

Comment

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 are responding to an article by Arulananthan et al. from July 2021 in *Oceans* [1]. Arulananthan et al. [1] report the discovery of 36 coral species new to Sri Lanka, confirmed by DNA barcoding. Hereby, the study provides interesting new information on the coral fauna of the poorly studied reefs of the Jaffna Peninsula, Northern Sri Lanka, and encouragingly reports relatively high coral cover values. The methods used to identify coral species, particularly where it concerns DNA barcoding, appear not suitable for accurately identifying the recorded corals to a species level. Consequently, we argue that it is premature for the authors to claim that they have identified 36 coral species previously not recorded from Sri Lanka and urge caution when using such claims as a basis for making decisions on conservation and management actions. As a solution, we propose a stepwise approach, which one could follow to assess the presence of new coral species with more certainty.

The authors used a combination of skeletal morphology and DNA barcoding, focusing on the Cytochrome Oxidase I marker (COI), to identify coral specimens. Table 1 shows which of these two methods was used by them. However, there are issues with both methods, which render these identifications unreliable. Concerning the morphological characteristics that were used, it is unclear how accurately this was done, as voucher material was not collected for most of the recorded species [2]. This is especially unfortunate in the current study, as most identifications were made primarily using field photos and field identification guides. Even though the cited Indo-Pacific Coral Finder [3] is an excellent tool kit for learning morphological characteristics for coral identification, it is designed for genus-level identification only. To overcome this, the authors indicate that they identified species by comparing characteristics based on species descriptions in *Corals of the World* [4]. To what degree this can accurately be carried out based on photos in the field, in most cases without collecting and preserving voucher material, remains questionable. This is indicated by species like *Acropora gemmifera*, which was recorded at more than 70% of the study sites. As discussed below, the COI sequences of two specimens identified by the authors as this species actually differ by two nucleotides from each other, indicating that these specimens probably belong to two different species. Whether or not these two species were found at all study sites, and which coral species they concern, remains uncertain.

Table 1. List of species recorded as ‘new’ by Arulananthan et al. [1], based on their identification and methodology, the availability of voucher material for cross-referencing, and the location of the type specimen repository. Abbreviations used under the repository location are as follows: USNM—United States National Museum of Natural History—Smithsonian Institution, Washington, D.C., USA; NHM—Natural History Museum, London, United Kingdom; NHMTU—Natural History Museum of Tohoku University, Japan; MTQ—Museum of Tropical Queensland, Townsville, Australia; MNHN—Museum National d’Histoire Naturelle, Paris, France; UP—University of the Philippines. Type specimen repositories marked with ‘?’ are based on the assumption that other known type specimens of the same collector/from the same expedition were deposited here.

Species Names with Authorities	Basis of the Identification	Availability of Voucher Material for Cross-Checking	Type Specimen Repository	Code/ Type No
Acroporidae Verrill, 1901				
<i>Acropora aspera</i> Dana, 1846	DNA marker COI	Not collected	USNM	287
<i>Acropora digitifera</i> Dana, 1846	DNA marker COI	Not collected	MTQ	G37980
<i>Acropora gemmifera</i> Brook, 1892	DNA marker COI	Not collected	NHM	1892.6.8.114
<i>Acropora latistella</i> Brook, 1891	Morphology based on [4]	Not collected	NHM	1892.6.8.275
<i>Acropora pulchra</i> Brook, 1891	Morphology based on [4]	Not collected	NHM	1884.2.16.1
<i>Alveopora allingi</i> Hoffmeister, 1925	Morphology based on [4]	Not collected	USNM	62494
<i>Astreopora myriophthalma</i> Lamarck, 1816	Morphology based on [4]	Not collected	MNHN	219c
<i>Astreopora listeri</i> Bernard, 1896	Morphology based on [4]	Not collected	NMH	1891-3-6-20
<i>Astreopora ocellata</i> Bernard, 1896	Morphology based on [4]	Not collected	NHM	1892-12-1-150
<i>Montipora flabellata</i> Studer, 1901	DNA marker COI	Not collected	N/A	Not available
<i>Montipora informis</i> Bernard, 1897	Morphology based on [4]	Not collected	NHM	1885-6-30-3
Merulinidae Verrill, 1865				
<i>Coelastrea palauensis</i> Yabe & Sugiyama, 1936	Morphology based on [4]	Not collected	NHMTU	56631
<i>Cyphastrea japonica</i> Yabe & Sugiyama, 1936	Morphology based on [4]	Not collected	NHMTU	40323
<i>Cyphastrea microphthalma</i> Lamarck, 1816	Morphology based on [4]	Not collected	MNHN?	Not available
<i>Platygyra acuta</i> Veron, 2000	Morphology based on [4]	Not collected	MQT	G55845
<i>Echinopora gemmacea</i> Lamarck, 1816	DNA marker COI	Not collected	MNHN?	Not available
<i>Dipsastraea amicum</i> Milne Edwards & Haime, 1850	Morphology based on [4]	Not collected	MNHN?	Not available
<i>Dipsastraea lizardensis</i> Veron, Pichon & Wijsman-Best, 1977	Morphology based on [4]	Not collected	NHM	1977.1.1.2
Pachyseridae Benzoni & Hoeksema, 2023				
<i>Pachyseris gemmae</i> Nemenzo, 1955	Morphology based on [4]	Not collected	UP	UP C-123
Oulastreidae Vaughan, 1919				
<i>Oulastrea crispata</i> Lamarck, 1816	Morphology based on [4]	Not collected	MNHN	scl15
Poritidae Gray, 1842				
<i>Porites evermanni</i> Vaughan, 1907	Morphology based on [4]	Not collected	USNM	21627
<i>Porites murrayensis</i> Vaughan, 1918	Morphology based on [4]	Not collected	USNM	47237
<i>Porites pukoensis</i> Vaughan, 1907	Morphology based on [4]	Not collected	USNM	22236
<i>Goniopora lobata</i> Milne Edwards, 1860	Morphology based on [4]	Not collected	MNHN?	Not available
<i>Goniopora minor</i> Crossland, 1952	Morphology based on [4]	Not collected	NHM	56
<i>Goniopora somaliensis</i> Vaughan, 1907	Morphology based on [4]	Not collected	MNHN	Not available
<i>Goniopora tenuidens</i> Quelch, 1886	Morphology based on [4]	Not collected	NHM	Not available
Dendrophylliidae Gray, 1847				
<i>Turbinaria frondens</i> Dana, 1846	Morphology based on [4]	Not collected	USNM	214
<i>Turbinaria reniformis</i> Bernard, 1896	Morphology based on [4]	Not collected	NHM	Not available
<i>Turbinaria stellata</i> Lamarck, 1816	Morphology based on [4]	Not collected	MNHN	Not available
Lobophylliidae Dai & Horng, 2009				
<i>Acanthastrea ishigakiensis</i> Veron, 1990	Morphology based on [4]	Not collected	MTQ	G32484
<i>Micromussa amakusensis</i> Veron, 1990	Morphology based on [4]	Not collected	MTQ	G32485
Euphylliidae Milne Edwards & Haime, 1857				
<i>Coeloseris mayeri</i> Vaughan, 1918	Morphology based on [4]	Not collected	USNM	45546 (syntype series (a series of multiple types) but no holotype)
Siderastreidae Vaughan & Wells, 1943				
<i>Siderastrea savignyana</i> Milne Edwards & Haime, 1849	Morphology based on [4]	Not collected	NHM	Not available

The modern application of molecular methods in coral taxonomy in recent years has consistently shown that many of the characteristics used to delineate species and genera, as described in *Corals of the World* [4], which was first published over 20 years ago, are not homologous. This can be partially attributed to the fact that the bulk of the work described was undertaken before molecular sequencing became available and because it primarily concentrated on macromorphological characteristics and undervalued the significance of micromorphological characteristics.

It is now well established that a combined analysis of morphological (informative characteristics determined at each taxonomic level by macromorphological, micromorphological, and microstructural examinations), molecular characteristics, and other sources of evidence (reproductive isolation, spawning synchronization, geographical boundaries, etc.) is key to establishing robust and reliable coral phylogenies [5–8]. Fukami et al. [9], for example, illustrate that one can assess *Acropora* diversity through DNA analyses of the mitochondrial marker mtCR (generally considered diagnostic for these coral species) in combination with present-day knowledge of their colony form, skeletal morphology, and spawning season [10]. This is also reflected, for example, by the newly released edition of the Coral Finder [11], which “no longer follows the *Corals of the World* framework. . . .but instead a new synthesis based on individual research publications”, using a robust taxonomic approach to delineate species boundaries in a range of coral clades.

Concerning the molecular methods used, Arulanathan et al. [1] imply in the title of their paper that the identities of all 36 species were recorded as new to Sri Lanka, confirmed by DNA barcoding. The paper only gives seven COI sequences, supposedly belonging to six different coral species. To identify these species, the authors used BLAST searches in GenBank to assess which sequences match most closely. The closest match of each sequence was then assumed to provide the identification. This “molecular identification” method can result in errors being made. Downloading the seven sequences of Arulanathan et al. [1] from GenBank (MN689059.2- MN689062.2, MN689065.2, MN689067.2, and MN689068.2) and aligning them in BioEdit [12] shows, for example, that the three sequences MN689059.2, MN689060.2, and MN689067.2 match each other for 100% in the regions where they overlap and can be aligned with each other. One cannot therefore conclude based on these sequences that they concern different species at all. These three sequences differ only in sequence length, i.e., having lengths of, respectively, 709, 717, and 715 nucleotides. These length differences explain why blasting on GenBank gave different results and let Arulanathan et al. [1] wrongfully believe that the three sequences concerned three different coral species, i.e., *Acropora aspera*, *A. gemmifera*, and *A. hyacinthus*.

Where the COI marker can be used as a barcode for most animal species, this marker is known to be unsuitable for resolving systematic relationships at a species level in Anthozoans because it is not variable enough [13–16]. It is certainly not appropriate for identifying species within species-rich groups such as *Acropora* [8]. Aligning GenBank sequences MN689060.2 and MN689062.2 with each other, both identified as *A. gemmifera* by Arulanathan et al. [1], two nucleotide differences become apparent. As genetic differences between coral species within COI are minimal, it is in this case quite likely that these sequences belong to two different species. This is confirmed by the fact that running the BLAST analysis on the matched sequence of *A. gemmifera* (MG383839.1) on GenBank shows that its COI sequence differs by only one nucleotide from the COI sequences of other *Acropora* species like *A. valida*, *A. tenuis*, and *A. awi*. It can be generally concluded that Arulanathan et al. unfortunately made two mistakes in their approach to identifying corals using DNA sequences: (1) The marker that was used is not variable enough to reliably identify the corals concerned to a species level. (2) One should not identify species based solely on the first or best match sequence resulting from a BLAST search on GenBank without considering that a BLAST search result is not only dependent on how similar sequences are but also on the query cover and therefore potentially the sequence length.

It is well established that the delimitation of closely related species in scleractinian genera such as *Acropora* is not possible using markers like COI [7,8,17–19]. Molecular analyses can aid in the identification of corals at the species level [8,20–23]. For example, Ramírez-Portilla et al. [8] examined three closely related, co-occurring tabular *Acropora* species in Japan and clearly demonstrated that these species can be distinguished from each other by using multiple lines of evidence: investigating their morphologies, carrying out cross-breeding trials, and using target capture-derived markers in conjunction with haplowebs [24]. As recent molecular studies [25,26] and cross-breeding trials [27] have indicated, a wider taxonomical structure linked to the geographical distribution of species across oceans is not evident for most coral species. Therefore, the identification of species may require a comparison to the original holotype specimen and other specimens collected at the type locality. To do this, Table 2 was made based on a search in WoRMS, the World list of Scleractinia [28], to cross-check the taxonomic descriptions and relevant authorities and locate the type localities [28] of the species recorded by Arulananthan et al. [1]. Considering these type localities, 10 out of the 36 species listed by Arulananthan et al. [1] appear to have subjective junior synonyms [28] (denoted by * in Table 2), which appear more likely to occur in Sri Lanka.

In general, we would like to propose the adoption of a stepwise integrated approach (Figure 1) when assigning names to corals in ecological and conservation studies, especially when labeling them as species new to a geographical area. This would make greater use of an open nomenclature, as indicated in the blue section, with specimens being labeled as closely familiar or having an affinity to a named species or as potentially a new record before being reported with apparent certainty.

It is unfortunate that Arulananthan et al. [1] (1) missed the present-day best available scientific knowledge, (2) used a marker that is considered not to be diagnostic at a species level for corals, and (3) relied on visual comparisons with *Corals of the World* [3] for their identifications at the species level without collecting voucher specimens. This strongly reduces the credibility of their results. While Veron's work [4] can serve as an initial reference, it is important to exercise caution in confirming the identity of many individual species. The study in its present form at most gives an indication of coral species that may be present at the reefs of the Jaffna Peninsula, Northern Sri Lanka, and for which further research, for example, for conservation purposes, is necessary to confirm their presence.

As Arulananthan et al. [1] stated, geographically distinct and long-neglected coastal regions can conceal remarkable biodiversity and therefore may provide a reservoir of genes that are capable of withstanding climate change-driven impacts. We agree that understanding the diversity of corals in Sri Lanka is important for the conservation of Sri Lanka's coral fauna. It is critical that taxonomic identifications are conducted in a robust way. Failure to correctly identify species can lead to misidentifying patterns of diversity and biogeography and potentially to the misdirection of scarce conservation resources. However, given the serious problems with both the morphological and molecular methods used to identify coral species by Arulananthan et al. [1], it is clearly premature to declare with certainty that this study reports on coral species previously not recorded in Sri Lanka.

Table 2. List of species recorded as ‘new’ by Arulananthan et al. [1], with type locality and potential subjective junior synonyms in Sri Lanka. Type localities marked with ‘?’ are based on the assumption that the collectors/authors of the older monographs have ambiguity over the type locality or the label attached to the type specimen.

Species Name with Authorities	Type Locality	Potential Junior Synonyms in Sri Lanka
Acroporidae Verrill, 1901		
<i>Acropora aspera</i> Dana, 1846 *	Fiji	<i>Acropora yeayamaensis</i> (Eguchi & Shirai, 1977), Japan; <i>Madrepora manni</i> (Quelch, 1886), Zamboanga, Philippines
<i>Acropora digitifera</i> Dana, 1846 *	Marshall Islands	<i>Acropora schmitti</i> (Wells, 1950), Cocos (Keeling) Islands,
<i>Acropora gemmifera</i> Brook, 1892	Rocky Islands, Great Barrier Reef, Australia	
<i>Acropora latistella</i> Brook, 1891 *	Port Denison, Great Barrier Reef, Australia	<i>Acropora imperfecta</i> (Nemenzo, 1971), Puerto Galera, Philippines; <i>Acropora loricata</i> (Nemenzo, 1967), Cebu, Philippines
<i>Acropora pulchra</i> Brook, 1891	Cocos (Keeling) Islands, Indian Ocean	
<i>Alveopora allingi</i> Hoffmeister, 1925	Pago Pago Harbour, American Samoa	
<i>Astreopora myriophthalma</i> Lamarck, 1816 *	Red Sea	<i>Astreopora stellae</i> (Nemenzo, 1964), Cebu, Philippines;
<i>Astreopora listeri</i> Bernard, 1896 *	Tonga	<i>Astreopora horizontalis</i> (Bernard, 1896), Seychelles
<i>Astreopora ocellata</i> Bernard, 1896	Torres Strait	
<i>Montipora flabellata</i> Studer, 1901	Hawaii	
<i>Montipora informis</i> Bernard, 1897	Torres Strait	
Merulinidae Verrill, 1865		
<i>Coelastrea palauensis</i> Yabe & Sugiyama, 1936	Palau	
<i>Cyphastrea japonica</i> Yabe & Sugiyama, 1936	Tanabe-wan, Wakayama-ken, subtropical Japan the Ocean around New Holland— possibly Western Australia	<i>Cyphastrea minuta</i> (Nemenzo & Ferraris, 1982), Cebu, Philippines
<i>Cyphastrea microphthalma</i> Lamarck, 1816 *	Mahe, Seychelles	
<i>Platygyra acuta</i> Veron, 2000	Indian Ocean?	
<i>Echinopora gemmacea</i> Lamarck, 1816	Tonga	<i>Barabattoia modesta</i> (Nemenzo, 1971), Cebu, Philippines
<i>Dipsastraea amicornum</i> Milne Edwards & Haime, 1850 *	Lizard Island, Great Barrier Reef	
<i>Dipsastraea lizardensis</i> Veron, Pichon & Wijsman-Best, 1977		
Pachyseridae Benzoni & Hoeksema, 2023 (Authors have listed Pachyseris gemmae Nemenzo, 1955 under Scleractinia incertae sedis but it is now under Pachyseridae Benzoni & Hoeksema, 2023 [28])		
<i>Pachyseris gemmae</i> Nemenzo, 1955	Bohol, Philippines	
Oulastreidae Vaughan, 1919		
<i>Oulastrea crispata</i> Lamarck, 1816	Indian Ocean	
Poritidae Gray, 1842		
<i>Porites evermanni</i> Vaughan, 1907	Oahu, Hawaii	
<i>Porites murrayensis</i> Vaughan, 1918	Mer Island, Torres Strait	
<i>Porites pukoensis</i> Vaughan, 1907	Molokai, Hawaii	
<i>Goniopora lobata</i> Milne Edwards, 1860	Red Sea	
<i>Goniopora minor</i> Crossland, 1952 (Considered as a junior subjective synonym of <i>G. pedunculata</i> Quoy and Gaimard, 1833 [28])	Great Barrier Reef	
<i>Goniopora somaliensis</i> Vaughan, 1907	French Somaliland	
<i>Goniopora tenuidens</i> Quelch, 1886	Zamboanga, Philippines	

Table 2. Cont.

Species Name with Authorities	Type Locality	Potential Junior Synonyms in Sri Lanka
Dendrophylliidae Gray, 1847		
<i>Turbinaria frondens</i> Dana, 1846 *	Fiji	<i>Turbinaria carcarenis</i> (Nemenzo, 1971), Cebu, Philippines; <i>Turbinaria contorta</i> (Bernard, 1896), South China Seas; <i>Turbinaria ramosa</i> (Yabe & Sugiyama, 1941), Meitu Minami-Naka-gun, Miyazaki-ken, Japan;
<i>Turbinaria reniformis</i> Bernard, 1896 *	Palm Islands, Great Barrier Reef	<i>Turbinaria rugosa</i> (Bernard, 1896), Formosa <i>Turbinaria disparata</i> (Bernard, 1896), Basay, Oriental Negros, Philippines;
<i>Turbinaria stelluta</i> Lamarck, 1816 *	American Ocean?	<i>Turbinaria globularis</i> (Bernard, 1896), Diego Garcia; <i>Turbinaria titizimaensis</i> (Yabe & Sugiyama, 1941), Ogasawa Islands, Japan; <i>Turbinaria nitida</i> (Nemenzo, 1960), Quezon, Philippines
Lobophylliidae Dai & Horng, 2009		
<i>Acanthastrea ishigakiensis</i> Veron, 1990 (Basionym <i>Acanthastrea ishigakiensis</i> is now accepted as <i>Lobophyllia ishigakiensis</i> [28])	Ishigaki Island, Japan	
<i>Micromussa amakusensis</i> Veron, 1990	Amaku Island, Japan	
Euphylliidae Milne Edwards & Haime, 1857 (Authors have listed <i>Coelosera mayeri</i> Vaughan, 1918 under the Family Agariciidae Gray, 1847 but it should be Euphylliidae Milne Edwards & Haime, 1857 [28])		
<i>Coelosera mayeri</i> Vaughan, 1918	Mer Island, Torres Strait	
Siderastreidae Vaughan & Wells, 1943 (Siderastreidae Vaughan & Wells, 1943 is now accepted as Rhizangiidae d'Orbigny, 1851 [28])		
<i>Siderastrea savignyana</i> Milne Edwards & Haime, 1849	Red Sea	

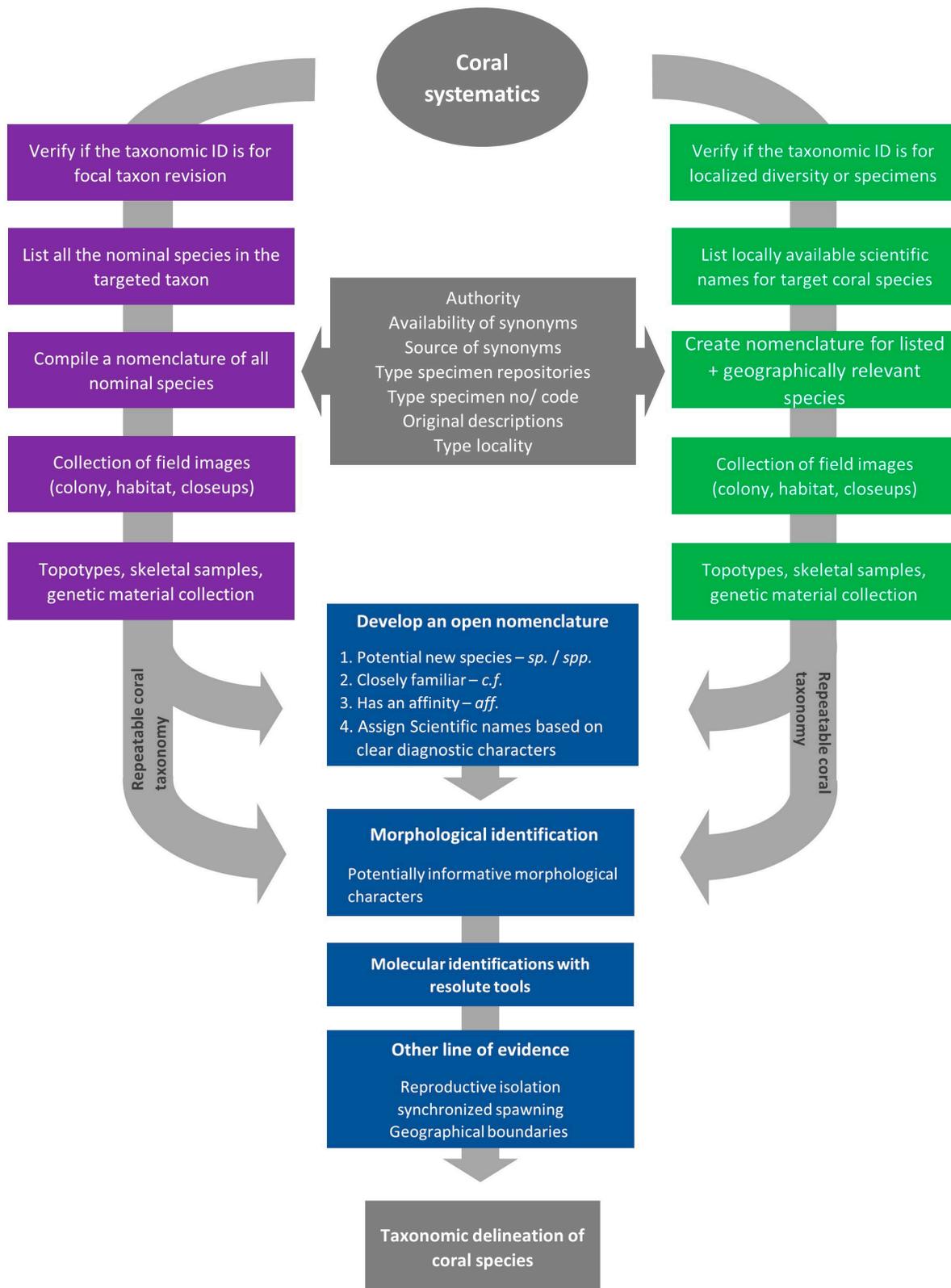


Figure 1. A schematic diagram illustrating a systematic, stepwise approach to coral taxonomy aimed at ensuring robustness, repeatability, and scalability. The two primary processes in coral taxonomy are indicated by distinct colors: purple represents taxonomic identification for focal taxon revisions, and green signifies taxonomic identification for localized diversity or specific specimens. The process indicated in blue is shared by both primary procedures.

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