Journal of Marine Science and Technology

Volume 21
Issue 7 *Vol 22, Supplemental*

Article 34

DNA BARCODING OF COASTAL LARVAL FISH COMMUNITIES OF DONGSHAR ISLAND, SOUTH CHINA SEA REVEALED BY MITOCHONDRIAL CO I SEQUENCES

I-Shiung Chen
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

Kwang-Tsao Shao
Biodiversity Research Centre, Academia Sinica, Taipei, Nankang, Taiwan, R.O.C., zoskt@gate.sinica.edu.tw

Shao-Liang Hsu
Institute of Ocean Technology and Marine Affairs, National Cheng-Kung University, Tainan, Taiwan, R.O.C

Gwo-Ching Gong
Institute of Marine Environmental Chemistry and Ecology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.
Taiwan Ocean Research Institute, National Applied Research Laboratories, Taiwan, R.O.C.

Yu-Chih Chen
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

See next page for additional authors

Recommended Citation

Chen, I-Shiung; Shao, Kwang-Tsao; Hsu, Shao-Liang; Gong, Gwo-Ching; Chen, Yu-Chih; and Liu, Ta-Kang (2013) "DNA BARCODING OF COASTAL LARVAL FISH COMMUNITIES OF DONGSHAR ISLAND, SOUTH CHINA SEA REVEALED BY MITOCHONDRIAL CO I SEQUENCES," *Journal of Marine Science and Technology*: Vol. 21 : Iss. 7 , Article 34.
DOI: 10.6119/JMST-013-1223-3
Available at: https://jmstt.ntou.edu.tw/journal/vol21/iss7/34

DNA BARCODING OF COASTAL LARVAL FISH COMMUNITIES OF DONGSHAR ISLAND, SOUTH CHINA SEA REVEALED BY MITOCHONDRIAL CO I SEQUENCES

Acknowledgements

We are very grateful for Mr. K. T. Chen, Mr. K. Hsu, Mr. S. P. Huang, and Mr. Y. H. Gong for their kindly helping on setting the light trap and preservation on the Dongsha Island, South China Sea during March 2011 to March 2012. The current research is partly granted from National Science Council, Taipei for first author. The research is partially also supported by the Marine Biodiversity team from CMBB & CEO, NTOU as well as the research project of Marine National Park Headquarters.

Authors

I-Shiung Chen, Kwang-Tsao Shao, Shao-Liang Hsu, Gwo-Ching Gong, Yu-Chih Chen, and Ta-Kang Liu

Journal of Marine Science and Technology, Vol. 21, Suppl., pp. 252-257 (2013)
DOI: 10.6119/JMST-013-1223-3

DNA BARCODING OF COASTAL LARVAL FISH COMMUNITIES OF DONGSHA ISLAND, SOUTH CHINA SEA REVEALED BY MITOCHONDRIAL CO I SEQUENCES

I-Shiung Chen[1], Kwang-Tsao Shao[2], Shao-Liang Hsu[3], Gwo-Ching Gong[4, 5], Yu-Chih Chen[1], and Ta-Kang Liu[3]

Key words: larval fish identification; DNA barcoding; mtDNA; CO I sequences.

ABSTRACT

The 70 different samples of larval fish morphotypes have been successfully amplified and sequenced in detail via PCR amplification for the partial sequences of mtDNA CO I gene. The overall genetic similarities ranged from 83% to 100%. Totally 26 different fish species can be detected and analyzed. There are 16 fish species can be identified into specific level. However, there are remaining 12 species can be expected just into suggested fish generic level. Apparently, two major groups of taxa would be the members of Gobiidae and Apogonidae which comprised 50% of total species diversity, and also comprised 67% of total fish samples. The promising method seems to gather reliable information for further development of larval fish identification in further fish resources study.

I. INTRODUCTION

Marine fish biodiversity have been addressed the tremendous species diversity especially on tropical and subtropical regions. The species identification of marine fishes would be very difficult for the different degree of larval stages for their own developmental process for surviving in the marine habitats due to lacking any further studies for developmental metamorphosis for most of marine fish species. Marine larval fish identification would be a very difficult task among huge species diversity of teloest fishes if the research is merely based on limited morphological observation of field sampling larval fish specimens [12, 13].

In the recent decade, the molecular biological analysis of mitochondrial genome for fish biological studies and systematic have yielded great progress for phylogenetic analysis as well as fish identification among their cryptic species within a certain systematic clade [6, 8, 12, 13]. Nowadays, the mitogenomic CO I sequences have been more intensive survey for gathering the important database for more huge variety of taxa in different animals and plants [1, 2, 4, 6, 12-14]. In order to realize the reproduction characteristics, sustainable fishery resources and migratory seasonality of marine coral-reef fish fauna, we start to employ the molecular sequence tools for clarifying and sorting the real fish biodiversity for the research region for detailed survey of larval fish fauna. To realize the true species diversity of larval fish communities faces the great difficulty for further morphological identification. No further information to know which kinds of or and when the larval fishes will approach to the coastal region.

The aim of this paper is try to document the real species diversity from coastal samples of larval fish communities via recent research based on the proof of mitochondrial DNA CO I sequences in the Dongsha island, South China Sea for further employment for full seasonal exchange for detecting the reproduction characteristics for marine fishes in the coral-reef habitats.

II. MATERIALS AND METHODS

1. Fish Sampling

All the larval fish fauna collected from coastal water of Dongsha Island, South China Sea by the light trap. The trap

Paper submitted 10/30/13; revised 12/10/13; accepted 12/23/13. Author for correspondence: Kwang-Tsao Shao (e-mail: zoskt@gate.sinica.edu.tw).
[1] *Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.*
[2] *Biodiversity Research Centre, Academia Sinica, Taipei, Nankang, Taiwan, R.O.C.*
[3] *Institute of Ocean Technology and Marine Affairs, National Cheng-Kung University, Tainan, Taiwan, R.O.C.*
[4] *Institute of Marine Environmental Chemistry and Ecology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.*
[5] *Taiwan Ocean Research Institute, National Applied Research Laboratories, Taiwan, R.O.C.*

Table 1. Mitogenomic similarity of CO I barcoding of sample data of larval fishes compared with known DNA sequence database.

Code	CO I similarity	Species name of Genbank	Abbreviation	Identification of taxa	
				Same genus	Same species
L01	96%	*Apogon guamensis*	APGU	V	
L03	83%	*Trimma flammeum*	TRFL	V	
L04	96%	*Apogon guamensis*	APGU	V	
L05	95%	*Apogon guamensis*	APGU	V	
L06	86%	*Salarias fasciatus*	SAFA	V	
L08	100%	*Salarias fasciatus*	SAFA	V	V
L09	85%	*Eviota melasma*	EVME	V	
L10	83%	*Trimma flammeum*	TRFL	V	
L11	100%	*Fowleria variegata*	FOVA	V	V
L12	99%	*Fowleria variegata*	FOVA	V	V
L13	85%	*Eviota melasma*	EVME	V	
L14	99%	*Scorpaenodes guamensis*	SCGU	V	V
L15	99%	*Bathygobius cocosensis*	BACO	V	V
L16	84%	*Cryptocentrus albidorsus*	CRAL	V	
L17	99%	*Fowleria variegata*	FOVA	V	V
L18	99%	*Lethrinus harak*	LEHA	V	V
L20	83%	*Trimma flammeum*	TRFL	V	
L21	83%	*Trimma flammeum*	TRFL	V	
L22	83%	*Trimma flammeum*	TRFL	V	
L23	83%	*Trimma flammeum*	TRFL	V	
L24	99%	*Bathygobius cocosensis*	BACO	V	V
L25	83%	*Trimma flammeum*	TRFL	V	
L26	83%	*Trimma flammeum*	TRFL	V	
L27	83%	*Trimma flammeum*	TRFL	V	
L28	83%	*Trimma flammeum*	TRFL	V	
L29	83%	*Trimma flammeum*	TRFL	V	
L30	84%	*Cryptocentrus albidorsus*	CRAL	V	
L32	99%	*Fowleria variegata*	FOVA	V	V
L33	99%	*Fowleria variegata*	FOVA	V	V
L34	96%	*Apogon guamensis*	APGU	V	
L35	86%	*Salarias fasciatus*	SAFA	V	
L36	100%	*Oligolepis acutipennis*	OLAC	V	V
L37	85%	*Eviota melasma*	EVME	V	
L38	100%	*Elops hawaiensis*	ELHA	V	V
L39	99%	*Fowleria variegata*	FOVA	V	V
L40	99%	*Fowleria variegata*	FOVA	V	V
L41	98%	*Apogon doryssa*	APVA	V	V
L42	99%	*Fowleria variegata*	FOGU	V	V
L44	96%	*Apogon guamensis*	APGU	V	
L45	99%	*Bathygobius cocosensis*	BACO	V	V
L46	84%	*Holacanthus tricolor*	HOTR	V	
L47	99%	*Bathygobius cocosensis*	BACO	V	V
L48	92%	*Apogon holotaenia*	APHO	V	
L49	99%	*Fowleria variegata*	FOVA	V	V
L50	100%	*Dascyllus aruanus*	DAAR	V	V
L51	85%	*Fowleria marmorata*	FOMA	V	
L52	92%	*Apogon cookii*	APCO	V	
L53	96%	*Apogon guamensis*	APGU	V	
L54	99%	*Chanos chanos*	CHCH	V	V
L55	99%	*Chanos chanos*	CHCH	V	V
L56	85%	*Fowleria marmorata*	FOMA	V	
L57	85%	*Eviota melasma*	EVME	V	
L58	100%	*Chanos chanos*	CHCH	V	V
L59	100%	*Chanos chanos*	CHCH	V	V
L60	100%	*Megalops cyprinoides*	MECY	V	V
L61	100%	*Lutjanus argentimaculatus*	LUAR	V	V
L62	92%	*Gymnapogon urospilotus*	GYUR	V	
L63	99%	*Valenciennea longipinnis*	VALO	V	V
L64	99%	*Fowleria variegata*	FOVA	V	V
L65	96%	*Apogon guamensis*	APGU	V	
L66	100%	*Fowleria variegata*	FOVA	V	V
L67	100%	*Lutjanus fulviflamma*	LUFU	V	V
L68	97%	*Pempheris vanicolensis*	PEVA	V	V
L69	83%	*Enneapterygius* sp.	ENSP	V	
L70	83%	*Enneapterygius* sp.	ENSP	V	
L71	83%	*Enneapterygius* sp.	ENSP	V	
L73	99%	*Scarus globiceps*	SCGL	V	V
L74	100%	*Scarus psittacus*	SCPS	V	V
L75	100%	*Scarus psittacus*	SCPS	V	V
L76	83%	*Enneapterygius* sp.	ENSP	V	

collected 2-3 hrs during the night-time in each month for March 2011 to March 2012. The collecting site was located on the southern coast of the Dongsha Island, South China Sea.

2. DNA Extraction

Total DNA of each sample was extracted from muscle tissue using the standard phenol-chloroform [7], and the quality of DNA was examined by gel electrophoresis on 0.5% agarose.

3. PCR Amplification of Mitochondrial DNA Sequences

Oligonucleotide primers for amplification of the mitochondrial CO1 gene by published sequences [12, 13] as the upstream primers: FishF1 (5'-TCAACCAACCACAAAGACAT TGGCAC-3') and FishF2 (5'-TCGACTAATCATAAAGATA TCGGCAC-3'), and the downstream primers: FishR1 (5'-TA GACTTCTGGGTGGCCAAAGAATCA-3') and FishR2 (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'). Each 50-uL reaction mixtures of comprise 5 U Pro Taq Plus DNA polymerase (Protech Technology Enterprise Co., Ltd), approximately 100-200 ng crude genomic DNA, 2.5 mM $MgCl_2$, 10X PCR buffer, 200 uM dNTPs, and 0.2 uM each primer. The PCR reaction was performed in Applied Biosystem 9700 themocyclers under the following parameters: initial denaturation for 5 mins at 94°C; 40-45 cycles of denaturation for 60 s at 94°C, annealing temperature for 60 s at 50 to 55°C, and extension for 2 mins at 72°C; and followed by a final elongation at 7 mins at 72°C. The PCR products were checked by 1.0% agarose gels and bands were visualized by ethidium bromide staining viewed and checked with an ultraviolet light source. The purified DNA sequences fragments sequenced on an ABI 3700 Automatic Sequencer.

4. DNA Sequence Alignment and Properties

We manually aligned the CO1 sequences using Bioedit sequence alignment editor [3]. Multiple alignment were carried out with Clustal X [11] and then slightly modified and confirmed by eyes. All alignment also based on the available, related CO1 sequences of mtDNA genomes from several teleost fishes found in Genbank. Average pairwise genetic distances were calculated using the Kimura two-parameter model (K2P: [5]) as implemented in MEGA 4 [10].

5. Molecular Phylogenetic Clustering

The algorithms used for phylogenetic analysis included the neighbor-joining (NJ), maximum parsimony (MP). We employed NJ analysis as complemented in implemented in MEGA 4 [10] and PAUP* 4.0 [9], to examine relationships among taxa. The NJ method has been shown to be computationally efficient, with a record of recovering trees that are good as those generated by alternate methods. After conducting all classification tests, we added all test sequences to the profile to allow for a more detailed examination of the factors that enable successful identification. MP analysis were performed with PAUP *5.0B. An MP analysis was first carried out on each gene region separately, and the data set was combined under a heuristic search strategy, with all sites weighted equally.

III. RESULTS AND DISCUSSIONS

1. DNA Identification of Sampled Larval Fish Fauna

The 70 different samples of larval fishes with either different developmental stages or distinct morphotypes have been successfully amplified and sequenced in detail via PCR amplification for the gathering partial sequences of mitogenomic CO I gene by employing the universal primers for all teleost fishes. The detailed comparison based on either BLAST program with available mtDNA CO I sequences data from Genbank or LAB fish DNA data have yielded the suggested fish list from the research of current molecular identification. The DNA dataset (about 600 bp) from samples were aligned with known CO I sequences which suggested from BLAST to compare with them.

The overall genetic similarity is ranging from 83% to 100% (Table 1). The criteria for suggestion of same fish species on the list is not less than 98%. Other value if less than 96% would just suggest for generic name and they are not immediately suggested full specific name at present stage.

2. Species Diversity from Larval Fish Community

The molecular phylogenetic clustering (shown in Fig. 1) from both NJ or MP criteria also reveal the same topology for clustering closely related species or well identified species lumping on the same clade, or sister clades. The bootstrap value shown on the branch. All individuals of larval fish samples represent the same haplotype or a few very similar haplotypes and they are still grouped into the same specific level compared with the suggested target species.

Among them, there are 16 fish species can be identified into specific level from suggested fish valid species (shown in Table 2). They are including *Apogon doryssa*, *Fowleria variegata* in Apogonidae; *Salarias fasciatus* in Blenniidae; *Chanos chanos* in Chanidae; *Elops hawaiensis* in Elopidae; *Bathygobius cocosensis*, *Oligolepis acutipennis*, and *Valenciennea longipinnis* in Gobiidae; *Lethrinus harak* in Lethrinidae; *Lutjanus argentimaculatus*, *Lutjanus fulviflamma* in Lutijanidae; *Megalops cyprinoide*s in Megalopidae; *Dascyllus aruanus* in Pomacentridae; *Scarus globiceps*, *Scarus psittacus* in Scaridae; and Scorpaenodes guamensis in Scorpaenidae. However, there are remaining 12 species can be expected into the suggested fish generic level with uncertain data for specific name via DNA proof including 4 species in Apogonidae, 3 species in Gobiidae, other reaming families merely with 1 uncertain species as Blenniidae, Pempheridae, Pomacanthidae, and Tripterygiidae.

Apparently, two major groups of taxa would be the members of Gobiidae and Apogonidae. Both of families comprised 50% of total larval fish species diversity, and also comprised 67% of total larval fish samples which analyzed.

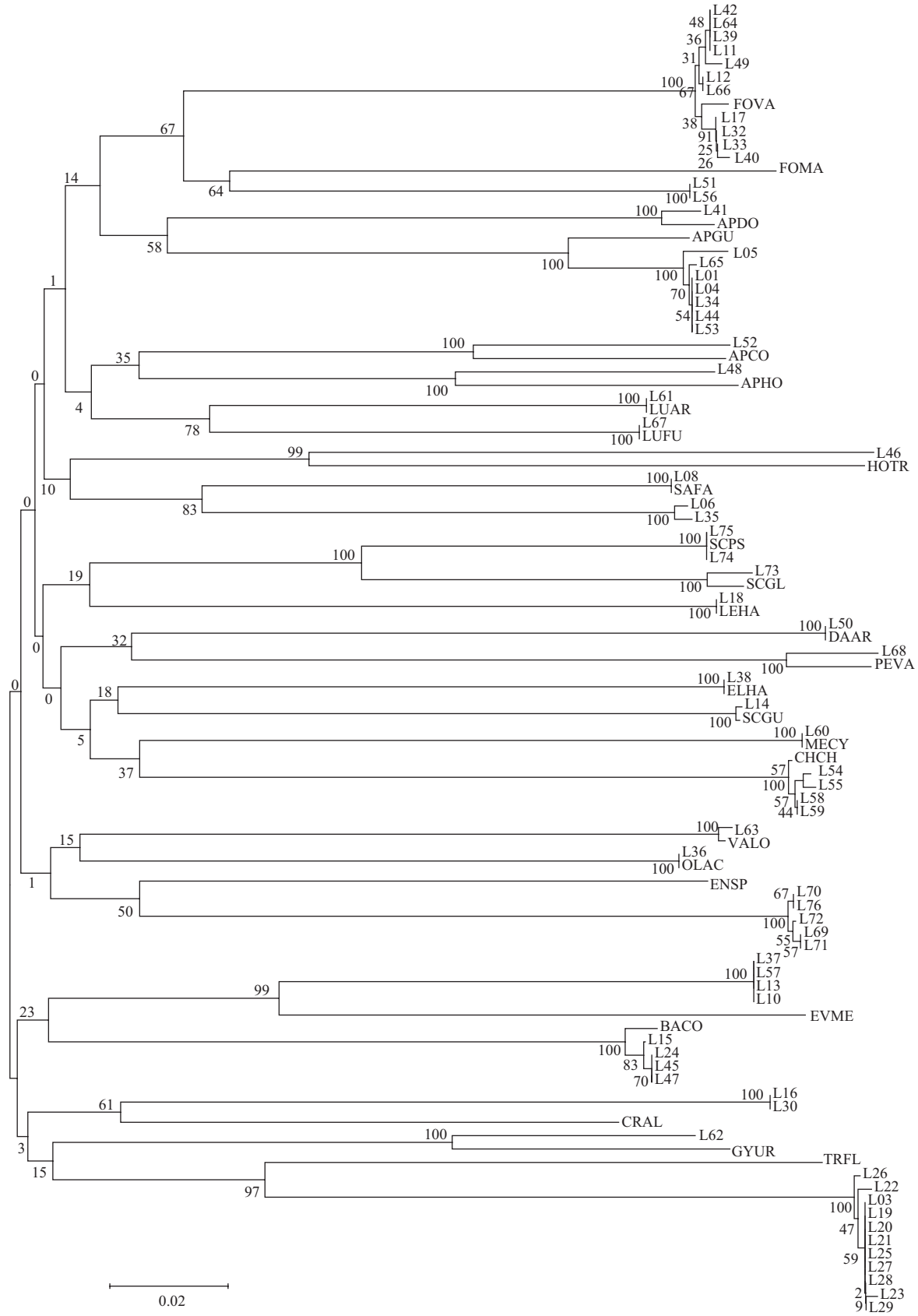

Fig. 1. The unrooted molecular phylogenetic tree of 70 sampled larval fishes by NJ criteria inferred from mitochondrial DNA CO I sequences. The value above or below branch indicate bootstrap support.

Table 2. Coastal fish fauna of larval fishes identified based on DNA barcoding.

Family	Species name
Apogonidae	*Apogon doryssa*
	Apogon sp. 1
	Apogon sp. 2
	Apogon sp. 3
	Fowleria sp.
	Fowleria variegata
	Gymnapogon sp.
Blenniidae	*Salarias fasciatus*
	Salarias sp.
Chanidae	*Chanos chanos*
Elopidae	*Elops hawaiensis*
Gobiidae	*Bathygobius cocosensis*
	Cryptocentrus sp.
	Eviota sp.
	Oligolepis acutipennis
	Trimma sp.
	Valenciennea longipinnis
Lethrinidae	*Lethrinus harak*
Lutijanidae	*Lutjanus argentimaculatus*
Lutijanidae	*Lutjanus fulviflamma*
Megalopidae	*Megalops cyprinoides*
Pempheridae	*Pempheris* sp.
Pomacanthidae	*Holacanthus* sp.
Pomacentridae	*Dascyllus aruanus*
Scaridae	*Scarus globiceps*
	Scarus psittacus
Scorpaenidae	*Scorpaenodes guamensis*
Tripterygiidae	*Enneapterygius* sp.

3. The Necessary for Comprehensive DNA Barcoding Data for Larval Fish Studies

From the totally 26 suggested valid fish species from 70 sampled larval fishes, there are still about less than half of them in lacking sufficient data for proving their own specific status, but merely up to generic identification for their own species confirmation. It is opening the query for stepping up for gathering more marine fish diversity with available Genbank CO I data for making better percentage of species identification for marine fish. However, they are at least providing the molecular evidences for different discrete species among the coral-reef fish community although the part of sample with the uncertainty of specific name with more reliable generic name suggested.

4. The Sample Collection of Larval Fishes via Light Trap

The samples of larval fishes obtained from the light trap collections seem to be with better swimming capability for approaching the light during the night-time survey. It would be more possible to gather most of samples with more latter phase of larval stage in overall average rather than the zooplankton net collection via fishery or research vessel. It seems to perform the more reliable morphological features to approach the morphological clues of adult features defined by general image or guidebook, even collected specimens. It is the best way to connect the different stage of larval fish morphotype data to make the further comments for larval fish reference on the huge marine fish species resources.

On the other hand, the estimated fish diversity merely based on morphotype usually happens to overlook or over estimation for real fish species diversity. For the example of current phase data, 70 current sampled morphotypes of larval fishes indeed merely belonging to 26 suggested discrete fish species. The nest stage for expending our research effort is to monitor the whole set of larval fish samples collected month by month, to make the more detail survey for gathering full story for seasonality of recruitment of larval fish resources in the near-shore coastal region of coral-reef region of Dongsha island, South China Sea.

ACKNOWLEDGMENTS

We are very grateful for Mr. K. T. Chen, Mr. K. Hsu, Mr. S. P. Huang, and Mr. Y. H. Gong for their kindly helping on setting the light trap and preservation on the Dongsha Island, South China Sea during March 2011 to March 2012. The current research is partly granted from National Science Council, Taipei for first author. The research is partially also supported by the Marine Biodiversity team from CMBB & CEO, NTOU as well as the research project of Marine National Park Headquarters.

REFERENCES

1. Arif, I. A., Khan, H. A., Shobrak, M., and Williams, J., "Cytochrome c oxidase subunit I barcoding of the green bee-eater (*Merops orientalis*)," *Genetics and Molecular Research*, Vol. 10, pp. 3992-3998 (2011).
2. Elmer, K., Almalki, A., Mohran, K. A., AL-Qahtan, K. N., and Almarri, M., "DNA barcoding of *Oryx leucoryx* using the mitochondrial cytochrome C oxidase gene," *Genetics and Molecular Research*, Vol. 11, pp. 539-547 (2012).
3. Hall, T. A., "Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT," *Nucleic Acid Symposium Series*, No. 41, pp. 95-98 (1999).
4. Hebert, P. D. and Gregory, T. R., "The promise of DNA barcoding for taxonomy," *Systematics Biology*, Vol. 54, pp. 852-859 (2005).
5. Kimura, M., "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences," *Journal of Molecular Evolution*, Vol. 16, pp. 111-120 (1980).
6. Min, X. J. and Hickey, D. A., "DNA barcodes provide a quick preview of mitochondrial genome composition," *PLOS One*, Vol. 2, pp. e325 (2007).
7. Sambrook, J. and Russel, D., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York (2001).
8. Steinke, D. and Hanner, R., "The FISH-BOL collaborators' protocol," *Mitochondrial DNA*, Vol. 22, Supplement 1, pp. 10-14 (2011).
9. Swofford, D., PAUP*: *Phylogenetic Analysis Using Parsimony (and Other Methods) Ver. 4*, Sinauer Associates, Sunderland (2002).
10. Tamura, K., Dudley, J., Nei, M., and Kumar, S., "MEGA4: Molecular

Evolutionary Genetics Analysis (MEGA) software version 4.0," *Molecular Biology and Evolution*, Vol. 24, pp. 1596-1599 (2007).
11. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G., "The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools," *Nucleic Acids Research*, Vol. 25, pp. 4876-4882 (1997).
12. Ward, R. D., Costa, F. O., Holmes, B. H., and Steinke, D., "DNA barcoding of shared fish species from the North Altantic and Australia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species," *Aquatic Biology*, Vol. 3, pp. 71-78 (2008).
13. Ward, R. D., Holmes, B. H., and Yearsley, G. K., "DNA barcoding reveals a likely second species of Asian sea bass (barramindi) (*Lates calcarifer*)," *Journal of Fish Biology*, Vol. 72, pp. 458-463 (2007).
14. Yu, D. B., Chen, R., Kaleri, H. A., Jiang, B. C., Xu, H. X., and Du, W. X., "Testing the utility of mitochondrial cytochrome oxidase subunit 1 sequences for phylogenetic estimates of relationships between crane (*Grus*) species," *Genetics and Molecular Research*, Vol. 10, pp. 4048-4062 (2011).