Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 5, 2015, pp. 2425-2430
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

STUDY OF UNIVERSAL PRIMERS OF MITOCHONDRIAL GENE COI AS POTENTIAL CANDIDATE FOR DNA BARCODING OF BUTTERFLIES FROM AMRAVATI REGION

P. B. Ingle[1], P.V. Thakare[2], and R. N. Khade[3]

[1]S.G.B. Amravati University, Amravati 444606.
[2] S.G.B. Amravati University, Amravati 444606.
[3]S. B. Jr. Science Collage Telhara. 444108.

INTRODUCTION

Humans are currently enduring the 6th mass extinction, losing between 1 - 10 % of biodiversity per decade. The reasons of it may be habitat loss, pest invasion, pollution, over harvesting of biodiversity, and infections. A required step prior to protection of biodiversity is its assessment, and is usually conducted at the species level. Therefore, species identification has a most importance. The term species means "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups.", but this morphological approach can be very tedious, and a matter of subjectivity. Moreover, phenotypic plasticity and genotypic variation in the features used for identification can easily lead to identification errors and cryptic species or differing life stages of species can add to the confusion of its identification (Hebert *et al,* 2003). Due to high diversity of species, it is critical to seek technological assistance for its description and recognition (Godfray 2002; Blaxter 2003). Johns & Avise (1998) demonstrated that closely related species of vertebrates regularly show more than 2% divergence at mitochondrial gene, cytochrome b. Hebert *et al* established that diversity in nucleotide sequences of the mitochondrial gene cytochrome oxido reductase (COI) region regularly permitted the discrimination of closely allied species of Lepidopterans. Their study addressed the extent of sequence diversity at COI among congeneric taxa in the major animal phyla. The sequencing pattern of this gene is assigned as barcode for given species. Concern has been expressed that efforts to base identification systems on mt DNA markers would fail because of the frequency of horizontal transfers of mitochondria between divergent lineages and also because closely allied species regularly share mitochondrial polymorphisms that were millions of years old (Mallet & Willmot 2003). But, it is evident that mitochondrial and nuclear genomes are linked, best evidenced by studies on cybrids. For example, mitochondria of chimpanzees and gorillas show 20% reductions in their oxidative capacity when placed in a human cytogenetic setting (Barrientos *et al*, 1998). Despite the close genetic similarity with their nuclear genomes, orangutan mitochondria has shown total collapse in respiratory capacity when they were placed in a human cell background (Barrientos *et al*. 2000). Comparative studies of the patterns of genetic diversity in mitochondrial and nuclear genomes have additionally provided evidence for the depletion of mt DNA diversity via selective sweeps, possibly mediated by the rise of mitochondrial variants with more effective nuclear interactions (Ballard 2000; Gerber *et al,* 2001).

In central India the butterfly species diversity was reported earlier by D'Abreeu (1931) he documented total 177 species occurring in the Central Provinces now Madhya Pradesh and

Vidarbha. He provided a special list of 92 species butterflies from Nagpur city. Later on Pandharipande (1990) recorded only 61 species of butterflies from the Nagpur city. 45 species of butterflies had been recorded in Melghat region by Mahabal (2005). Survey of the butterfly diversity of the Melghat Tiger Reserve from January 2005 to December 2007 showed overall 101 species of butterflies belonging to 8 families and 19 subfamilies (Wadatkar and Kasambe, 2008). Total 145 species of butterflies were recorded at the eight study sites, of which 62 species were new records for the Nagpur city. (Tipale *et al* 2009). For present study we have used five species of butterflies from different genera of two families. Four species are from family Nymphalidae and one from Papilionidae.

MATERIALS AND METHODS

Five species of Butteflies *Junonia lemonias, Graphium agamemnon, Danaus chrysippus, Hypolimnas misippus, Euploea core* were collected. Total DNA was isolated from thorasic muscles of butterfly by salting out technique. Thoraces were crushed in the liquid nitrogen. To the crushed tissues 300 µl 1X TEN (250mM NaCl, 50mM Tris HCl, 10mM EDTA, pH 8.0) and 30 µl of 2% SDS was added. 30 µl of 1 mg/ml Proteinase-K was also added to the mixture. Homogenized tissue was left for 3 h at 55^0C. After incubation 100 µl of 5M NaCl was added. The mixture was then vortexed and centrifuged for 10 min. DNA was precipitated from the supernatant by addition of 1ml of ice-cold absolute ethanol. Samples were centrifuged at high speed for 10 min to produce a DNA pellet. Excess ethanol was removed and pellets were washed twice with 1ml of 70% ethanol. After the final wash, all remaining ethanol was removed and the samples were air-dried and resuspended in 50 µl of nuclease free water.

Amplification by PCR.

For PCR amplification of COI gene primers 2µl each, Taq polymerase 2 units, template DNA 5µl, PCR master mix 25µl and Nuclease free water 16µl were taken. PCR was set at 6 minutes of 94^0 c and six cycles of 45^o c for 90 seconds, 72^o c for 72 seconds, 1 minute at 72^o c. In second step cycling conditions were set by 1 minute at 94^o c followed 40 cycles of 51^o c for 90 seconds and 72^o c for 75 seconds and final extension at 4^oc. PCR product was stored at 4^o c for further use.

RESULTS

In present study mitochondrial gene cytochrome c oxidase I (*COI*) is amplified from all five species of butterflies. Total DNA was isolated from thorasic muscles of butterfly with salting out method. The COI gene of mitochondrial origin was amplified by universal primers. The amplicon of size was fractionated by agarose gel electrophoresis (1% w/v). The sequencing of COI gene of *Graphium agamemnon* was perfomed by Chromus Biotech ltd. Which can be summarized in shown in figure

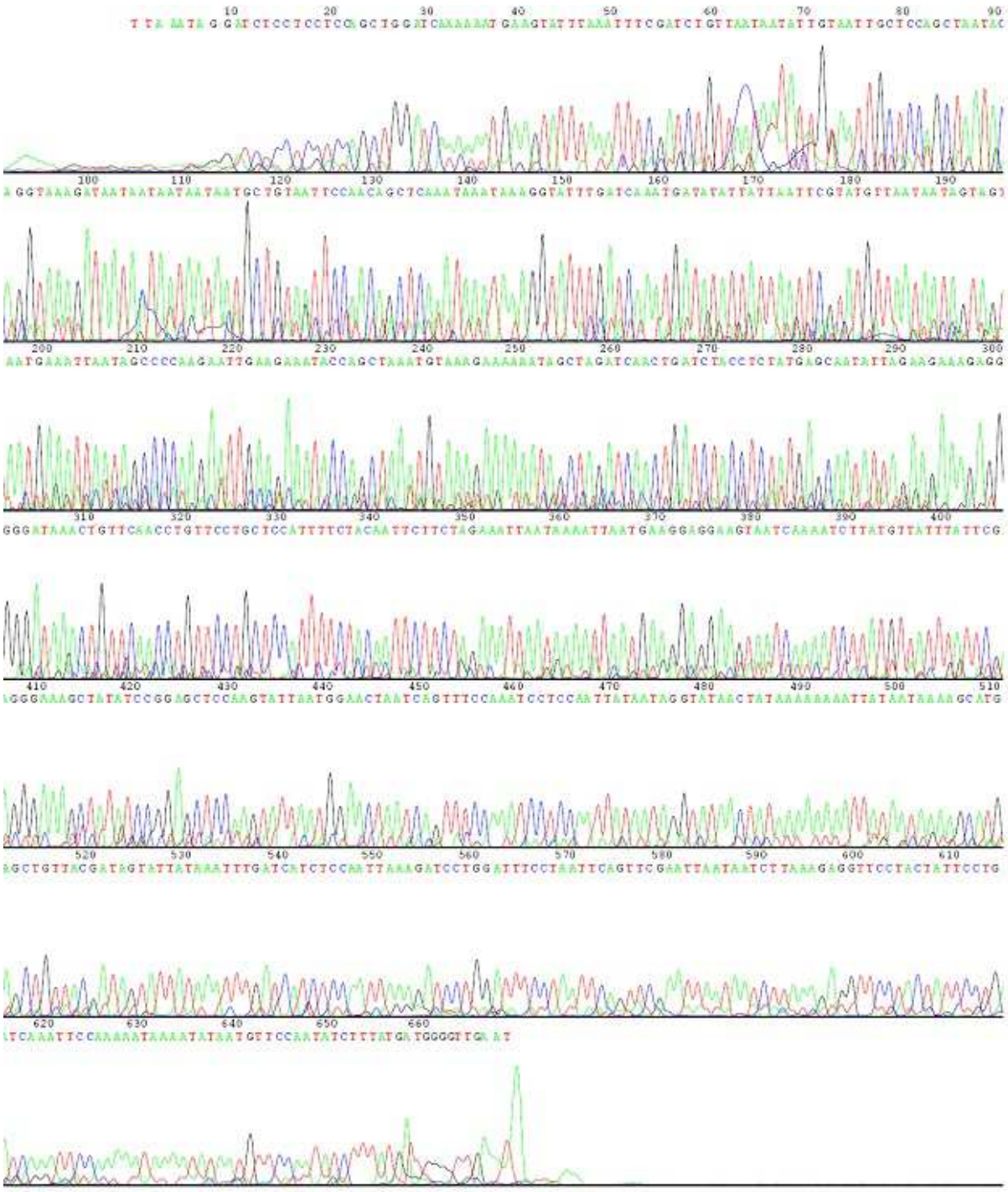

Figure 1 showing sequence of *Graphium agamemnon*

Sequence of *Graphium agamemnon* was searched for similar sequence using BLAST. Out of all results *Graphium agamemnon* cytochrome c oxidase I (COI) gene, partial cds; tRNA-Leu gene, complete sequence; and cytochrome c oxidase II (COII) gene, complete cds; mitochondrial genes for mitochondrial products showed 97% similarity as Score = 1120 bits (606), Expect = 0.0

Identities = 640/657 (97%), Gaps = 0/657 (0%) . The comparision of sequence is given in figure 2

```
>lcl|34737  Graphium agamemnon
Length=2290
Score = 1120 bits (606),  Expect = 0.0
Identities = 640/657 (97%), Gaps = 0/657 (0%)
Strand=Plus/Minus
Query  3     AAATAGGATCTCCTCCTCCAGCTGGATCAAAAAATGAAGTATTTAAATTTCGATCTGTta  62
             ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  688   AAATAGGATCTCCTCCTCCAGCAGGATCAAAAAATGAAGTATTTAAATTTCGATCTGTTA  629

Query  63    ataatattgtaattgctccagctaatacaggtaaagataataataataatgctgtaa  122
             |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  628   ATAATATTGTAATTGCTCCAGCTAATACTGGTAAAGATAATAATAATAATAATGCTGTAA  569

Query  123   tTCCAACAGCTCAAATAAATAAAGGTATTTGATCAAATGATATATTATTAATTCGTATGT  182
             ||||  ||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  568   TTCCTACAGCTCAAATAAATAAAGGTATTTGATCAAATGATATATTATTAATTCGTATAT  509

Query  183   TAATAATAGTAGTAATGAAATTAATAGCCCCAAGAATTGAAGAAATACCAGCTAAATGTA  242
             |||||||||||||||| ||||| || ||||||||||||||||||||||||||||||||||
Sbjct  508   TAATAATAGTAGTAATAAAATTGATTGCCCCAAGAATTGAAGAAATACCAGCTAAATGTA  449

Query  243   AAGAAAAAATAGCTAGATCAACTGATCTACCTCTATGAGCAATATTAGAAGAAAGAGGGG  302
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448   AAGAAAAAATAGCTAGATCAACTGATCTACCTCTATGAGCAATATTAGAAGAAAGAGGGG  389

Query  303   GATAAACTGTTCAACCTGTTCCTGCTCCATTTTCTACAATTCTTCTAGAAATTAATAAAA  362
             |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  388   GATAAACTGTTCAACCTGTTCCTGCCCCATTTTCTACAATTCTTCTAGAAATTAATAAAA  329

Query  363   TTAATGAAGGAGGAAGTAATCAAAATCTTATGTTATTTATTCGAGGGAAAGCTATATCCG  422
             |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||| |
Sbjct  328   TTAATGAAGGAGGAAGTAATCAAAATCTTATATTATTTATTCGAGGGAAAGCTATATCTG  269

Query  423   GAGCTCCAAGTATTAATGGAACTAATCAGTTTCCAAATCCTCCAATTATAATAGGTATAA  482
             | ||||| || ||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  268   GGGCTCCGAGCATTAATGGAACTAATCAATTTCCAAATCCTCCAATTATAATAGGTATAA  209

Query  483   CTATaaaaaaaaTTATAATAAAAGCATGAGCTGTTACGATAGTATTATAAATTTGATCAT  542
             | ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  208   CCATAAAAAAAATTATAATAAAAGCATGAGCTGTTACAATAGTATTATAAATTTGATCAT  149

Query  543   CTCCAATTAAAGATCCTGGATTTCCTAATTCAGTTCGAATTAATAATCTTAAAGAGGTTC  602
             |||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  148   CTCCAATTAAAGATCCTGGATTTCCTAATTCAGTTCGAATTAATAATCTTAAAGAAGTTC  89

Query  603   CTACTATTCCTGATCAAATTCCAAAAATAAAATATAATGTTCCAATATCTTTATGAT  659
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  88    CTACTATTCCTGATCAAATTCCAAAAATAAAATATAATGTTCCAATATCTTTATGAT  32
```

Figure 2

Original bases	Substituted	Number
T	A	26
A	T	91
A	T	127
G	A	181
G	A	199
A	G	205
A	T	208
T	C	329
G	A	394
C	T	421
A	G	424
A	G	430
T	C	433
G	A	451
T	C	484
G	A	520
G	A	597

DISCUSSION

DNA barcoding has emerged at a valuable time for taxonomy. Economic development and increased international commerce are leading to higher extinction rates and the introduction of invasive and pest species. As a result local, National, and International user populations are demanding more and faster species identification services and better information about their biodiversity than ever before. Barcoding is emerging as a cost-effective standard for rapid species identification.

The 650 bp mitochondrial cytochrome *c* oxidase 1 (CO1) DNA barcode is easily sequenced and provides good species level specificity. Our results with butterflies species showed that mitochondrial gene COI fulfill criteria for candidate gene for DNA barcoding. This study showed that universal primers available for mitochondrial gene COI, can be used easily to amplify this gene.

ACKNOWLEDGEMENT

The authors are grateful to Jayant Wadatkar who shared valuable knowledge for field characters of butterflies. The authors are also thankful to all the teaching and non-teaching staff, researchers and students of Department of Biotechnology Sant Gadge Baba Amravati University for their support.

REFERENCES

Ballard, J, W. O. (2000) Comparative genomics of mitochondrial DNA in Drosophila simulans. J. Mol. Evol. 51, 64–75.

Barrientos, A., Kenyon, L. & Moraes, C. T. (1998) Human xenomitochondrial cybrids. Cellular models of mitochondrial complex Ideficiency. J. Biol. Chem. 273, 14 210–14 217.

Barrientos, A., Muller, S., Dey, R., Weinberg, J. & Moraes, C. T. (2000) Cytochrome c oxidase assembly in primates is sensitive to small evolutionary variations in amino acid sequence. Mol. Biol. Evol. 17, 1508–1519.

Blaxter, M. (2003) Counting angels with DNA. Nature 421, 122–124.

D'Abreu, E.A., (1931). The central provinces butterfly list. Records of the Nagpur museum number VII. Government printing city press.

Godfray, H. C. J. (2002) Challenges for taxonomy. Nature 417, 17–19.

Gerber, A. S., Loggins, R., Kumar, S. & Dowling, T. E. (2001) Does non-neutral evolution shape observed patterns of DNA variation in animal mitochondrial genomes? A. Rev. Genet. 35, 539–566.

Hebert P. D., Ratnasingham S, de Waard J. R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. Ser. B 270 (Supplememt 1): 96-99.

Johns, G. C. & Avise, J. C. (1998) A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. Mol. Biol. Evol. 15, 1481–1490.

Mallet, J. & Willmot, K. (2003) Taxonomy: renaissance or Tower of Babel? Trends Ecol. Evol. 18, 57–59.

Mahabal A. (2005), An overview. Fauna of Melghar Tiger Resereve, conservation area series 24. Zoological survey of India Kolkata, 1-10 pp.

Pandharipande, T.N., (1990) Butterflies from Nagpur City, Central India (Lepidoptera: Rhopalocera). J. Res. Lepid., 29(1/2): 157-160.

Pimm, S.L., Russell, G.J., Gittleman, J.L., Brooks, T.M. (1995) The future of biodiversity. Science, 269, 347-350.

Tipale A. D, and Khurad A. M. (2009) Butterfly Species Diversity, Habitats and Seasonal Distribution in and Around Nagpur City, Central India. World Journal of Zoology 4 (3): 153-162.

Wadatkar J.S. and Kasambe R. (2008) Butterflies of melghat tiger reserve, Maharashtra with notes on their abundance, status and larval host plants. The Ecoscan 2 (2):165-171.