Indian Journal of Science and Technology Vol.2 No 5 (May 2009) ISSN: 0974- 6846

Ethnobotany genomics – use of DNA barcoding to explore cryptic diversity in economically important plants

Steven G. Newmaster and Subramanyam Ragupathy
Biodiversity Institute of Ontario Herbarium, University of Guelph, Guelph, Ontario, Canada. N1G 2W1.
snewmast@uoguelph.ca; ragu@uoguelph.ca

Abstract: The ethnobotany genomics concept is founded on the idea of 'assemblage' of biodiversity knowledge. This includes a coming together of different ways of knowing and valorizing species variation in a novel approach seeking to add value to both traditional knowledge (TK) and scientific knowledge (SK). Ethnobotany genomics is defined as exploring the variation in genomic sequences from many species, and here we present some of our recent work that demonstrates the potential benefits of this approach for ethnobotanical research with economic implications. DNA barcoding was used to identify Acacia and nutmeg taxa that are economically important to society-at-large. Furthermore we identified considerable variation that is recognized by several indigenous cultures. The impacts of ethnobotany genomics will extend well beyond biodiversity science. Explorations of the genomic properties across the expanse of life are now possible using DNA barcoding to assemble sequence information for a standard portion of the genome from large assemblages of species. Perhaps the most important contribution is major barcode projects will leave an important legacy; a comprehensive repository of high-quality DNA extracts that will facilitate future genomic investigations.

Keywords: DNA barcoding, economic botany, biodiversity, plant diversity, ethnopharmacology, ethnomedicine, ethnobiology, biotechnology.

Introduction

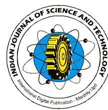
Comprehensive sampling of genomic biodiversity is fast becoming a reality for some genomic regions with complete organelle genomes and broad sampling of taxa for specific regions of a genome. Ethnobotany genomics is defined as exploring the variation in genomic sequences from many species, and here we present some of our recent work that demonstrates the potential benefits of this approach for ethnobotanical research with economic implications. Ethnobotany genomics is particularly central to research relating to Traditional Knowledge (TK) and Scientific Knowledge (SK) classification systems and our understanding of biodiversity and patterns of evolution. Recent genomic advances are increasing the efficiency of acquiring genomic biodiversity, and suggest that we may be able to document global diversity in a timely manner.

The ethnobotany genomics concept is founded on the idea of 'assemblage' of biodiversity knowledge. This includes a coming together of different ways of knowing and valorizing species variation in a novel approach seeking to add value to both traditional knowledge (TK) and scientific knowledge (SK). Ethnobotany genomics draws on an ancient body of knowledge concerning the variation in the biological diversity that surrounds different cultures; combined with modern genomic tools such as DNA barcoding it also explores the natural genetic variation found among organism. This genomic variation is explored along a gradient of variation found within organisms on the landscape. The motivation for this new approach is a quest to understand the diversity of life that surrounds us and how we can utilize such diversity to serve society-at-large with nutrition, medicine and more. The problem is a taxonomic impediment to discovering the earth's biodiversity.

Most children are born taxonomists. Exploring, discovering, and naming the living things in one's environment, whether it's a backyard or a park we naturally tend to classify the diversity that surrounds us. Some of the first scientists, such as Aristotle, focused intense efforts on exploring and cataloguing the living world. At the height of global exploration from the 15th to 19th centuries, taxonomists were in great demand, as new lands and species were discovered, many of which had significant economic value. Notable Western taxonomists include Ernst Haeckel, Carolus Linnaeus, and Charles Darwin.

Today, traditional taxonomic practices are inadequate on their own to deal with the need for accurate and accessible taxonomic information. The perception that taxonomy is not relevant has led to a significant drop in the funding and training of professional taxonomists. As taxonomist retire, there are fewer and fewer biologists who practice traditional taxonomy; the collection, description, naming and categorization of organisms through intense study of their physical attributes. These traditional taxonomists, who rely on fieldwork and morphological study as core aspects of their taxonomic work, appear to be slowly going extinct (Grant, 2009), global phenomena that is reaching all corners of the globe including India and China. Consequently, many herbaria and museums of natural history have closed because of substantial funding cuts for natural science programs in general. This has led to recurring publications predicting a crisis in taxonomy and of the need for a rejuvenation of the field (Godfray, 2002a, 2002b; Tautz *et al.*, 2002, 2003). The need for alpha taxonomy (new discovery and description) is critical as we still do not have a good answer to the simple question, "how many species are found on the earth" (Lipscomb *et al.*, 2003; Wheeler *et al.*, 2004; Will & Rubinoff, 2004; Hebert & Gregory, 2005; Newmaster *et al.*, 2009a). In fact, it is surprising and disturbing that after 250 years of modern systematic biology it is estimated that we have named less than 10% of the species on earth

©Indian Society for Education and Environment (iSee)

Indian Journal of Science and Technology  Vol.2 No 5 (May 2009) ISSN: 0974- 6846

(Hawksworth & Kalin-Arroyo, 1995). The total number of species on earth remains unknown with estimates ranging from 10 million to more than 100 million (May, 1988; Hammond, 1992; Hawksworth & Kalin-Arroyo, 1995). The number of species remaining to be discovered is well beyond the current capacity of our descriptive taxonomists and systematists; current estimates of the losses of biodiversity are greater than our ability to recognise new species (Godfray, 2002a; Blaxter, 2004). There is an urgent need to develop technology that will expedite our ability to catalogue species (Godfray, 2002b; Blaxter & Floyd, 2003; Godfray & Knapp, 2004; Newmaster *et al*., 2009a). In response, many researchers have called for an automated identification system that will aid taxonomists in routine identifications (Gaston & O'Neill, 2004; Wheeler *et al*., 2004) and alpha-taxonomic research (Hebert & Gregory, 2005; Newmaster *et al*., 2006, 2009a). Newmaster *et al*. (2009a) presents novel identification technology including state-of-the-art DNA bar coding in the development of an automated identification system for plants. The concept is not entirely new because the field of taxonomy, or systematics as it is often called, has been leaning towards the molecular end of the spectrum since genetic technology matured in the late 1970s and 1980s. Many modern taxonomists blend traditional methods, such as morphological and ecological study, with modern molecular techniques, such as DNA sequencing.

DNA barcoding is a critical technique employed in ethnobotany genomics. DNA barcoding is a method of species identification using specific regions of DNA sequence data (Hebert *et al*., 2003; Ratnasingham & Hebert, 2007). Hebert *et al.* (2003) has developed barcoding in animals, which is well documented and can be reviewed online via the Canadian Barcode of Life (http://www.bolnet.ca) and the Consortium for the Barcode of Life (CBOL, http://www.barcoding.si.edu). Although the difficulties in barcoding plants have been debated (Chase *et al*., 2005; Kress *et al*., 2005; Cowan *et al*., 2006; Pennisi 2007), detailed studies (Newmaster *et al*., 2006b, 2008b' 2009a; Newmaster & Ragupathy *et al*., 2009a, 2009b; Kress & Erickson, 2007, 2008; Ragupathy *et al*., 2009; Fazekas *et al*., 2008, 2009; Lahaye *et al*., 2008) have demonstrated the utility of barcoding as an effective tool for plant identification. Recently DNA barcoding has been used as a modern genomics tool for identifying several cryptic plant species (Newmaster *et al*., 2009a, 2008a, 2008b; Newmaster & Ragupathy 2009; Ragupathy *et al*., 2009).

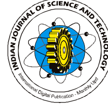
DNA barcoding addresses one of the gaps in molecular biology. That is there are few molecular data sets in which individuals from multiple populations from several species within a genus are sampled. This sampling design is largely lacking in the literature because it falls between typical studies in plant systematics and population genetic sampling. Plant systematics projects sample many species each with low or no population-level replication; population genetic sampling focuses is more intensive with many populations for one or small number of species. One study that has bridge these two research areas has discovered that only by more extensive population sampling can the hypothesis of species-level monophyly be adequately tested (Funk & Omland, 2003). DNA barcoding in plants is required in order to explore the prevalence of non-monophyly and the very nature of species boundaries. We expect that DNA barcoding in plants will not only be an important identification tool, but also provide taxonomic resolution and insight into the diversity of plants.

Materials and methods

Ethnobotany surveys: Floristic explorations were made within respective study areas within India, Africa, Australia and South America (Newmaster & Ragupathy, 2009a; Newmaster *et al*., 2008a, 2008b; Ragupathy *et al*., 2009, 2008a, 2008b; Ragupathy & Newmaster, 2009). Collections were made from April 2004- January 2009 and included all seasons in order to collect any ephemerals or specialized phenotypes. Six collections or "specimens" from each population were collected, labelled with locations and collection numbers. Corresponding field data included details of the specimens (habit, flower colour, phenology and presence or absence of latex) and environmental variables (habitat, latitude, longitude, altitude, soil type and plant associations). Multiple populations were sampled along transects separated by 2 km in order to insure that we were collecting distinct populations and not vegetative colonies. This also accounted for local morphological variants within the different ecosites. The survey used that of earlier methodologies (Newmaster *et al*., 2006a, 2007; Ragupathy *et al*., 2008a; Ragupathy & Newmaster, 2009) to identify local experts in traditional botanical knowledge. Vouchers were collected and labelled for all taxa identified. The data were gathered in a series of structured, semi-structured and unstructured interviews, and participatory approach regarding plant uses, identification, and nomenclature. To elucidate cultural domains and determine differences in knowledge or taxonomy among aboriginals, a cross check was made with other aboriginal respondents by using various research protocols such as free recall lists, pile sorts, and consensus analysis.

Plant vouchers: Plant samples were collected from the aboriginal community and preserved for both herbaria and DNA barcode analysis. Leaf, stem and flower parts collected in situ were fixed in silica gel, FAA (50% ethanol, 5% acetic acid, 10% formalin, 35% water) and stored in 70% ethanol for morphological study ex situ. Herbarium specimens were prepared as per Jain and Rao's (1977) manual and deposited in the herbarium of Kongunadu Arts and Science College, Coimbatore. The isotypes of new taxa and other taxonomically significant

©Indian Society for Education and Environment (iSee)



plant species were deposited at Madras Herbarium (MH), Southern Circle, Botanical Survey of India, Coimbatore and Ontario Agricultural College (OAC) Herbarium, Biodiversity Institute of Ontario, University of Guelph, Canada.

Identification analysis: Calculation of a Consensus Factor (Fic), and pile sorting relative frequency (RF) was used to test homogeneity of knowledge (SK & TK) in identifying specimens, revealing cryptic taxa or limitations of the classification without the use of molecular data. Voucher samples collected from five collection sites were systematically identified by the taxonomists and aboriginal informants. The relative frequency (RF) of each specimen from the interviews were calculated to determine a quantitative value for choosing a plant name (latin binomial or aboriginal ethno-taxon) from the pool of collected vouchers and placing it in a species concept (Newmaster *et al.*, 2006a, 2007). RF is the simple calculation of the percentage of specimens associated with a taxon when taxonomists or aboriginal informants are presented with a pool of vouchers and asked to perform "pile sorting". Trotter and Logan (1986) provide the calculation of a Consensus Factor [Fic=Nur-Nt/(Nur-1)], which is adopted to evaluate the degree of partition into categories (Heinrich, 2000). We have adopted this to include 'aboriginal utility' by the aboriginal informants (Ragupathy *et al.*, 2008a, 2009; Ragupathy & Newmaster, 2009), where Nur is the number of use-reports of informants for particular category (TK plant use) factor, where a use-report is a single record for use of a plant mentioned by an individual, and Nt refers to the number of species used for that particular category for all informants (Ragupathy *et al.*, 2008a).

DNA barcoding: Three DNA regions (*rbcl*, *matK* and *trnL-F*) were selected based on the previous plant barcoding studies (Newmaster *et al.*, 2006b, 2008b; Fazekas *et al.*, 2009, 2008; Ragupathy *et al.*, 2009). We isolated total genomic DNA from approximately 10 mg of dried leaf material from each sample using the kit, NucleoSpin® 96 Plant II (MACHEREY-NAGEL). Extracted DNA was stored in sterile microcentifuge tubes at -20ºC. The selected loci were amplified by PCR (see primers in Table 1) on a PTC-100 thermocycler (Bio-Rad). DNA was amplified in 20 µl reaction mixtures containing 1 U AmpliTaq Gold Polymerase with GeneAmp 106PCR Buffer II (100 mM Tris-HCl pH 8.3, 500 mM KCl) and 2.5 mM MgCl2 (Applied Biosystems, Foster City, CA), 0.2 mM dNTPs, 0.1 mM of each primer (0.5 mM for *matK*), and 20 ng template DNA. Amplified products were sequenced in both directions with the primers used for amplification, following the protocols of the University of Guelph Genomics facility. Products from each specimen were cleaned using Sephadex columns and run on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA). Bidirectional sequence reads were obtained for all the PCR products. Sequences were assembled using Sequencher 4.5 (Gene Codes Corp, Ann Arbor, MI), and aligned manually using Bioedit version 7.0.9. The sequences were used in combination with the morphometric analysis to produce classification trees.

Morphometric data collection and analyses: Morphological data variables were recorded for all specimen collections. A matrix of specimens and morphological characters were used in a multivariate phenetic analysis. Canonical ordination was used to detect groups of specimens and to estimate the contribution of each variable to the analysis. A cluster analysis was used to classify the specimens because it is better at representing distances among similar specimens (Sneath & Sokal, 1973). Cluster analysis was carried out using NTSYS (Rohlf, 2000). A distance matrix was generated from the specimens and characters using an arithmetic average (UPGMA) clustering algorithm and standardized data based on average taxonomic distance subjected to the unweighted pair-group method. The resulting distance matrix from the cluster analysis used in combination with the sequence data above to produce classification trees.

Results and discussion

Acacia ethnobotany genomics

The genus *Acacia* is an economically important species that comprises approximately 1350 species of which there are many cryptic sister species with pantropical distributions (Maslin *et al.*, 2003). *Acacia* species are well adapted to dry forest conditions (Ross, 1981) and have great utility in the forest industry; timber, fuel wood, fibre, medicine, food, handicrafts, domestic utensils, environmental amelioration, soil fertility, shade, game refuge, livestock fodder, ornamental planning, gum, and tannins (Wickens *et al.*, 1995; Ragupathy *et al.*, 1997; McDonald *et al.*, 2001; Midgley & Turnbull, 2003). However, there exists a taxonomic impediment because many *Acacia* species are quite difficult to differentiate using morphological characters (Bentham, 1842; Wardill *et al.*, 2005). Identification is important in order to distinguish invasive weedy species (Kriticos *et al.*, 2003) from rare species (Byrne *et al.*, 2001) or those of economic importance (Midgley & Turnbull, 2003).

DNA barcoding may be used to identify *Acacia* taxa that are important to society-at-large. Prickly acacia (*A. nilotica* subsp. *indica*) is a highly invasive weed in northern Australia. It is thought to have been introduced to Australia from India but the current distribution is not known and our samples indicate it is quite variable throughout India. This variability may include potential new species, which could be invasive weeds or new food and medicine. The aboriginal cultures recognize several ethnotaxa of this species, which they use as timber, tools, furniture, fodder for sheep and goats and personal hygiene; the young braches used as tooth brush by doing this infected teeth gums get cured properly. A similar

Indian Journal of Science and Technology Vol.2 No 5 (May 2009) ISSN: 0974- 6846

ethnotaxa (*Acacia leucophloea*) is extensively used for making illicit liquor.

Given the important economic value of acacias, it would be very useful to have a reliable identification tool that can differentiate *Acacia* species using only the leaves. Our classification tree from DNA barcoding sequence data (*rbcl*, *matK* and *trnH-psbA*) clearly resolved 12 *Acacia* species and identified considerable intraspecific variation (Fig. 1). In our study we chose sister species of *Acacia* that are difficult to tell apart. The defining characters of many acacias are found in the small flowers that appear during short periods of time during the year. Vegetation characters are more variable and less reliable for identification. In our study, we revealed the misidentification of 10 herbarium specimens were those that only had vegetative characters, underscoring the difficulty of identifying these species. Other studies have utilized DNA to classify previously undetermined specimens due to lack of available morphological characters and as a classification tool where specimens have proven difficult to classify (Wardill *et al.*, 2005). Many of these studies use fragments of DNA from various regions such as *ITS1* and *trnL*, which are useful for subspecies identification (Brenan, 1983; Fagg & Greaves, 1990). Wardill *et al.* (2005) created an *ITS1* genotype library that was used as an identification tool to be matched exactly to genotypes of other herbarium specimens identified by taxonomists. Although this *ITS1* genotype library is a useful tool for acacias, this is not a good region for DNA barcoding because it is not possible to sequence this region for many different groups of plants (Erickson *et al.*, 2008).

Our research confirmed a recent taxonomic split in the genus *Acacia.* In the classification DNA barcode using *rbcl*, *matK* or *trnH-psbA* can distinguish the new genus *Vachellia* species from that of the *Acacia* species (Fig. 1). Variation in *rbcl* alone could be used to differentiate *Vachellia* species from that of the *Acacia* species. These results are also supported by previous phenetic analysis in our lab (Newmaster *et al.*, 2009b). These results are supported by other phylogenetic studies in which *Vachellia* species are placed in a separate clade (100% bootstrap support); all species other than those of *Vachellia* are placed in a different clade (66% bootstrap support), indicating that *Vachellia* is relatively distantly related to other members of *Acacia* s.l. (Luckow *et al.*, 2003; Miller *et al.*, 2003; Seigler *et al.*, 2006). *Vachellia* (Acacieae, *Acacia* subg. *Acacia*) was actually recognized as a distinct genus from the 'true' *Acacia* early in the taxonomic *Acacia* literature (Wight & Arnott, 1834; Bentham, 1840). Our barcoding results support the earlier classification that recognizes *Vachellia*, which is the earliest legitimate generic name for species currently ascribed to *Acacia* subg. *Acacia*. This supports a growing body of morphological and genetic differences separating the subgenera of *Acacia* s.l. and molecular evidence that the genus *Acacia* s.l. is

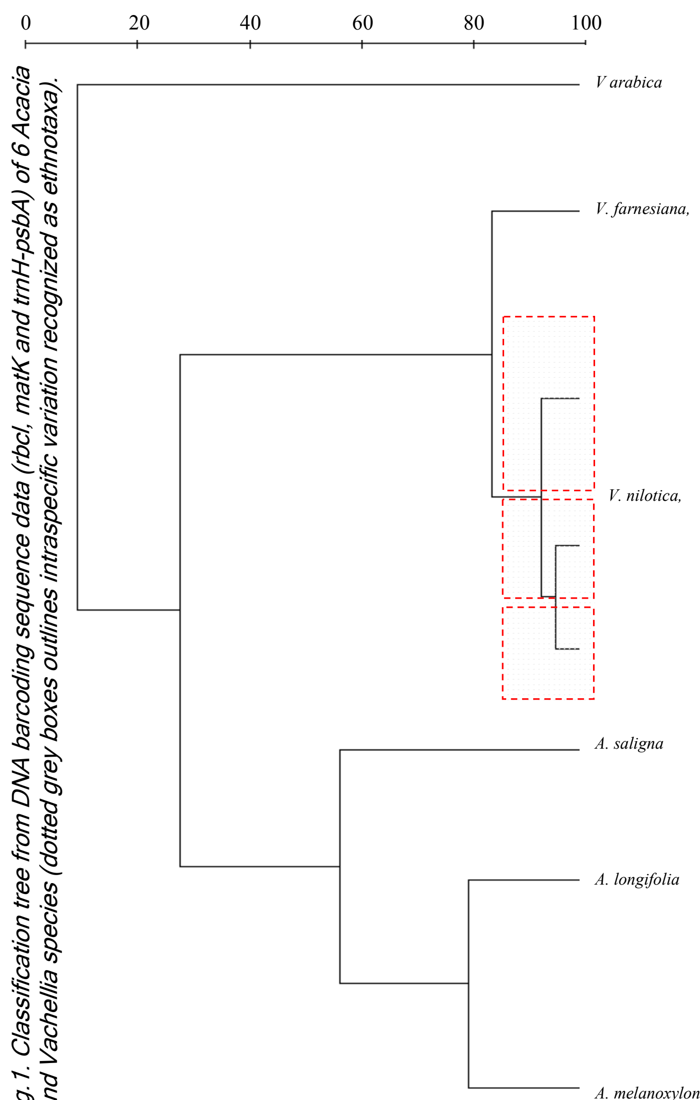


Fig.1. Classification tree from DNA barcoding sequence data (rbcl, matK and trnH-psbA) of 6 Acacia and Vachellia species (dotted grey boxes outlines intraspecific variation recognized as ethnotaxa).

polyphyletic, which has prompted new generic combinations (Miller & Bayer, 2001; Maslin *et al.*, 2003; Miller *et al.*, 2003; Seigler *et al.*, 2006; Newmaster & Ragupathy, 2009a). In current scenario, the majority of the Australian taxa would remain as *Acacia* Mill. with a significant number of name changes to Senegalia (203 spp.) and *Vachellia* Wight & Arn. (161 spp.) in Asia, Africa, Australia and in the Americas (Maslin *et al.*, 2003). The result of this reclassification will clarify the taxonomy of many economically important species.

Nutmeg ethnobotany genomics

The Myristicaceae, or nutmeg family, is an older group within the angiosperms that contains recently evolved species. The nutmeg family is comprised of ~500 species of canopy to subcanopy trees native to tropical rainforest environments (Smith, 1937; Janovec & Harrison, 2002). Nutmeg, *Myristica fragrans* Houtt. (Myristicaceae), is a coomon member of the nutmeg family that is endemic to

©Indian Society for Education and Environment (iSee)

the Maluku Province of Indonesia (formerly known as the Spice Islands). It has long been of importance both as a spice and as a commodity that was once of geopolitical significance. Historical and current indigenous uses of the fruit and seed are described in some detail by Van Gils and Cox (1994). Although the botany, cultivation, and history of *Myristica fragrans* have been studied, ethnobotanical studies of other species nutmeg are noticeably lacking. For example, members of the genus *Virola* and *Compsoneura* are harvested extensively in many South American countries as a source of wood for veneer and timber. In some Neotropical countries, exports of *Virola* sp. are rivalled in economic importance only by big-leaf mahogany (Rodan *et al.*, 1992; Macedo & Anderson, 1993). *Virola* and *Compsoneura* are species with considerable intraspecific genomic variation (Newmaster *et al.*, 2008b).

Identification of these economically important species are difficult. Many nutmeg species share similar leaf morphologies, identification of species relies heavily upon characteristics of the small flowers (1-4 mm) that are only present on adult trees for a few weeks every year. The genus Compsoneura, is an ideal group for testing barcoding in plants as they present a taxonomic impediment and the family has been found to have low levels of molecular variation compared to other closely related families (Sauquet *et al.*, 2003). Compsoneura contains some recently described taxa (Janovec & Neill, 2002) and a new species split (Janovec & Harrison, 2002). A recent ethnobotany genomic study (Newmaster *et al.*, 2008b) investigated the utility of six coding (Universal Plastid Amplicon – *UPA*, *rpoB*, *rpoc1*, *accD*, *rbcl*, *matK*) and one noncoding (*trnH-psbA*) chloroplast loci for barcoding in the genus *Compsoneura* using both single region and multiregion approaches. Five of the regions we tested were predominantly invariant across species (*UPA*, *rpoB*, *rpoC1*, *accD*, *rbcl*). Two of the regions (*matK* and *trnH-psbA*) had significant variation and show promise for barcoding in nutmegs. This study demonstrated that a two-gene approach utilizing a moderately variable region (*matK*) and a more variable region (*trnH-psbA*) provides resolution among all the *Compsonuera* species sampled including the recently evolved *C. sprucei* and *C. mexicana.* A classification analyses based on nonmetric multidimensional scaling ordination, concluded that the use of two regions results in a decreased range of intraspecific variation relative to the distribution of interspecific divergence with 95% of the samples correctly identified in a sequence identification analysis.

Further research presented here (Fig. 2) revealed cryptic diversity within the current species concepts, which has been recognized by various aboriginal cultures. The classification tree from recent DNA barcoding sequence data (*rbcl*, *matK* and *trnH-psbA*) reveals considerable intraspecific variation (Fig. 2). Further sampling is needed to determine whether they

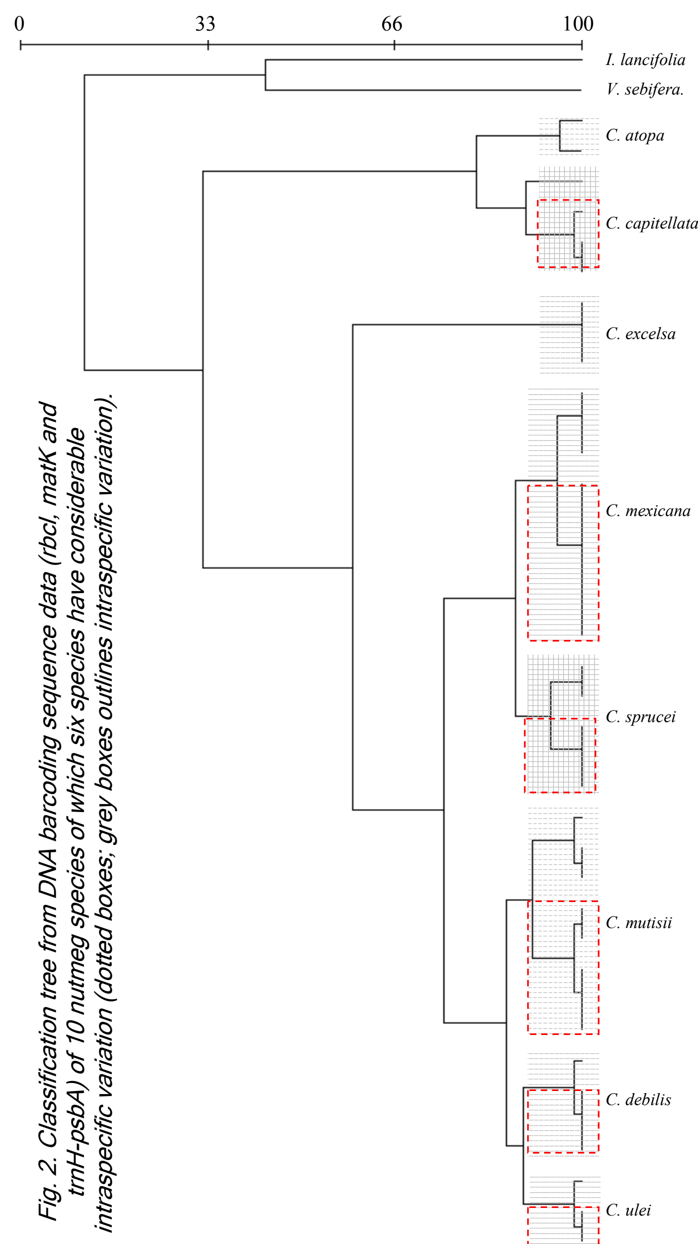


Fig. 2. Classification tree from DNA barcoding sequence data (rbcl, matK and trnH-psbA) of 10 nutmeg species of which six species have considerable intraspecific variation (dotted boxes; grey boxes outlines intraspecific variation).

warrant new species designations. As with all new species discoveries, this would have to be validated by the taxonomic evidence including morphology and potentially additional molecular data. What will need to be investigated is the consistency of characters used for a barcode and whether these characters hold true with increasing sample size. We are conducting further investigations concerning the interpretation of this variation by different aboriginal cultures. This barcoding study on nutmegs corroborates the recent description of *C. mexicana*, and supports the generic split into sections *Hadrocarpa* and *Compsoneura*, which was suggested previously based on a suite of morphological character evidence (Janovec & Harrison, 2002). Our research was limited to Central and South America and to our

Indian Journal of Science and Technology Vol.2 No 5 (May 2009) ISSN: 0974- 6846

knowledge, there are no published comprehensive studies that test the ability of plant barcodes to discriminate multiple populations of sister species that span several contents such as a pantropical distribution.

Conclusion

Ethnobotany genomics employs DNA barcoding for routine and reliable identifications that is becoming an automated process. Modern automated identification technology (AIT), such as DNA barcoding, may provide a quick, repeatable and reliable tool for identifying ethnotaxa and variation in cryptic species. Recent development of an AIT system for plants indicates that the efficacy of an AIT system equates with savings in time and resources, while providing quick, reliable identifications (Newmaster *et al.*, 2009a). We are currently using DNA barcoding to discriminate the cryptic ethno-taxa for *Tripogon* (Newmaster *et al.*, 2008a; Ragupathy *et al.*, 2009) and *Biophytum* (Newmaster *et al.*, 2009b) within regional floras. We propose that a DNA barcode may be a quick and reliable tool to identify ethnotaxa, which will further legitimize the validity of TK, rendering it testable and ultimately generalisable, mobile and globally meaningful.

The impacts of ethnobotany genomics will extend well beyond biodiversity science. Explorations of the genomic properties across the expanse of life are now possible using DNA barcoding to assemble sequence information for a standard portion of the genome from large assemblages of species. This is in contrast to the usual focus of large-scale genomics projects which acquire sequence information for all genes in single taxa. The barcode region is a genomic sentinel in which nucleotide composition of the plant barcode region closely mirror those in the rest of the genome. As our library of species expands we will be able to flag species whose genomes show unusual nucleotide composition, allowing them to be probed in more detail. Shifts in sequence composition may also reveal idiosyncratic rates of sequence and amino acid change. Perhaps the most important contribution is major barcode projects will leave an important legacy; a comprehensive repository of high-quality DNA extracts that will facilitate future genomic investigations.

Acknowledgement

We would like to thank members of the Biodiversity Institute of Ontario Herbarium. Jose Maloles for editing an earlier version and Dr Aron Fazekas and Royce Steeves for field and lab work with respect to the nutmeg samples. John Janovec at the Botanical Research Institute of Texas provided valuable samples and advice on nutmeg. Bruce Maslin at the W.A. Herbarium, Department of Environment and Conservation in Western Australia and Joe Miller CSIRO, Australia offered valuable comments and advice regarding Acacia.

References

1. Bentham G (1840) Contributions towards a flora of South America – enumeration of plants collected by Mr. Schomburgk in British Guiana. *J. Botany (Hooker)*. 2, 127-146.
2. Bentham G (1842) Notes on Mimoseae, with a short synopsis of species. *Journal of Botany (Hooker)*. 4, 323-418.
3. Blaxter ML (2004) The promise of a DNA taxonomy. *Phil.l Trans. Royal Soc. London Series B. Biol. Sci*. 359, 669-679.
4. Blaxter ML and Floyd R (2003) Molecular taxonomics for biodiversity surveys: already a reality. *Trends in Ecol. Evol*. 18, 268-269.
5. Brenan JPM (1983) *Manual on Taxonomy of Acacia Species: Present Taxonomy of Four Species of Acacia (A. albida, A. senegal, A. nilotica and A. tortilis)*, pp. 20-35. FAO, Rome, Italy.
6. Byrne M, Tischler G, Macdonald B, Coates DJ and McComb J (2001) Phylogenetic relationships between two rare acacias and their common, widespread relatives in south-western Australia. *Conservation Genetics*. 2, 157-166.
7. Chase MW, Salamin N, Wilkinson M *et al.* (2005) Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Soc. B: Biol.Sci.* 360, 1889-1895.
8. Cowan RS, Chase MW, Kress WJ and Savolainen V (2006) 300 000 species to identify: problems, progress, and prospects in DNA barcoding of land plants. *Taxon*. 55, 611-616.
9. Erickson DL, Spouge J, Resch A, Weigt LE and Kress WJ (2008) DNA barcoding in land plants: developing standards to quantify and maximize success. *Taxon*. 57, 1304-1316.
10. Fagg CW and Greaves A (1990) Acacia Nilotica 1869-1988, Annotated Bibliography No. F42 (ed. Langdon K), CAB International, published in collaboration with the Oxford Forestry Institute, Wallingford, UK.
11. Fazekas AJ, Burgess KS, Kesanakurti PR, Percy DM, Hajibabaei M, Graham SW, Husband BC, Barrett SCH and Newmaster SG (2008) Assessing the utility of coding and non-coding genomic regions for plant DNA barcoding. *PLOS ONE*. 3, 1-12.
12. Fazekas AJ, Kesanakurti PR, Burgess KS, Percy DM, Graham SW, Barrett SCH, Newmaster SG, Hajibabaei M and Husband BC (2009) Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? *Mol. Ecol. Resources*. 9, 130-139.
13. Funk DJ and Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes and consequences, with insights from animal mitochondrial DNA. *Ann. Rev. Ecol., Evol. Systematics*. 34, 397-423.
14. Gaston KJ and O'Neill MA (2004) Automated species identification: Why not? *Phil. Trans. Royal Soc. London Series B*. 359, 340-387.
15. Godfray HCJ (2002a) Challenges for taxonomy. *Nature*. 417, 17-19.

©Indian Society for Education and Environment (iSee)

16. Godfray HCJ (2002b) Towards taxonomy's 'glorious revolution'. *Nature*. 420, 461.
17. Godfray HCJ and Knapp S (2004) Introduction. in taxonomy for the twenty-first century-special issue. *Phil. Trans. Royal Soc. London Series B*. 359, 559-569.
18. Grant B (2009) A Fading Field Traditional taxonomists are an endangered species. Could their unique brand of knowledge disappear, too? *Scientist*. 23(6), 32-33.
19. Hammond, P. (1992) 'Species inventory', in Groombridge, B. (Ed.): Global Biodiversity: Status of the Earth's Living Resources, pp.17-39. Chapman and Hall, London,
20. Hawksworth DL and Kalin-Arroyo MT (1995) Magnitude and distribution of biodiversity in Heywood, V.H. (Ed.). In *Global biodiversity Assessment*, pp.107-191. Cambridge University Press, UK.
21. Hebert PDN and Gregory TR (2005) The Promise of DNA barcoding for taxonomy. *Sys. Biol*. 54(5), 852-859.
22. Hebert PDN, Cywinska A, Ball SL and DeWaard JR (2003) Biological identification through DNA barcodes. *Proc. Royal Soc. B: Biol. Sci.* 270, 313-321.
23. Heinrich M (2000) Ethnobotany and its role in drug development. *Phytotherapy Research* 14, 479-488.
24. Jain SK and Rao RR (1977) *A Handbook of field and Herbarium Methods.* Today and Tomorrow's Printers & Publishers, New Delhi.
25. Janovec JP and Harrison JS (2002) A morphological analysis of the Compsoneura sprucei complex (Myristicaceae), with a new combination for the Central American species Compsoneura mexicana. *Sys. Bot*. 27, 662-673.
26. Janovec JP and Neill AK (2002) Studies of the Myristicaceae: an overview of the *Compsoneura atopa* complex, with descriptions of new species from Columbia. *Brittonia*. 54, 251-261.
27. Kress J and Erickson DL (2008) DNA barcodes: genes, genomics, and bioinformatics. *Proc. Natl. Acad. Sci., USA*. 105, 2761-2762.
28. Kress WJ and Erickson DL (2007) A two-locus global DNA barcode for land plants: the coding *rbcl* gene complements the non-coding trnH-psbA spacer region. *Public Library of Science ONE*. 2, e508. doi: 10.1371/journal.pone.0000508.
29. Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences*, USA, 102, 8369-8374.
30. Kriticos DJ, Sutherst RW, Brown JR, Adkins SW and Maywald GF (2003) Climate change and the potential distribution of an invasive alien plant: Acacia nilotica ssp. indica in Australia. *J. Appl. Ecol*. 40, 111-124.
31. Lahaye R, van der Bank M, Bogarin D *et al.* (2008) DNA barcoding the floras of biodiversity hotspots. *Proc. Natl. Acad. Sci., USA*. 105, 2923-2928. doi: 10.1073/pnas.0709936105.
32. Lipscomb S, Platnick N and Wheeler Q (2003) The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends in Ecol. Evol*. 18, 65-66.
33. Luckow M, Miller JT, Murphy DJ, Livshultz T (2003) A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data. In: *Advances in Legume Systematics, Part 10, Higher Level Systematics* (eds. Klitgaard BB & Bruneau A), pp. 197-220. Royal Botanical Gardens, Kew, UK.
34. Macedo DS and Anderson AB (1993) Early Ecological Changes Associated with Logging in an Amazon Floodplain. *Biotropica*. 25(2), 151-163.
35. Maslin BR, Miller JT and Seiger DS (2003) Overview of the generic status of Acacia (Leguminosae: Mimosoideae). *Aus. Sys. Bot*. 16, 1-18.
36. May RM (1988) How many species are there on Earth? *Science*. 241, 1441-1449.
37. McDonald MW, Maslin BR and Butcher PA (2001) Utilisation of acacias. In: *Flora of Australia, Volume 11A, Mimosaceae, Acacia Part 1* (eds. Orchard AE, Wilson AJG), pp. 30-40. ABRS/CSIRO Publishing, Melbourne, Australia.
38. Midgley SJ and Turnbull JW (2003) Domestication and use of Australian Acacias: an overview. *Aus. Sys. Bot*. 16(1), 89-102.
39. Miller JT and Bayer RJ (2001) Molecular phylogenetics of Acacia (Fabaceae: Mimosoideae) based on the chloroplast *matK* coding sequence and flanking trnK intron spacer region. *Am. J. Bot*. 88(4), 697-705.
40. Miller JT and Bayer RJ (2003) Molecular Phylogenetics of Acacia subgenera Acacia and Aculeiferum (Fabaceae: Mimosoideae) based on the chloroplast *matK* coding sequence and flanking trnK intron spacer regions. *Aus. Sys. Bot*. 16, 27-33.
41. Miller JT, Grimes JW, Murphy DJ, Bayer RJ and Ladiges PY (2003) A phylogenetic analysis of the Acacieae and Ingeae (Mimosoideae: Fabaceae) based on trnK, *matK*, psbA-trnH, -and trnL/trnF sequence data. *Sys. Bot*. 28, 558-566.
42. Newmaster SG and Ragupathy S (2009a) Testing Plant Barcoding in a Sister Species Complex of Pantropical Acacias (Mimosoideae, Fabaceae). *Molecular Ecological* Resources. 9(Suppl. 1), 172-180. (doi: 10.1111/j.1755-0998.2009.02642.x).
43. Newmaster SG and Ragupathy S (2009b) Ethnobotany Genomics - Discovery and Innovation in a New Era of Exploratory Research. *J. Ethnobiol. Ethnomedicine*. (in review).
44. Newmaster SG, Fazekas A and Ragupathy S (2006b) DNA barcoding in the land plants: evaluation of *rbcl* in a multigene tiered approach. *Can. J. Bot*. 84, 335-341.
45. Newmaster SG, Fazekas A, Steeves R and Janovec J (2008b) Testing Candidate Plant Barcode Regions in the Myristicaceae. *Mol. Ecol. Res*. 8, 480-490.
46. Newmaster SG, Murugesan M, Ragupathy S, Nagaraj N and Velusamy B (2009b) Ethnobotany Genomics study reveals three new species from the Velliangiri Hills in the Nilgiri Biosphere Reserve, Western Ghats, India. *Ethnobot.* 21, 1-28.
47. Newmaster SG, Ragupathy S and Janovec J (2009a) A Botanical Renaissance: State-of-the-art DNA Barcoding Facilitates an Automated Identification Technology (AIT) System for Plants. *International Journal of Computer Applications in Technology*. (Invited article, in press).

48. Newmaster SG, Ragupathy S, Balasubramaniam NC, and Ivanoff RF (2007) The Multi-mechanistic Taxonomy of The Irulas In Tamil Nadu, South India. *J. Ethnobiol.* 27, 31-44.
49. Newmaster SG, Ragupathy S, Ivanoff RF, and Nirmala CB (2006a) Mechanisms of Ethnobiological Classification. *Ethnobot.*. 18(1,2), 4-26.
50. Newmaster SG, Velusamy B, Murugesan M and Ragupathy S (2008a) *Tripogon cope*, a new species of Tripogon (Poaceae: Chloridoideae) in India with a Morphometric analysis and synopsis of Tripogon in India. *Sys. Bot*. 33(4), 695–701.
51. Pennisi E (2007) Wanted: a barcode for plants. *Science*. 318, 190–191.
52. Ragupathy S and Newmaster SG (2009b) Valorizing the 'Irulas' traditional knowledge of medicinal plants in the Kodiakkarai Reserve Forest, India. *J. Ethnobiol. Ethnomedicine*. 5(10), 1-13.
53. Ragupathy S, Ashwath N and Mahadevan A (1997) Acacias and mine rehabilitation: the need for inoculating acacias with mycorrhizal fungi, Forestry Program, ACIAR Acacia Publication, Australia. In: *Turnbull, J. W., H.R. Crompton and K. Pinyopusarerk. eds. Recent Developments in Acacia Planting.* Proceedings of an International workshop held in Hanoi, Vietnam, Australian Centre for International Agricultural Research, Canberra. pp. 274-280.
54. Ragupathy S, Newmaster SG, Gopinadhan P and Newmaster C (2008b) Exploring Ethnobiological Classifications for Novel Alternative Medicine: a case study of Cardiospermum halicacabum L. (Modakathon, Balloon Vine) as a traditional herb for treating rheumatoid arthritis. *Ethnobot*. 19, 1-20.
55. Ragupathy S, Newmaster SG, Murugesan M, Velusamy B and Huda M (2008) Consensus of the 'Malasars' traditional aboriginal knowledge of medicinal plants in the Velliangiri holy hills, India. *J. Ethnobiol. Ethnomedicine*. 4(8), 1-14.
56. Ragupathy S, Newmaster SG, Velusamy B and Murugesan M (2009a) DNA barcoding discriminates a new cryptic grass species revealed in an ethnobotany study by the hill tribes of the Western Ghats in southern India. *Mol. Ecol. Res*. 9(Suppl. I), 172–180.
57. Ratnasingham S and Hebert PDN (2007) BOLD: the Barcode of Life Data System (www.barcodinglife.org). *Mol. Ecol. Notes*. 7(3), 355-364.
58. Rodan BD, Newton AC and Verissimo A (1992) Mahogany conservation: status and policy initiatives. *Environ. Conservation*. 19, 331-338.
59. Rohlf F (2000) NTSYS: Numerical Taxonomy and Multivariate Analysis System, Version 2.1. Exeter Software, New York.
60. Ross JH (1981) An analysis of the African Acacia species: their distribution, possible origins and relationships. *Bothalia*. 13, 389–413.
61. Sauquet H, Doyle JA, Scharaschkin T *et al.* (2003) Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implications for character evolution. *Bot. J. Linnean Soc.* 142, 125–186.
62. Seigler DS, Ebinger JE and Miller JT (2006) The genus Senegalia (Fabaceae: Mimosoideae) from the New World. *Phytologia*. 88(1), 38-93.
63. Smith AC (1937) The American species of the Myristicaceae. *Brittonia*. 2, 393–510.
64. Sneath P and Sokal R (1973) Numerical taxonomy. W. H. Freeman, San Francisco, California, USA
65. Tautz D, Arctander P, Minelli A, Thomas RH and Vogler AP (2003) A plea for DNA taxonomy. *Trends in Ecol. Evol*. 18, 70–74.
66. Tautz D, Arctander P, Minelli, A, Thomas RH and Vogler AP (2002) DNA points the way ahead in taxonomy. *Nature*. 418, 479.
67. Trotter R and Logan M (1986) Informant consensus: a new approach for identifying potentially effective medicinal plants. In: *Plants in indigenous medicine and diet: biobehavioural approaches* (ed. Etkin NL), pp.234 91-112. Redgrave Publishers, Bedfort Hills, NY.
68. Van Gils C and Cox PA (1994) Ethnobotany of nutmeg in the Spice Islands. *J. Ethnopharmacol.* 42, 117-124.
69. Wardill TJ, Graham GC, Zalucki M et al. (2005) The importance of species identity in the biocontrol process: identifying the subspecies of Acacia nilotica (Leguminosae: Mimosoideae) by genetic distance and the implications for biological control. *J. Biogeography*. 32, 2145–2159.
70. Wheeler QD, Raven PH and Wilson EO (2004) Taxonomy: Impediment or Expedient? *Science*. 303(5656), 285-286.
71. Wickens GE, Seif-El-Din AG, Sita G and Nahal I (1995) *Role of Acacia species in the rural economy of dry Africa and the Near East.* FAO Conservation Guide No. 27. Food and Agriculture Organization, Rome, Italy.
72. Wight R and Arnott GAW (1834) *Prodromus Florae Peninsulae Indiae Orientalis*. London.
73. Will KW and Rubinoff D (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*. 20, 47-55.

©Indian Society for Education and Environment (iSee)