussa amakusensis</i> Veron, 1990	3, 28	Western Indian Ocean, Federal Republic of Somalia
29	Poritidae Gray, 1842	<i>Porites</i> Link, 1807	<i>Porites evermanni</i> Vaughan, 1907	3, 28	Andaman and Nicobar Islands, Indo Pacific

Table 1. Cont.

Sp No.	Family	Genus	Species	Updates on Taxon Details	Geographic Distribution [2,3,28–36]
30			<i>Porites murrayensis</i> Vaughan, 1918	3, 28	Red Sea, Indian Ocean, Indo Pacific, South Africa
31			<i>Porites pukoensis</i> Vaughan, 1907	3, 28	Indian Ocean, Madagascar
32		<i>Goniopora</i> de Blainville, 1830	<i>Goniopora lobata</i> Milne Edwards, 1860	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, South Pacific Ocean, Federal Republic of Somalia
33			<i>Goniopora minor</i> Crossl &, 1952	3, 28	Red Sea, Central Indo Pacific, Indian Ocean
34			<i>Goniopora somaliensis</i> Vaughan, 1907	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, South Africa, South Pacific Ocean, Kenya, Federal Republic of Somalia
35			<i>Goniopora tenuidens</i> Quelch, 1886	3, 28	Indian Ocean. Indo Pacific, South Pacific Ocean
36	Siderastreidae Vaughan & Wells, 1943	<i>Siderastrea</i> Blainville, 1830	<i>Siderastrea savignyana</i> Milne Edwards & Haime, 1850	3, 28	Red Sea, Indian Ocean, Indo Pacific, Mozambique, Madagascar, South Pacific Ocean

In summary, our study holds significant value in its exploration of previously unstudied taxa and geographic regions, as well as in shedding light on the potential existence of new species records, albeit with acknowledged methodological limitations. Moreover, through this initial investigation, our goal was to raise awareness regarding the area's potential significance and to propose further research with enhanced methodologies for robustly validating our discoveries. We also recognize the challenges associated with conducting comprehensive species occurrence inventories and the circumstances where their approaches may prove beneficial.

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