**BIODIVERSITAS**
Volume 24, Number 1, January 2023
Pages: 47-54



ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240107

# DNA barcoding of the most common marine ornamental fish species spilled over from a small-sized marine protected area, Bali Barat National Park, Indonesia

**DEWA GEDE RAKA WIADNYA[1,♥], NIA KURNIAWAN[2], ANIK MARTINAH HARIATI[3], SEPTIANA SRI ASTUTI[4], AKHSAN FIKRILLAH PARICAHYA[2], MUHAMMAD DAILAMI[3], WAHYU ENDRA KUSUMA[3]**

[1]Department of Fisheries, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia. Tel.: +62-341-553512, Fax.: +62-341-557837, ♥email: dgr_wiadnya@ub.ac.id

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

[4]Graduate Program, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

Manuscript received: 30 September 2022. Revision accepted: 26 December 2022.

**Abstract.** *Wiadnya DGR, Kurniawan N, Hariati AM, Astuti SS, Paricahya AF, Dailami M, Kusuma WE. 2023. DNA barcoding of the most common marine ornamental fish species spilled over from a small-sized marine protected area, Bali Barat National Park, Indonesia. Biodiversitas 243: 47-54.* Healthy coral reefs within a protected area may function as a source of recruitment to the surroundings through spill-over and export larvae. This study aims to generate a list of common species of marine ornamental fishes for the aquarium trade in Indonesia, mainly collected adjacent to Bali Barat National Park (BBNP). Lived-fish samples were collected from six fish collectors in two villages surrounding BBNP, Sumber Kima, Bali, and Bangsring, Banyuwangi. All species were checked by three active fishermen in both villages for confirmation of the most common species and not belonging to the target. A total of 82 separate groups out of 145 specimens were identified based on their morphological characters. All samples were then sequenced based on mitochondrial cytochrome oxidase subunit I (COI) gene amplification. The sequence read lengths for all the specimens were between 655 and 658 bp. All the findings from this DNA barcoding approach were in line with morphological species identification. Wrasses (Family: Labridae) were the most dominant (41 species) representing the group, followed by Pomacentridae (24 species), Acanthuridae (14 species), Pomacanthidae (14 species) and Gobiidae (10 species). Among the species, only green sergeant, *Amblyglyphidodon batunaorum*, belonged to the Vulnerable (VU) category based on IUCN criteria. The rest were either Least Concern (LC), Data Deficient (DD), or Not Evaluated (NE). It implied that no catch species were under fishing pressure except for the green sergeant. Considering a total of 28 target species by local fishermen (recorded in an earlier article), small-scale fishermen can easily catch more than 100 species in one trip. The results significantly contributed to the need to generate a list of common ornamental fish species specifically exported from Indonesia as an aquarium trade commodity.

**Keywords:** Aquarium trade, fish diversity, fish-export commodities, reef fishery

## INTRODUCTION

With more than 39,500 km$^2$ of coral reefs, Indonesia's marine waters cover more than 15% of global coral reef areas (Burke et al. 2012). The reefs generally form around and protect more than 13,500 islands within the country and are categorized as fringing reefs (Madden et al. 2013). Australia is believed to have the most significant coral reef habitats in the world (approximately 42,000 km$^2$) but is not included as a priority area for marine biodiversity (Asaad et al. 2018). Together with five other countries (Malaysia, the Philippines, Timor Leste, Papua New Guinea, and the Solomon Islands), most of Indonesia's territorial waters are located within the Coral Triangle, a priority region for marine biodiversity conservation due to ecosystem and species richness, and endemicity as well (Asaad et al. 2018).

Indonesia's marine waters are home to more than 3600 marine-fish species which more than 70% are reef-associated (Froese and Pauly 2022). This strategically positioned coral reefs as an essential ecosystem to support capture fisheries in Indonesia as one of the most important sources of livelihood for coastal communities. Despite the importance, more than 90% of Indonesia's coral reefs are directly or indirectly threatened by human activities (Burke et al. 2012). Destructive and illegal, together with legal fishing practices, are the main threats to the coral reef ecosystem that cause over-fishing and degradation of supporting habitats for fisheries (Razak et al. 2022). Often attributed to other factors, a coral predator, *Acanthaster planci*, the outbreak is one of the main threats to coral reefs (Baird et al. 2013). Coastal developments, marine pollution, and sedimentations in many areas are non-point threats to coral reefs (Burke et al. 2012). Lastly, climate change through global warming can induce coral bleaching, causing coral death in many sites (Ferse et al. 2014). The national and local governments should provide resources for education on sustainable exploitation practices and protecting and conserving coral reefs by local communities who depend on them.

During the 3rd World National Park Congress in 1982, organized in Bali, the Government of Indonesia announced the formal enactment of 11 National Parks, including the smallest, Bali Barat (Cochrane 2013). The Bali Barat National Park (BBNP) covers a small coastal marine area of approximately 3415 ha (Suparno et al. 2019), predominantly covered by coral reefs, sea grass beds, and mangrove habitats (Doherty et al. 2013). The coral reefs inside the park experienced low coral cover due to a severe coral bleaching event in 1997 (Suparno et al. 2019) and local stressors such as physical damage to coral colonies and fishing gear (Boakes et al. 2022). However, surveys conducted in 2011 showed an increase in live coral cover. Furthermore, it resulted in double the fish biomass compared to the adjacent areas (Doherty et al. 2013). All coral reefs and other essential habitats inside BBNP must be protected from extractive use, as they should be. The more significant presence of park management inside the no-take zone and fishermen shifting into tourism activities may have contributed to the recently reduced threats in the park. Healthier fish communities in the park can act as a potential source of recruitment for the surrounding area (Russ et al. 2004; Goñi et al. 2010; Colléter et al. 2014), including ornamental reef fishes.

Ornamental fishes are included in the category of pets for non-consumption purposes, but with attractive appearance, behavior, shapes, and color, kept in transparent aquariums or similar open facilities. It has become a vital component of the highly commercial aquarium trade from the natural sources industry (Chen et al. 2019). Nowadays, fishermen predominantly from two villages, Sumber Kima in Bali and Bangsring in Banyuwangi East Java, continue to exploit coral reefs in the surrounding marine areas of BBNP for the ornamental reef (also called aquarium) fish trade (Lilley and Lilley 2007). These ornamental fish species are mainly intended for export needs and local hobbyists, to some extent. The live fish were packed and exported through Surabaya (East Java) or Denpasar (Bali) international airport (out of a total of eight international ports of embarkation in Indonesia). The final destinations of the fish are mainly Japan, the European Union, and the USA (Leal et al. 2016). Most export declarations needed to be completed with a species list (Biondo 2017).

Together with the Philippines, Indonesia is one of the world's biggest exporters of ornamental reef fish species (Biondo 2017). However, data monitoring systems still needed to be implemented, especially concerning the number and species involved in the trade. Information on species, through a combination of morphological and DNA barcoding approaches, is essential for management authority, especially the quarantine institution at the embarkation port that provides proper documentation for the export. Knowledge of reef fishes in Indonesia is mainly based on the work of two senior fish taxonomists in the country and region (Allen et al. 2018). Researches and records on DNA barcoding of marine ornamental fish species in Indonesia are minimal (Fadli et al. 2020), and none so far have come from the seven existing marine protected areas in Indonesia, those managed under the Ministry of Environment and Forestry. BBNP is a mainly terrestrial protected area and includes a small marine area. This study aims to investigate the most common ornamental fish species for aquarium trade through DNA barcoding and a morphological approach. The main reasonings for the study include: (i) Marine ornamental fishery is economically considered a living jewel means highly commercial species that significantly contributed to the income of producer countries (Bamaniya et al. 2016); (ii) Indonesia is one of the biggest exporters of marine ornamental fish species, but without monitoring system, especially species involved in the trade (Biondo 2017) that most likely due to limited knowledge on the species; (iii) continue recruitment of fish species can be attained from the benefit of marine protected area management, such as a national park - a small-sized national park (BBNP) can also attain benefit although only for local fishermen (Russ et al. 2004; Goñi et al. 2010; Colléter et al. 2014); (iv) Researches and records on DNA barcoding of marine reef fishes in Indonesia, especially ornamental species are very limited (Fadli et al. 2020). This study may significantly contribute to the need to generate a list of marine ornamental species for the aquarium trade.

## MATERIALS AND METHODS

### Sampling live fishes

A preliminary survey was conducted in April 2019 to fishermen in two villages (three fishers from Sumber Kima in Bali and the other three from Bangsring in Banyuwangi), together with catch collectors who bring the live fish to exporters. The local name for the most common species and price for each species category in the catches were listed based on information provided by local fishermen. The common (English) name for each local name was noted by local collectors or frequently called middlemen. Samplings for live fish were conducted from July 2019 to January 2020 in both villages (Figure 1). Lived-fish specimens were selected from three catch collectors (middlemen) in each village. All six collectors were presumed to be the biggest and genuinely represent catch species in the area. The selection of the collectors and fishermen in Sumber Kima Bali was based on advice provided by Gigih Setya Wibawa, a government official who knew the area very well. The selection of catch collectors and active fishers in Bangsring was based on advice given by Kusairi, a local indigenous person working as a dive instructor and naturalist in the area. Fishermen caught the fish in two ways: snorkeling with a canoe operated by one fisherman or a hookah compressor with a motorized boat operated by at least two fishermen. Both systems used the same gear, a fine mesh of a supple net. The catches were kept in plastic bags and maintained alive until they reached the collector.

A total of 145 specimens selected from collectors were separated into 82 groups based on their morphological characters and stored in 82 separate plastic bags with water-saturated oxygen. All the live fish specimens were transported with care for about eight hours before they reached the laboratory.

### Specimen digital photograph, morphological identification, fin biopsy, and preservation

In the laboratory, all fish specimens were teemed and killed in two steps, anesthetic (rotenone) and gradually decreasing the water temperature by adding ice outside the plastic bags (Bennett et al. 2016). When fishes finally died (no gill cover movement), a digital photograph was taken of each specimen to clearly show the main morphological characteristics of the species and as a supporting document for the morphological deposit specimen. Each specimen was placed on an acrylic marble slate (a modified fish-Foldio) with the head facing the left-hand side of the photo-taker, labeled with its specimen code and color scale. Morpho species for each specimen were determined and presumed based on the available taxonomic keys of the marine fishes (Carpenter 1999a, 1999b, 2001; Kuiter and Tonozuka 2001; Froese and Pauly 2022). Following tissue biopsy for DNA analysis, the whole-body specimens were wrapped with plastic bags, labeled, and stored frozen at around -25ºC. DNA and whole-body specimens were deposited in DIB (Depository Ichthyologicum Brawijaya), Faculty of Fisheries and Marine Science, Universitas Brawijaya. The dean formally approved the proposal, including all field and laboratory activities (4366/UN/F06/KS/2019). It also passed all the requirements for ethical clearance for animal study from Universitas Brawijaya (No. 102-KEP-UB-2020; signatory: Prof. Dr. Aulanni'am, DVM, DES).

### DNA extraction

Sample tissue, approximately 10-20 mg, was taken from the right pectoral fin. It was preserved in 96% alcohol and stored in a 1.5 mL microcentrifuge extraction tube. DNA extraction was carried out using the Wizard® Genomic DNA Purification Kit protocol (Promega A1120-LOT0000417067). A 120 µL EDTA 0.5 M (pH 8.0) and 500 µL Nuclei Lysis Solution were added into the 1.5 mL microtube, which was cooled down to -4ºC. Sample tissue (10-20 mg) was prepared in 1.5 mL microtubes by cutting them into small pieces and adding 600 µL EDTA/Nuclei Lysis Solution. A measure of 17.5 µL of proteinase K (Bio-37084, LOT-ES537-B088860) was added to accelerate cell lysis. Incubation was carried out overnight at an average temperature of 55ºC. A total of 600 µL of Protein Precipitation Solution (Promega Ref.A795A-LOT0000366672) was added following incubation, and then centrifugation was carried out for 4 minutes (13,000-16,000 x g). The supernatant DNA formed in the upper layer was transferred slowly to a new tube containing isopropanol (600 µL), and centrifuged for one minute (13,000-16,000 x g). A measure of 600 µL of 70% ethanol was added into the microcentrifuge tube containing DNA pellets, gently inverted several times to wash the DNA, and centrifuged for 1 minute (13,000-16,000 x g) at room temperature (27ºC). Finally, 100 µL of DNA Rehydration Solution (Promega: Ref. A796A-LOT0000397641) was added into the tube, followed by the rehydration process by incubating the DNA at 65°C for 1 hour.

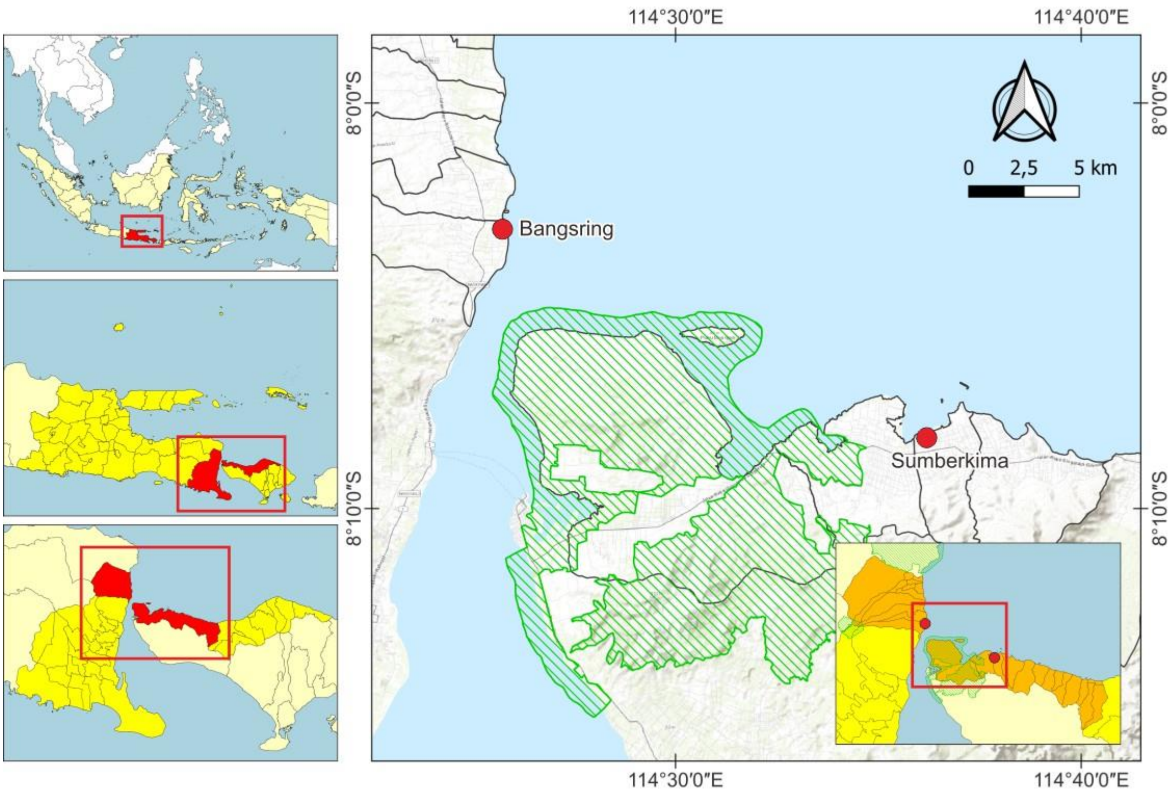


**Figure 1.** Terrestrial-marine outer boundary of Bali Barat National Park, Indonesia; i.e., Sumber Kima and Bangsring, two community villages that collect ornamental fishes adjacent to the park

**Polymerase Chain Reaction (PCR) amplification, electrophoresis, and DNA sequencing**

DNA amplification was carried out using mitochondrial DNA region COI. The PCR components consisted of GoTaq® Green Master Mix (Taq DNA polymerase, MgCl2, dNTPs) 5 µL, ddH2O 3.6 µL, and primer universal FishF1 -5'TCAACCAACCACAAAG-ACATTGGCAC3' and FishR1 -5'TAGACTTCTGGGTGGCCAAAGAATCA3' (Ward 2012). The thermal cycle of PCR consisted of a 2 min (94ºC) initial step with 35 cycles of 30 sec (94ºC), annealing for 50 sec (54ºC), elongation for 90 sec (72ºC), and final extension for 600 sec (72ºC). PCR products were visualized with 1.5% agarose gel. Electrophoresis gel consisted of 1.5% agarose, TBE 1X and 1 µL EtBr. Electrophoresis was conducted for about 30 minutes (90 volt) using a 3 µl PCR product. PCR product visualization was performed using a UV transilluminator. The sequencing process for the PCR product was carried out using the services of the genetic company Genetika Science Indonesia.

**Data sequence analysis**

Forward and reverse nucleotide sequences for each specimen were checked and edited based on Chromas software (www.technelysium.com.au) and EuGene (www.eugene.toulouse.inra.fr) to ensure accurate alignment and contig or consensus between forward and reverse and mesquite (www.mesquitproject.org) for the detection of a stop codon if present. The fix-aligned sequences were compared with sequences uploaded in GenBank (www.ncbi.nlm.nih.gov) through BLAST (Basic Local Alignment Search Tool) in combination with the Barcode of Life Data (BOLD) system (www.v4.boldsystems.org). The original (scientific) names and authorship of each species were based on the latest valid names, authorship, and dates confirmed on Eschmeyer's Catalog of Fishes (Eschmeyer et al. 2010). All the sequences were deposited in GenBank (www.ncbi.nlm.nih.gov).

**Target species, IUCN threatened, and CITES Appendix**

All the confirmed species through morphological examination and BLAST were listed (Table 1) and compared with the target list of catch species formerly published in Lilley and Lilley (2007). A photograph of each species was also shown to three fishermen from each village, and they finally judged whether a species belonged to the target or just a common catch. All fishermen can quickly determine the target specie because of their higher price at local collectors. Lastly, an analysis of IUCN red data list criteria (www.iucnredlist.org) and CITES Appendixes (www.cites.org) was performed for each species to evaluate their global threatened status (Froese and Pauly).

## RESULTS AND DISCUSSION

**Results**

In total, 143 specimens were successfully sequenced (on average, 1.7 sequences per species), with sequence read lengths of 655-659 bp. The average nucleotide composition was as follows: A: 23.55%; T: 29.52%; G: 18.48% and C: 28.44%. The base compositions showed that the AT content (53.08%) was slightly higher than the GC content (46.92%). No insertions, deletions, or codon stops were found during the initial sequence analysis. Based on the results from BLAST in GenBank (www.ncbi.nlm.nih.gov) and BOLD (www.v4.bildsystems.org), it showed that all sequences confirmed the species with >98.5% similarity.

This study confirmed 82 ornamental reef species consisting of 52 genera and 18 different families using morphological examination and DNA sequence analysis (Table 1). Labridae was the family with the most dominant species found in the catch (41 species), followed by Pomacentridae (24 species), Pomacanthidae and Acanthuridae (each with 14 different species), and Gobiidae (10 species). The scientific name for Batuna's damselfish, *Amblyglyphidodon batunai*, was replaced and newly accepted as *Amblyglyphidodon batunaorum* (Parenti 2021). Bicolor chromis, *Chromis margaritifer*, still used in GenBank, was renamed and accepted as *Pycnochromis margaritifer* (Tang et al. 2021). Lastly, the Spine-cheeck anemonfish, *Premnas biaculeatus*, was updated to *Amphiprion biaculeatus* (Tang et al. 2021). For all the scientific names remained the same for the rest of the list (Table 1). Fishermen and collectors stated that these 82 species are the most common ornamental fishes for aquarium trade caught from the surrounding marine waters of BBNP.

Among the 82 species confirmed in this study, there were no species included in the CITES Appendix (www.checklist.cites.org), accessed on November 2$^{nd}$, 2021). *Amblyglyphidodon batunaorum*, formerly *A. batunai*, falls under the Vulnerable (VU) category within the IUCN red data list status under criteria A2bc (decreased population size, declined number of mature individuals, and trend of habitat degradation (www.iucnredlist.org, accessed on July 2022). Most species (79.3%) are in the Least Concern (LC) category, 15.9% have never been evaluated (NE), and 3.7% have been evaluated, but the results were not sufficient (DD) to determine the category.

**Table 1**. DNA deposit specimen, number of sequenced specimens (N), GenBank accession number and species scientific name of the ornamental reef fishes caught from the surrounding marine waters of Bali Barat National Park, Indonesia

| DNA deposit | N | Acession number | Species name |
|---|---|---|---|
| AEI_01 | 1 | OP062130 | *Abudefduf vaigiensis* |
| ABN_01; ACJ_01 | 2 | OP062108; OP062119 | *Acanthurus auranticavus* |
| AAN_01 | 1 | OP062088 | *Acanthurus blochii* |
| ABD_05 | 1 | OP062100 | *Acanthurus nigricauda* |
| OES_01 | 1 | OP062154 | *Acanthurus olivaceus* |
| CSF_01 | 1 | OP062144 | *Acanthurus pyroferus* |
| ACI_01 | 1 | OP062124 | *Acanthurus triostegus* |
| AAM_01 | 1 | OP062087 | *Amblyeleotris guttata* |
| ADL_01; 03 | 2 | OP062165; OP062166 | *Amblyglyphidodon batunaorum* |
| ABB_01-02 | 2 | OP062161; OP062162 | *Amblygobius decussatus* |
| AAV_01-02 | 2 | OP062095; OP062096 | *Amphiprion biaculeatus* |
| YSW_01-02 | 2 | OP062219; OP062220 | *Anampses meleagrides* |
| AAD_01-02 | 2 | OP062082; OP062083 | *Apolemichthys trimaculatus* |
| SBH_01 | 1 | OP062203 | *Bodianus bilunulatus* |
| GRF_06-10 | 5 | OP062181-5(*) | *Bodianus bimaculatus* |
| AAO_01; EHF_01; 03-04 | 4 | OP062089; OP062171-3(*) | *Bodianus mesothorax* |
| ACU_01 | 1 | OP062127 | *Caesio teres* |
| YBF_01 | 1 | OP062215 | *Caesio xanthonota* |
| LTB_03-04 | 2 | OP062186; OP062187 | *Canthigaster bennetti* |
| ACK_01 | 1 | OP062125 | *Centropyge eibli* |
| MAF_02 | 1 | OP062152 | *Centropyge fisheri* |
| MSA_02 | 1 | OP062153 | *Centropyge nox* |
| ABM_01; PSA_01-02 | 3 | OP062107; OP062196; OP062197 | *Centropyge vrolikii* |
| AAX_01-02 | 2 | OP062097; OP062098 | *Chaetodon bennetti* |
| AAI_01 | 1 | OP062085 | *Chaetodon melannotus* |
| ACF_01 | 1 | OP062122 | *Chaetodontoplus mesoleucus* |
| AAK_01; 06-07 | 3 | OP062155; OP062156; OP062157 | *Chelmon rostratus* |
| SFW_01-02; 04-05 | 4 | OP062188-91(*) | *Cirrhilabrus cyanopleura* |
| FWF_01 | 1 | OP062176 | *Cirrhilabrus flavidorsalis* |
| FWF_02 | 1 | OP062177 | *Cirrhilabrus lubbocki* |
| RBW_01 | 1 | OP062198 | *Cirrhilabrus rubrimarginatus* |
| FFW_01-02 | 2 | OP062147; OP062148 | *Cirrhilabrus rubripinnis* |
| RCW_01-02 | 2 | OP062199; OP062100 | *Coris gaimard* |
| ABZ_01-02 | 2 | OP062114; OP062114 | *Cromileptes altivelis* |
| AAU_04 | 1 | OP062158 | *Cryptocentrus cinctus* |
| TSS_01; SCG_01-02 | 3 | OP062210; OP062206; OP062207 | *Ctenochaetus binotatus* |
| ACB_01-02 | 2 | OP062116; OP062117 | *Ctenochaetus striatus* |
| ABJ_01 | 1 | OP062105 | *Cyprinocirrhites polyactis* |
| AAR_01-02; AEM_03 | 3 | OP062091; OP062092; OP062132 | *Dascyllus aruanus* |
| AEL_01; BTH_01-02 | 3 | OP062132; OP062142; OP062143 | *Dascyllus melanurus* |
| RTD_01-02 | 2 | OP062201; OP062202 | *Dascyllus reticulatus* |
| ZFL_01; 03 | 2 | OP062223; OP062224 | *Dendrochirus zebra* |
| ABI_01 | 1 | OP062104 | *Dischistodus melanotus* |
| AAC_01 | 1 | OP062081 | *Genicanthus lamarck* |
| ADF_01-02 | 2 | OP062163; OP062164 | *Halichoeres chloropterus* |
| ABQ_01-02; CWS_01 | 3 | OP062111; OP062112; OP062145 | *Halichoeres hortulanus* |
| ACC_01 | 1 | OP062145 | *Halichoeres melanurus* |
| TTW_01-02 | 2 | OP062211; OP062212 | *Halichoeres prosopeion* |
| AAJ_01 | 1 | OP062086 | *Hemitaurichthys polylepis* |
| LBF_01; 04 | 2 | OP062150; OP062151 | *Heniochus diphreutes* |
| BLW_02 | 1 | OP062139 | *Macropharyngodon negrosensis* |
| AMW_04-05 | 2 | OP062139; OP062168 | *Macropharyngodon ornatus* |
| FCF_01 | 1 | OP062146 | *Malacanthus latovittatus* |
| AAY_01-02 | 2 | OP062159; OP062160 | *Meiacanthus grammistes* |
| ABK_01 | 1 | OP062106 | *Neoglyphidodon melas* |
| ACM_01 | 1 | OP062126 | *Novaculichthys taeniourus* |
| ACE_01-02 | 2 | OP062120; OP062121 | *Paracanthurus hepatus* |
| YFW_02-03 | 2 | OP062217; OP062118 | *Paracheilinus flavianalis* |
| DWF_01 | 1 | OP0622179 | *Pentapodus caninus* |
| CMF_01-02 | 2 | OP062169; OP062170 | *Plagiotremus rhinorhynchos* |
| SBS_01 | 1 | OP062204 | *Plectorhinchus albovittatus* |
| SBS_03 | 1 | OP062205 | *Plectorhinchus vittatus* |

| | | | |
|---|---|---|---|
| ABS_01; ACS_01 | 2 | OP062113; OP062103 | *Pomacanthus imperator* |
| ABE_01-02 | 2 | OP062101-2; OP062102 | *Pomacanthus sexstriatus* |
| GBD_01; 03-04 | 3 | OP062178; OP062179; OP062180 | *Pomacentrus caeruleus* |
| YDF_01; 03 | 2 | OP062213; OP062214 | *Pomacentrus moluccensis* |
| ADE_01-02 | 2 | OP062128; OP062129 | *Pomacentrus xanthosternus* |
| FBF_01-02 | 2 | OP062174; OP062174 | *Pseudanthias dispar* |
| HAF_02 | 1 | OP062149 | *Pseudanthias huchtii* |
| AAS_05-06 | 2 | OP062093; OP062094 | *Pseudanthias rubrizonatus* |
| SWC_02-03 | 2 | OP062194; OP069125 | *Pseudocheilinus evanidus* |
| AAH_01; BGD_01; 03 | 3 | OP062084; OP062135; OP062136 | *Ptereleotris heteroptera* |
| SFL_01-02 | 2 | OP062208 | *Pterois antennata* |
| BCF_01-02 | 2 | OP062133; OP062134 | *Pycnochromis margaritifer* |
| ABO_03-04 | 2 | OP062109; OP062110 | *Rhinecanthus verrucosus* |
| ABA_02 | 1 | OP062099 | *Salarias fasciatus* |
| ACH_01 | 1 | OP062123 | *Siganus puellus* |
| AAP_01 | 1 | OP062090 | *Symphorichthys spilurus* |
| BHW_03-04 | 2 | OP062137 | *Thalassoma amblycephalum* |
| SSG_01-02 | 2 | OP062192; OP062193 | *Valenciennea sexguttata* |
| BSG_03-04 | 2 | OP062140; OP062141 | *Valenciennea strigata* |
| YEL_01 | 1 | OP062216 | *Variola louti* |

Note: (*) indicate sequential accession numbers, if it would have been written as such, cannot be accommodated in the column-table, Ex: OP062181-5(*) means that it consists of five sequential accession numbers: OP062181; OP062182; OP062183; OP062184, and OP062185

**Discussions**

This study has confirmed 82 marine ornamental fish species recruited from BBNP to the surrounding areas. A similar study based on 54 specimens collected from local markets of Pelabuhan Ratu and Ujung Genteng West Java, found a total number of 46 species (Nuryanto et al. 2021) from which only four species were found to be similar to marine ornamental fishes in the surrounding BBNP. The species were: *Abudefduf vaigiensis* (Pomacentridae), *Centropyge eibli* (Pomacanthidae), *Chelmon rostratus* (Chaetodontidae), and *Dendrochirus zebra* (Scorpaenidae). Another barcoding study was also very recently conducted by Sektiana et al. (2022) based on 53 specimens collected from Pulau Tabuhan, just outside BBNP. BLAST analysis has resulted in 49 confirmed species, where 23 out of 49 overlapped with this study's findings. This higher number of similarities is reasonable because the study area overlapped. On the contrary, the survey by Nuryanto et al. (2021) revealed only four species were found similar to this study as the distance was far from this current study. All the findings from these studies proved the high species diversity of marine ornamental fish species in Indonesia, which support the country as one of the biggest exporters of ornamental fish species (Biondo 2017).

The global market for marine ornamental fishes has existed since the 1930s; however, the number of species involved still needs to be determined (Palmtag 2017). The global trade of most marine ornamental fishes is made possible through wild-caught animals from coral reefs (Pouil et al. 2020). The impact of this removal cannot be understood well due to a lack of data on commerce, such as a species list and the number of each species sold. Marine aquarium trade can be a direct threat to the reef ecosystem. This global trade deals with the wild capture of over 1800 ornamental reef fishes from 40 countries (Biondo 2017; Biondo and Burki 2020). Indonesia, the Philippines, and Sri Lanka are the three leading exporters of marine ornamental fishes (King 2019).

As mentioned above, BBNP (Figure 1) covers a small coastal marine area of approximately 3415 ha (Suparno et al. 2019) with healthy corals and ornamental reef fishes. Tourism activities in the park led to an increased presence of park management officials (Weeks et al. 2014) and, consequently, reduced the number of violations of the marine no-take zone regulations. Higher compliance can expect healthy reefs and their fishes to act as a source of recruitment to the adjacent area, especially ornamental reef fishes for the aquarium trade (Di-Lorenzo et al. 2016). Coral reefs surrounding the western part of BBNP are exploited by fishermen from Bangsring Village, mainly for the aquarium trade. In the eastern part, the fishing community from Sumber Kima is the primary user with a similar goal. More than 80% of villagers in Sumber Kima earn their living from ornamental reef fisheries near BBNP (Lilley and Lilley 2007). There is no published study on the actual figure for Bangsring, but observation showed an almost comparable situation. The potential contribution of the park as a source of income for locals is crucial and plays a pivotal role in sustainably maintaining the local economy.

Fishermen from Sumber Kima listed 28 ornamental fish species as their main target (Lilley and Lilley 2007) composed of 10 different families and most dominated by Balistidae (7 species) followed by Pomacanthidae (5 species) and Syngnathidae (4 species). Three target species were found to overlap with the common catch species in the findings of this study. Combining the common catch (82 species) in this study with target species of fishermen in Sumber Kima Bali (28 species) (Lilley and Lilley 2007), although considering the overlapping between the two, it can be effortless to have more than 100 species of marine ornamental fishes from Sumber Kima alone.

Analysis of the IUCN rareness status of all 82 species resulted in only one species, *A. batunaorum*, that falls under the vulnerable (VU) category (Froese and Pauly 2022) based on A2bc criteria. This status concluded a population reduction during the last three generations for a value of more than 30% (www.iucnredlist.org, criteria A2). Furthermore, scientists also observed a declining area of occupancy (criteria b) and a decrease in habitat quality (criteria c). The species is closely associated with *Acropora* staghorn-type coral, and typical habitat used to lay their eggs and guard it (Kuiter and Tonozuka 2003). Massive coral mortality, especially *Acropora*, and degradation of coral reef habitat occurred mainly in Papua New Guinea (Haywood et al. 2016). This limited area, although massive, has led IUCN to place *A. batunaorum* as vulnerable. The remaining 81 species belonged to Least Concern (LC) category (63 species), data deficient (DD) (three species), and even not evaluated (NE) yet (13 species).

*Amblyglyphidodon batunaorum* (green sergeant, also called green damsel) was included in the list of 82 common species of ornamental reef fishes caught adjacent (outside) to BBNP (this study) but was not in the list of target species, especially for fishermen from Sumber Kima Village. Its Exclusion from target species implied that the local population may still be stable and with no additional stress factors other than common species in the catch. Fishermen from both villages did not recognize any market preference (indicated by a better price) for *A. batunaorum* and thus did not list it as a target species (Lilley and Lilley 2007). The local common names for this Batuna's damselfish were betok, betok laut, padi-padi, and tibok, referring to damselfish (Froese and Pauly 2022). This is a well-known species with a low price amongst local collectors and fishermen from both villages, who have yet to experience a better price for *A. batunaorum*.

*Cromileptes altivelis* and *Variola louti* were two species in the common catch that also targeted for live-reef fish trade (Kindsvater et al. 2017). Additionally, *Siganus*, *Plectorhinchus,* and *Caesio* were listed in the national statistics of fisheries (edible food). Ornamental reef fisheries for the aquarium trade may threaten the global live-reef fish trade (Khasanah et al. 2019) and national (food) commercial fisheries. Marine Protected Areas (MPAs) such as BBNP may be a good way to restore the source of recruitment when over-fishing occurs outside MPAs (Russ et al. 2004; Goñi et al. 2010; Colléter et al. 2014).

Over 82 reef ornamental fishes were confirmed as common catch species for aquarium trade collected adjacent to a small-sized marine protected area, BBNP. Considering the 28 targeted species by local fishermen, only three were found to overlap with common species. So, it would not be surprising to have more than 100 species spilled over from approximately 3415 ha of coastal marine waters of BBNP. Among 82 species, only green sergeant, *A. batunaorum*, globally belongs to the Vulnerable (VU) category based on IUCN A2bc criteria. However, the species was frequently caught by fishermen from both villages and was not listed as a target species. If the reef health inside the park's no-take zone can be maintained, in the long run, it can be a source of recruitment to the surrounding marine waters.

## ACKNOWLEDGEMENTS

We appreciate all the support provided by Gigih Setya Wibawa, who accompanied and connected the authors to local fishermen and fish collectors. We also thank Andreas Hary Muljadi, a local fish expert, for helping in the determination of some species that the authors were not sure of.

## REFERENCES

Allen GR, Erdmann MV, Hidayat NI. 2018. *Pomacentrus Bellipictus*, a new microendemic species of damselfish (Pisces: Pomacentridae) from the Fakfak Peninsula, West Papua, Indonesia. J Ocean Sci Found 30: 1-10. DOI: 10.5281/zenodo.1246885.

Asaad I, Lundquist CJ, Erdmann MV, Costello MJ. 2018. Delineating priority areas for marine biodiversity conservation in the coral triangle. Biol Conserv 222: 198-211. DOI: 10.1016/j.biocon.2018.03.037.

Baird AH, Pratchett MS, Hoey AS, Herdiana Y, Campbell SJ. 2013. Acanthaster planci is a major cause of coral mortality in Indonesia. Coral Reefs 32 (3): 803-812. DOI: 10.1007/s00338-013-1025-1.

Bamaniya DC, Pavan-Kumar A, Gireesh-Babu P, Sharma N, Reang D, Krishna G, Lakra WS. 2016. DNA barcoding of marine ornamental fishes from India. Mitochondrial DNA Part A 27 (5): 3093-3097. DOI: 10.3109/19401736.2014.1003923.

Bennett RH, Ellender BR, Mäkinen T, Miya T, Pattrick P, Wasserman RJ, Woodford DJ, Weyl OLF. 2016. Ethical considerations for field research on fishes. Koedoe 58 (1): 1-15. DOI: 10.4102/koedoe.v58i1.1353.

Biondo MV. 2017. Quantifying the Trade in Marine Ornamental Fishes into Switzerland and an estimation of imports from the European Union Glob Ecol Conserv 11: 95-105. DOI: 10.1016/j.gecco.2017.05.006.

Biondo MV, Burki RP. 2020. A systematic review of the ornamental fish trade with emphasis on coral reef fishes-An impossible task. Animals 10 (11): 2014. DOI: 10.3390/ani10112014.

Boakes Z, Hall AE, Ampou EE, Jones GCA, Suryaputra IGNA, Mahyuni LP, Prasetijo R, Stafford R. 2022. Coral reef conservation in bali in light of international best practice, a literature review. J Nat Conserv 67: 126190. DOI: 10.1016/j.jnc.2022.126190.

Burke L, Reytar K, Spalding K, Perry A. 2012. The Nature Conservancy. World-Fish Center, International Coral Reef Action Network. UNEP World Conservation Monitoring Centre and Global Coral Reef Monitoring Network,Washington, DC.

Carpenter KE, Niem VH. 1999a. The Living Marine Resources of the Western Central Pacific, Volume 4: Bony Fishes Part 2 (Mugilidae to Carangidae). FAO, Rome, Italy.

Carpenter KE, Niem VH. 1999b. 3 FAO Species Identification Guide for Fishery Purposes. FAO, Rome, Italy.

Carpenter KE, Niem VH. 2001. The Living Marine Resources of the Western Central Pacific, Volume 5: Bony Fishes Part 3 (Menidae to Pomacentridae). FAO, Rome, Italy.

Chen JY, Zeng C, Jerry DR, Cobcroft JM. 2019. Recent advances of marine ornamental fish larviculture: Broodstock reproduction, live prey and feeding regimes, and comparison between demersal and pelagic spawners. Rev Aquac 12 (3): 12394. DOI: 10.1111/raq.12394.

Cochrane J. 2013. Exit the dragon? Collapse of co-management at Komodo National Park, Indonesia. Tour Recreat Res 38 (2): 127-143. DOI: 10.1080/02508281.2013.11081740.

Colléter M, Gascuel D, Albouy C, Francour P, de Morais L, Valls A, Le Loc'h F. 2014. Fishing inside or outside? A case studies analysis of potential spillover effect from marine protected areas, using food web models. J Mar Syst 139: 383-395. DOI: 10.1016/j.jmarsys.2014.07.023.

Di-Lorenzo M, Claudet J, Guidetti P. 2016. Spillover from marine protected areas to adjacent fisheries has an ecological and a fishery component. J Nat Conserv 32: 62-66. DOI: 10.1016/j.jnc.2016.04.004

Doherty O, Milner C, Dustan P, Campbell S, Pardede S, Kartawijaya T, Alling A. 2013. Report on Menjangan Island's coral reef: A Bali Barat National Park marine protected area. Atoll Res Bull 599 (599): 1-18. DOI: 10.5479/si.00775630.599.

Eschmeyer WN, Fricke R, Fong JD, Polack D. 2010. Marine fish diversity: History of knowledge and discovery (Pisces). Zootaxa 2525 (1): 19. DOI: 10.11646/zootaxa.2525.1.2.

Fadli N, Nor SAM, Othman AS, Sofyan H, Muchlisin ZA. 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. PeerJ 8: e9641. DOI: 10.7717/peerj.9641.

Ferse SCA, Glaser M, Neil M, Schwerdtner MK. 2014. To cope or to sustain? Eroding long-term sustainability in an indonesian coral reef fishery. Reg Environ Chang 14 (6): 2053-2065. DOI: 10.1007/s10113-012-0342-1.

Froese R, Pauly D. 2022. Fishbase: World Wide Web Electronic Publication. www.Fishbase.org.

Goñi R, Hilborn R, Díaz D, Mallol S, Adlerstein S. 2010. Net contribution of spillover from a marine reserve to fishery catches. Mar Ecol Prog Ser 400: 233-243. DOI: 10.3354/meps08419.

Haywood MDE, Dennis D, Thomson DO, Pillans RD. 2016. Mine waste disposal leads to lower coral cover, reduced species richness and a predominance of simple coral growth forms on a fringing coral reef in Papua New Guinea. Mar Environ Res 115: 36-48. DOI: 10.1016/j.marenvres.2016.02.003.

Khasanah M, Kadir NN, Jompa J. 2019. Reproductive biology of three important threatened/near-threatened groupers (*Plectropomus leopardus*, *Epinephelus polyphekadion* and *Plectropomus areolatus*) in Eastern Indonesia and implications for management. Animals 9 (9): 643. DOI: 10.3390/ani9090643.

Kindsvater HK, Reynolds JD, de Mitcheson YS, Mangel M. 2017. Selectivity Matters: Rules of thumb for management of plate-sized, sex-changing fish in the live reef food fish trade. Fish Fish 18 (5): 821-836. DOI: 10.1111/faf.12208.

King TA. 2019. Wild caught ornamental fish: A perspective from the UK ornamental aquatic industry on the sustainability of aquatic organisms and livelihoods. J Fish Biol 94 (6): 925-936. DOI: 10.1111/jfb.13900.

Kuiter RH, Tonozuka T. 2001. Pictorial Guide to Indonesian Reef Fishes. PT Dive & Dive's, Bali.

Kuiter RH, Tonozuka T. 2003. Pictorial Guide to: Indonesian Reef Fishes Part 1, 2, 3. PT. Dive & Dive's, Bali.

Leal MC, Vaz MCM, Puga J, Rocha RJM, Brown C, Rosa R, Calado R. 2016. Marine ornamental fish imports in the European Union: An economic perspective. Fish Fish 17 (2): 459-468. DOI: 10.1111/faf.12120.

Lilley GR, Lilley R. 2007. Towards a sustainable marine aquarium trade: An indonesian perspective. SPC Live Reef Fish Inf Bull 17: 11-19.

Madden RHC, Wilson MEJ, O'Shea M. 2013. Modern fringing reef carbonates from equatorial SE Asia: An integrated environmental, sediment and satellite characterisation study. Mar Geol 344: 163-185. DOI: 10.1016/j.margeo.2013.07.001.

Nuryanto A, Kusbiyanto K, Bhagawati D. 2021. Molecular barcoding of marine ornamental fish from the southern coast of West Java validates conventional identification. E3S Web Conf 322: 01004. DOI: 10.1051/e3sconf/202132201004.

Palmtag MR. 2017. The marine ornamental species aquaculture trade 3. In: Calado R, Olivotto I, Oliver MP, Holt GJ (Eds). Marine Ornamental Species Aquaculture. John Wiley & Sons, Inc., New York.

Parenti P. 2021. An annotated checklist of damselfishes, Family Pomacentridae Bonaparte, 1831. J Anim Divers 3 (1): 37-109. DOI: 10.52547/JAD.2021.3.1.6.

Pouil S, Tlusty MF, Rhyne AL, Metian M. 2020. Aquaculture of marine ornamental fish: Overview of the production trends and the role of academia in research progress. Rev Aquac 12 (2): 1217-1230. DOI: 10.1111/raq.12381.

Razak TB, Boström-Einarsson L, Alisa CAG, Vida RT, Lamont TAC. 2022. Coral reef restoration in Indonesia: A review of policies and projects. Mar Policy 137: 104940. DOI: 10.1016/j.marpol.2021.104940.

Russ GR, Alcala AC, Maypa AP, Calumpong HP, White AT. 2004. Marine reserve benefits local fisheries. Ecol Appl 14 (2): 597-606. DOI: 10.1890/03-5076.

Sektiana SP, Abdillah AA, Alam MJ, Isroni W, Dewi NN, Kim HW, Andriyono S. 2022. Diversity of the Tabuhan Island coral reef fish revealed by DNA barcoding and implication on conservation strategy in Banyuwangi, Indonesia. Biodiversitas 23 (9): 4844-4851. DOI: 10.13057/biodiv/d230953.

Suparno N, Currier K, Milner C, Alling A, Dustan P. 2019. Ecological changes in the coral reef communities of Indonesia's Bali Barat National Park, 2011-2016. Atoll Res Bull 620: 1-35. DOI:

Tang KL, Stiassny MLJ, Mayden RL, DeSalle R. 2021. Systematics of damselfishes. Ichthyol Herpetol 109 (1): 258-318. DOI: 10.1643/i2020105.

Ward RD. 2012. FISH-BOL, A case study for DNA barcodes. In: Kress DL, Erickson WJ (Eds). DNA Barcodes. Springer, Berlin.

Weeks R, Aliño PM, Atkinson S, Beldia P, Binson A, Campos WL, Djohani R, Green AL, Hamilton R, Horigue V, Jumin R, Kalim K, Kasasiah A, Kereseka J, Klein C, Laroya L, Magupin S, Masike B, Mohan C, Da Silva PRM, Vave-Karamui A, Villanoy C, Welly M, White AT. 2014. Developing marine protected area networks in the coral triangle: Good practices for expanding the coral triangle marine protected area system. Coast Manag 42 (2): 183-205. DOI: 10.1080/08920753.2014.877768.