

DNA Barcoding: the Molecular Detective

^{1,2}Mohd Nor Siti-Azizah

¹School of Biological Sciences Universiti Sains Malaysia, Penang; ²Centre of Marine and Coastal Studies, Universiti Sains Malaysia, Penang. Email: sazizah@usm.my

Abstract

The existing taxonomic identification of most taxa has mainly been through morphological characters. However, there are limitations to relying primarily on morphology when attempting to identify species during various stages of their development or when examining fragmentary or processed remains. DNA barcoding is a diagnostic tool in which short DNA sequences are utilised for species identification. We describe here some of the works that have been conducted in our laboratory as well with collaborators on the utilisation of this technique

Keywords: DNA barcoding, taxonomic, COI

Introduction to DNA Barcoding

This paper provides an overview of the molecular technique, DNA barcoding in taxonomic identification and other applications. Existing taxonomic identification of most taxa has mainly been through morphological characters. However, there are limitations to relying primarily on morphology when attempting to identify species during various stages of their development or when examining fragmentary or processed remains. DNA barcoding is a diagnostic tool in which short DNA sequences are utilised for species identification. Basically, it utilises an approximately 650 base pair segment at the 5' end of the cytochrome c oxidase subunit 1 mitochondrial region (COI) as the standard barcode region for almost all groups of life forms (major exceptions being plant, microbes and Cnidaria). It was first introduced in 2003 under the global initiative of the Consortium of Barcode of Life (CBOL) aimed to identify all life forms based on this gene (Herbert et al., 2013). The DNA barcodes comprising of DNA sequence data records are linked to an online database called **Barcode of Life Data Systems (BOLD)** which is linked to all major molecular databases including; GenBank, the European Molecular Biological Lab and the DNA Databank of Japan. **It assembles molecular, morphological and distributional data in collaboration with other scientific databases such as NCBI, MitoFish, ITIS and BioOne which are easily accessible to the public globally.** The uniqueness of the BOLD system is it is very comprehensive. The BOLD system requirements include pictures of specimens and links to where the specimens and tissues are vouchered in addition to trace files of sequences. Therefore, it allows easy reference for future studies.

Applications of DNA Barcoding

Since its introduction as a taxonomic tool, it has found importance in addressing many conservation and sustainable issues. Thus once a database has been established, it can be used for a variety of applications. These include;

- Documenting biodiversity together with other database projects
- Linking life history stages
- Differentiating cryptic species, identifying gut contents, human disease vectors and agricultural pests
- Environmental sustainability of natural resources, protecting and conservation of endangered species

- Monitoring water quality, detecting and monitoring biological invasions
- Consumer protection through precise identification of seafood products against substitutions and health risk.

We describe here some of the work that have been conducted in our laboratory as well with collaborators on the utilisation of this technique.

Building a reference library- Cryptic and new species discovery

In Malaysia, the family Carangidae is one of the major marine fishery and also one of the most diverse group. Moderate frequency of potentially cryptic species within commercially exploited Indo-Malay Archipelago (IMA) Carangidae was observed in a DNA barcoding study in this region which highlighted the possibility of further species discovery (Tun Nurul Aimi Mat Jaafar et al., 2012). Misidentification of economically important species in cryptic species-complexes can result in inaccurate data collection potentially leading to the overexploitation of stocks. Furthermore, phylogeographic pattern of several species within this family was elucidated. Allopatric divergence between the Sabah and the West Peninsular Malaysia were observed which would require that management of this fishery be independently conducted. This is part of our on-going efforts to molecularly identify all the major commercially important marine fish species in the surrounding seas of Malaysia, namely in the Straits of Malacca, South China Sea, Sulu Sea and Sulawesi Sea. To date we have barcoded more than 100 fish species commonly obtained at fish landing sites.

Evidence of introduced and endangered species

In the second example, the common occurrences of translocation and biological invasions of ecosystems is described. Such information is very important in the management of wild and cultured populations. A study on the commercially important oyster genus *Crassostrea* of wild and cultured populations revealed the presence of invasive species in aquaculture premises which has also found its way into the natural environment (Suzana et al., 2011). This is a common occurrence in most water bodies. Similarly, through a DNA barcoding investigation, presence of an invasive jellyfish species was recorded in the coastal waters of Peninsular Malaysia (Anita et al., 2012). A barcoding study conducted in Lake Laut Tawar in Aceh Province Sumatra revealed the taxonomic ambiguity between the genus *Puntius* and *Poropuntius* which are morphologically very similar and generated DNA barcodes of the introduced, threatened and endangered species (Muchlisin et al., 2013). Identification of threatened, endangered and protected species would assist in the conservation and management of these species.

DNA barcoding for consumer protection

In the third example consumer protection against mislabeling and substitution for lower valued as well as food poisoning is illustrated by the DNA barcoding of seafood product and the puffer fishes. Identification of fish, fillets, fins, and fragments will enable efficient detection of catch substitutions, where low value species are substituted for high value species in the market and will also extend to the detection of quota substitutions. A database of the pufferfish is also being developed. This group is notoriously known to have members that produce toxins. Several of these species are morphologically very similar to non-toxic relatives. Instances of fatalities have been reported among consumers as a result of mistaken identity among the two groups.

New approaches in DNA barcoding

New innovative approaches such as metabarcoding and ecological barcoding of multispecies barcode can now be utilized to identify rich spawning grounds of economically important species through larval identification as well as for ecosystem monitoring of

invasives or endangered species, the latter through traces of fluids or body tissues left behind in the environment. Identification of fish eggs and larvae and phyto or zooplanktons is highly beneficial in ecosystem research, direct and indirect fisheries management. Precise identification of larvae or to the species level is virtually impossible. Such information is a necessity for the effective management of fisheries resources in this country.

Working together to ensure sustainable resources through DNA Barcoding

In general, application of DNA barcoding to fishes in the biodiversity hotspot of the Indo-Malay Archipelago (IMA) has not been applied widely. Considering our position in a biodiversity hotspot region it is imperative that we should also be actively involved in the molecular documentation, management and conservation of our native species. It is hoped that collaborative research could be initiated based on this relatively simple approach to identify more species in this biodiversity rich region. This database would be highly beneficial to the management, conservation and sustainable utilisation of our rich resources.

Acknowledgements

I would like to express my appreciation to all members of Molecular Ecology Lab 308 Universiti Sains Malaysia, Penang, Malaysia and Syiah Kuala University, Banda Aceh, Indonesia for their technical assistance and financial support from both universities including the RU and Delivering Excellence APEX University (1002/PBiologi/910317) grants.

References

1. Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R. 2003. Biological identifications through DNA barcodes. *Proc Roy Soc Lond B* 270: 313–321.
2. Tun Nurul Aimi Mat Jaafar, Martin I. Taylor, Siti Azizah Mohd Nor, Mark de Bruyn, Gary R. Carvalho 2012. DNA Barcoding Reveals Cryptic Diversity within Commercially Exploited Indo-Malay Carangidae (Teleostei: Perciformes) *PLOS ONE*. November 2012 7 (11) | e49623
3. Muchlisin Z. A., Thomy Z., Fadli N., Sarong M.A., Siti-Azizah M.N. 2013, DNA barcoding of freshwater fishes from Lake Laut Tawar, Aceh Province, Indonesia *Acta Ithyologica Et Piscatoria* Vol. XLIII 43 (1): 21–29
4. Suzanna M., Mohd Lutfi A., Abdul Hadi A., Devakie M.N., and Siti Azizah M.N. 2011 Genetic variation in Malaysian oysters: taxonomic ambiguities and evidence of biological invasion. *Biol Invasion* 13:1893–1900.
5. Anita T., Elham M., Khairun Y., Sim Y.K. and Siti Azizah M.N. 2012. Diversity and genetic identification of the jellyfish (Scyphozoa) along the east coast of Peninsular Malaysia based on Morphological and 16S rRNA gene. *International Fisheries Symposium 2012*. 6-8 Dec 2012 Can Tho University, Vietnam