

BOTANICAL IDENTIFICATION OF MEDICINAL PLANTS: A BIOSYSTEMATIC CONTEMPLATION

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ABSTRACT

Identification of a species is the most critical task which necessitates application of instinct, knowledge and skill. A correctly identified species is given recognition with the help of an appropriate name. Nomenclature of organisms is thus a means to afford communication about them and to standardize and organize an unambiguous reference system about them. Inadequacy and inaccuracy of base-line information regarding delimitation of natural biotic entities and their recognition are likely to magnify small gross errors of correlation and extrapolation to large dimensions while digitalizing data for species distribution maps and their utilitarian perspectives. The events of misidentification of plant species, inadvertent use of totally unrelated species or by closely related inferior quality species can hinder their medicinal use, the adverse effects of which may even kill a consumer. The medicinally useful plants and plant products must have their specific identity correctly ascertained with standardized circumscription and nomenclature for quality control and prevention of adulteration of drugs. This serious challenge has to be taken up by biosystematics, the Post-Linnaean Systematics most appropriate to recognize species and variations within species for its in-built taxonomic philosophy and genecological principles to read both micro- and macro- biomolecules emitting diagnostic and phylogenetic signals. Through this field-based natural science life forms can be discovered, identified, described, named, classified and catalogued, with their diversity, ecology, phylogeny and distributions. In view of the virtues and prospect greater concern of biosystematics is an absolute necessity for augmenting success and prosperity of traditional as well as modern systems of medicines utilizing herbal medicines. In essence, biosystematics provides indispensable bioresources data-base to support many fields of beneficial research including medical science.

KEYWORDS: Identification, Species, Nomenclature, Medicinal plants, Genecology, Biosystematics

Identification of species: A contemplation

Identification of a species needs application of instinct, knowledge and skill. Identification is thus the art and science of recognizing a living entity and distinguishing it from others using an appropriate name. Standardized nomenclature of each organism enables communication about a species by a single or unique name and organization of an unambiguous reference system about the elements that constitute different plant-, animal- and microbial components of biodiversity and bioresources. Identification of a species and its under-categories really poses a serious challenge to the stock-taking and assessment of biodiversity and resources availed there from. Moreover the contemporary biologists show inadequate concern in matters of identification of the biological specimens and samples they work on. Negligence to classical disciplines in general and taxonomy or systematics has been yielding inadequacy and inaccuracy of base-line information regarding delimitation of natural biotic entities and their recognition are likely to magnify small gross errors of correlation and extrapolation to large dimensions while digitalizing data for species distribution maps and their

utilitarian perspectives. One must consider that nomenclature as well as conceptualization and delimitation of taxa have far reaching implications for diversity assessment, conservation, phylogeographic deductions, optimum utilization and inferences. The events of misidentification of species yielding non-wood forest products (Mukherjee, 2012a) especially crude drugs can often prove detrimental producing adverse effects even to kill a patient. Advertent adulteration by totally unrelated species or by closely related inferior quality species as well as inadvertent misidentification of species can be damaging to the efficacy and quality of the product. Each medicinally useful species as well as other forest dwelling species used as source of food, spices, forage, fibre, floss, resins, gums etc. must have individual identity correctly ascertained with standardized circumscription and nomenclature. This is necessary for quality control and prevention of adulteration of the product. This serious challenge of the hour has to be taken up primarily by such classical discipline of biological science as taxonomy or systematics hand in hand with its modern form, the biosystematics.

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Species: A comprehension

Defining a species with a single or a few sentences is possibly the most difficult task. Even *Charles Darwin* was of the opinion that “No one definition has yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species”. It is rather a concept that can be developed from several definitions and explanations that ‘species’ perpetually receives.

“*Species*” is the Latin word derived from *specere*, a present infinitive expression for a “kind” I observe (present active *speciō*). Linnaeus considered species as a ‘kind’ or group of individuals clustered according to their physical resemblance. It implies that organisms can be grouped into discrete (static) recognizable entities. Linnaeus considered the species to be fixed entities i.e. not capable of undergoing evolution. Linnaean concept of species and binomial system of nomenclature proposed by him got accepted by taxonomists and are still in vogue. Scientists have issuing identity cards to every species based on the standard laid down by Linnaeus. For all plants the officially accepted starting point of nomenclature is 1753 being the year of publication of *Species Plantarum* and for all animals the nomenclature starts from 1758, the year of publication of the 10th edition of *Systema Naturae*. Mainly for these two publications he would remain immortal in all the days to come. He is aptly regarded as the intellectual father of taxonomy.

A species has been defined diversely which can be steered in two conceptual directions:

(i) *Taxonomic concept*: This includes application of orthodox, morphological and typological concepts, with the foundation laid by Linnaeus (1753; 1758, 1759). The concept at present is put under three basic categories, viz., classical, phenetic and cladistic or phylogenetic. The classical concept (alpha taxonomy) is based totally on utilization of external characters or morphology of the organism for its identification as a species. The phenetic concept (Sneath and Sokal, 1963; Sneath and Sokal, 1973) is in principle based on objectivity and repeatability and in practice it uses the multivariate analytical techniques packed in software-programmes to assess the overall similarity in organisms or measure distances between biotic units. Phenetics always considers a species as a natural biotic unit or a phenon with absolute over-all similarity. This concept or

method is not sensitive to sibling species since in such a case the member-organisms, although morphologically identical or indistinguishable from each other, are genetically distinct or reproductively isolated. The cladistics or the phylogenetic systematics (Hennig, 1966, Wiley and Mayden, 1985) principally adhering to the evolutionary concept considers species as that monophyletic biotic unit which have an objectively (quantitatively assessed absolute sharing of a common ancestry and lineage i.e. line of descent.

(ii) *Biological concept*: According to Ernest Mayr (1953): “Species are groups of actually (or potentially) interbreeding natural populations, which are reproductively isolated from other such groups. Establishment of this concept necessitates application of conventional as well as molecular biosystematics.

There is basically no conflict between the taxonomic and biological species concept, especially in case of sexually reproducing organisms. However, especially for the large group of strictly autogamous and vegetatively reproducing plants, the biological species concept does not work naturally and needs application of genetics and molecular taxonomy. Again the classical taxonomy fails to distinguish sibling species which are composed of morphologically indistinguishable but reproductively isolated individuals (Mayr, 1970). For understanding more about species the data sources had to be expanded from morphology to anatomy, cytogenetics, ecology, embryology, serology, paleontology, molecular biology etc. Even the Beta-taxonomists, who critically validate recognition of the species on morphological attributes, have been presently using DNA-data in addition. In spite of such realization, the deeper insight into cytological, genetic and molecular attributes is considered by many to be of little importance in providing practical utility in ready recognition of species. However one has to be optimistic in utilization of biosystematic tools especially in recognition of the prokaryotes, animal- and plant- parts, tissues or residues, where morphology has no role to play. In such cases one has to use molecular data sources, especially sequence of DNA and RNA molecules, to identify the species. Discrimination of species is possible by analysis of a short fragment of DNA and tagging the label showing the characteristic short base sequence very much like the bar coded price statement attached to commercial commodities (Hebert, 2003). Accordingly species is a unit of living entity in which a specific short tandem repeat (nucleotide sequence

up to 25 bp or so in length) in their genomes (nuclear/ mitochondrial/ chloroplastidial [plants]) is conservative among its members (absolutely constant) and variable (distinct) from those of others. The specific short sequence of nitrogenous bases transmitting diagnostic signal for a species is called DNA bar-code (Kress *et al.*, 2005; Kress and Erickson, 2008). Thus, being characteristic for a species DNA bar-code provides the best tool for its recognition. *DNA bar-coding* is a biosystematic method which uses a short genetic marker in the genome of an organism to identify it as a specific species.

Infraspecific categories

The taxonomic hierarchy internationally followed puts Regnum or Kingdom at the top and species at the base and three infraspecific categories below the rank of the species, viz. *Subspecies*: A population of some biotype forming regional facies of a species.

Variety: A population of some biotype forming local facies of a species

Form: A category with sporadic variation in one or two characters.

Nomenclature

Appropriate and standard nomenclature of a species is no less important than its identification. Biological nomenclature is the art and science of naming a species abiding by certain principles, rules and recommendations set up internationally as the Codes (Rules) of Nomenclature. There are many advantages of biological nomenclature. An organism's scientific name hands over the key to literature concerning its characteristics, occurrence, relationship, values etc. The scientific names provide a means of channeling the outputs of systematic research for use by all conveyors of benevolence. Thus biological nomenclature is relevant not only to the taxonomists but also to all who (economic botanists, herbalists, pathologists, pharmacologists, pharmacists, molecular biologists, physiologists, ecologists and others) need to communicate internationally about organisms unburdened by the problems of the plethora of languages and dialects (Mukherjee, 2012b).

Names of each type of organism are also extremely diverse. It was thus considered necessary to standardize nomenclature by certain rules which had its

activation for the first time in 1867 to eventually get established in form of a rule book called International Code of Nomenclature. For each category of Biota separate sets of Rules have been framed viz., Bacteria, Plants and animals. Nomenclature of Plants of natural habitats is governed worldwide by International Code of Botanical Nomenclature (ICBN). It covers Fungi, Lichens, Algae (including the prokaryotic Cyanobacteria), Bryophytes, Pteridophytes, Gymnosperms and Angiosperms.

Biological characteristics and relationships of different groups of organisms vary widely. Viruses and Bacteria which have very attenuated morphological characteristics are identified and classified by biochemical and molecular attributes and so each group requires a separate Code of Nomenclature different from those of the eukaryotes. Cyanobacteria were sought to be separated from algae as they are similar to bacteria but there was no general agreement and are still retained within Plantae. The rules and recommendations in the context of nomenclature of plants can be availed from ICBN(2006) familiar as the *Vienna Code*. However the Melbourne *Code* (2012) has replaced the *Vienna Code* of 2005. At the 18th International Botanical Congress held in Melbourne, Australia from 23rd to 30th July, 2011, the name International Code of Botanical Nomenclature (ICBN) was changed to *International Code of Nomenclature for algae, fungi, and plants* (ICN) which in essence is the set of rules and recommendations dealing with the formal botanical names that are given to plants, fungi and a few other groups of organisms, all those "traditionally treated as plants" (McNeill *et al* 2006). Subsequent to Melbourne Congress (McNeill *et al*, 2012) it will no longer be necessary for new names of plants, fungi, and algae (and designations of types) to appear in printed matter in order to be effectively published—effective publication being a fundamental requirement of the Code for acceptance of any nomenclatural act" (McNeill and Turland, October, 2011). As an alternative, publication online in Portable Document Format (PDF) in a publication with an International Standard Serial Number (ISSN) or International Standard Book Number (ISBN) will be permitted. The Special Committee had proposed 1 January 2013 as the starting date for the new rules (the beginning of the year following the expected publication of the new Code), but the Section believed implementation so important that it decided to bring the date forward to 1 January 2012. As with previous codes, it takes effect as soon as ratified by the congress i.e. on

and from Saturday 23 July 2011, but the documentation of the code in its final took some more time to get published in 2012. For names published on or after 1 January 2012, the description and/or diagnosis must be in either English or Latin. This will apply to names of new taxa in all groups covered by the Code. It is already the requirement for names of new fossil-taxa published on or after 1 January 1996.

For over 100 years, the Code has permitted separate names for asexual and sexual phases of those fungi whose life history involves morphological expressions so different that, until recently, it was commonly impossible to link one to the other. Molecular studies have changed this situation very substantially, and more and more connections are being made, so that the asexual phase (the anamorph) and the sexual phase (the teleomorph) of the one fungal species are increasingly being identified. As such, currently, the name applied to the whole fungus (the holomorph) has to be one that is based on a teleomorphic element, an additional new set of rules was accepted that will allow lists of widely used names to be protected en masse, or lists of names of uncertain application to be rejected en masse, so as to minimize the nomenclatural disruption that would otherwise be caused by applying the rule of priority strictly.

Since 2004, the online database MycoBank (www.mycobank.org) has become increasingly used by mycologists to register new fungal names and associated data, such as descriptions and illustrations. Upon registration, MycoBank issues a unique number which can be cited in the publication where the name appears. This number is also used by the nomenclatural database Index Fungorum and serves as a Life Science Identifier (LSID). The Nomenclature Section in Melbourne approved a new rule in the Code whereby, on or after 1 January 2013, the publication of a new fungal name (names of new taxa, new combinations, replacement names, and names at new rank) must include a citation of "an identifier issued by a recognized repository" in order to be validly published (i.e., to have any status under the Code).

Bacteriological Code (BC) first developed in 1953 (published in 1958), and started essentially as a derivative of the *ICBN* and 1973 developed what amounted to a new starting date through the establishment of an "Approved List of Bacterial Names". The naming of

viruses and subviral agents (prions etc.) will be covered by the draft *International Code of Virus Classification and Nomenclature*, currently being developed from the current *Rules of Virus Classification and Nomenclature* by the International Committee for the Taxonomy of Viruses (ICTV) of the International Union of Microbiological Societies (IUMS).

Cultivated plants do not have a natural population structure like the wild plants. These plants have been developed through a variety of artificial breeding processes. The *International Code of Nomenclature for Cultivated Plants* (ICNCP) originated in 1953 and represents a set of rules subordinate to those of the *ICBN* and applicable specifically to cultivated plants below the level of subspecies. Thus up to the level of the Subspecies *ICBN* applies to cultivated plants too, as wild species were domesticated to develop cultivated species and their varieties. Below the level of subspecies and for the varieties in cultivation the International Code of Nomenclature for Cultivated Plants (ICNCP) published by the International Society of Horticultural Science is applicable. The ICNCP regulates the names of *cultigens* i.e. plants whose origin or selection is primarily due to intentional human activity (Brickell *et al.*, 2009). For the most part, these are plants with names in the classification categories cultivar, Group and grex, within the scope of the *ICNCP* (2009 version edited by Brickell *et al.*).

Nomenclatural problems

Many plants have inadvertently been named several times, giving rise to a list of several millions of plant species. The same plant was given different names because of slight differences resulting from habitats in different climates, while others were named by scientists unaware the plant had already been described and named. Even different plants received the same name. Standardization of nomenclature is thus absolutely necessary.

Scientists have known for a long time that the databases contained many duplicates, but until now no one has been certain of how many because the information has been contained in several databases around the world. Now, for the first time, a single working list of all flowering plant species has been created, and it has been made possible by an automated rule-based system. Creation of a single "Plant List" was the result of the Convention on Biological Diversity at

their 2002. As per the Catalogue of Life dated 17th August 2012, which contains contributions from 115 databases as many as many as 1,315,754 species, 113,716 infraspecific taxa, 870,920 synonyms and 351,941 common names have been put into record which covers viruses and subviral agents, bacteria and archaea, chromista, algae fungi, protozoa, plants and animals. The expected number of species and infraspecific categories are still far from our present knowledge.

Problems in identification of a species

- i. An identified species may be a part of another species.
- ii. Different plant species may belong to the same species complex.
- iii. Two morphologically alike entities may belong to different species
- iv. The identification of a species may not tally with the scientific recognition of that species.

Since the identification of a species must be at par with scientific recognition, it has always been a subject of criticism and revisions. Natural selection often drives the evolution of new species as traits favoured within populations may incidentally create reproductive isolation between populations. The species problem seems to be in a large measure related to a combination of genetic and ecological issues which needs to be addressed with certain experimental procedures. While at present the purely genetic perspective of the issue is fairly well comprehended, a simultaneous understanding of the ecological concern is necessary. Thus much appears certain, however, that the Linnean species are units of the same importance ecologically as their constituent elements are genetically. In view of the necessity of keeping the relation between ecological and purely genetic units in mind, the terms ecotype, ecospecies, cenospecies were proposed and used by Turesson (1922 a, b; 1925) to cover the variations shown by populations of the same species as they are realized in different ecological condition prevailing in nature. Efforts of Turesson paved the pathway towards development of a new form of taxonomy i.e. biosystematics to resolve and delimit every biotic entities (Camp and Gilly, 1943) using spectacles with glasses of ecology held in the frame of genetics. Aspects of rapprochement between taxonomy and ecology concern considerably to the philosophical framing of biosystematics (Mukherjee, 2009).

Biosystematic approaches to address the problem

Biosystematics has been defined as the most dynamic form of taxonomy which is concerned with establishment of variation pattern based primarily on population sampling, ecological observations and genetic experiments.

Biosystematics, the Post-Linnaean Systematics, is the most appropriate subject to recognize species and variations within species since it is taxonomy in its philosophy and genecology in principle which concerns both micro- and macro- biomolecules emitting diagnostic and phylogenetic signals (Mukherjee and Acharya, 2011). It is traditional in one hand and modern on the other. This is the field-based natural science through which life forms are discovered, identified, described, named, classified and catalogued, with their diversity, life histories, living habits, roles in an ecosystem, and spatial and geographical distributions recorded. In essence, it is biosystematics, the neo-taxonomy that provides indispensable information to support many fields of research and beneficial applied programmes related to medical science.

Objectives of biosystematics are to:

1. delimit every natural biotic entity composing biodiversity.
2. provide a more meaningful system of nomenclature.
3. resolve microevolution leading to speciation.
4. ascertain genetic relationships among organisms.
5. provide a more scientific basis for recognition of the infraspecific categories.
6. overall improvement of traditional taxonomy and optimization of services to other disciplines of science.

Biosystematic methods

1. Field Study

- (i) Sampling of population systems showing variation.
- (ii) Ecological observations on population systems showing variation.
- (iii) Collection of specimens for herbarium preservation and of diaspores (seeds, rhizomes, tubers etc.) for culture in experimental garden.

2. Herbarium study

- (i) Recording of range of variation within species.
- (ii) Recording of geographical distribution and detection of discontinuity, if any (disjunction & vicariance) from specimens.

3. Experimental Work

- a. In Garden:
 - (i) Observation on variations in controlled environment
 - (ii) Sympatric Trial through hybridization between population systems.
 - (iii) Observation on vitality and vigour of hybrids.
- b. In Laboratory:
 - (i) Observation on homology of chromosomes in hybrids at meiosis.
 - (ii) Observation on miscellaneous aspects viz. anatomy, embryology, palynology etc. for relationship evaluation in case of non-hybridizing populations.
 - (iii) Perform molecular Biosystematic experiments

4. Compilation Work

Above findings are compiled with base-line data from comparative morphology and geographical distribution.

Molecular Biosystematics

Molecular Biosystematics, although field based, uses both micro- and macro-molecules with virtues of taxonomic markers being polymorphic and conservative to resolve distinctions as well as micro-evolution concerning species. A precise idea of such molecules can be gathered from the following inventory. However modern biosystematics should not be confused with molecular taxonomy. Although related, biosystematics always resolves variation pattern in population systems in the field first which is eventually evaluated either by in field -genetic experiments or by in- lab molecular studies.

A. Micromolecules (mol mass<1kDa)

- 1. Primary metabolites:
 - a. Universal- Citric acid, aconitic acid etc.
 - b. Non universal- Non-protein aminoacids
- 2. Secondary metabolites :

- a. Phenolic compounds : Flavonoids, Phenolic acids, Coumarins, Xanthones, Quinones etc.
- b. Terpenes: Monoterpenes (Geraniol, menthol, pinene, camphor, Carvone etc.); Diterpenes (phytol), Sesquiterpenes (Farnesol), Triterpene (Squalene), Tetraterpene (Carotenoids), Polyterpene (Natural rubber)
- c. Tannins: Hydrolysable (Ellagitannins), Condensed (Proanthocyanin, Leucocin)
- d. Alkaloids
- e. Glucosinolates etc.

B. Macromolecules (mol mass>1kDa)

Non-Semantides : Large molecules of Starch, cellulose, lipids containing no hereditary information.

Semantides: Molecules containing hereditary information which are *Primary*(DNA:Nuclear, Chloroplast, mitochondrial), *Secondary*(RNAs (m-RNA, r-RNA, t-RNA, Sn-RNA, RNA I, Cp-RNA & Mt-RNA) and *Tertiary*(Proteins)

Aspects studied and methods used in Molecular Biosystematics

1. Proteins: [Material: RUBISCO, Seed Albumin, Plastocyanins, Cytochrome C, Plastoquinone etc. Methods: *Immuno-electrophoresis, Comparative amino acid sequencing, isoelectric focusing, electrophoresis*]

2. Nucleotides: [Material: DNA (Nuclear, mitochondrial and chloroplastial), RNA (ribosomal) ;Methods: DNA-DNA Hybridization, DNA-RNA Hybridization, Nucleotide sequencing]

Nucleotides useful in Biosystematic study

- (i) Nuclear: rDNA -18S (encoding the Smaller subunit of rRNA); ITS I & II + 5.8S to a lesser extent 26S gene (along with 5.8S genes encoding the larger subunit of rRNA), gap C gene encoding cytosolic Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
- (ii) Chloroplast: (rbcL gene of cpDNA encoding RUBISCO, matK gene within trnk introns, ndhF etc.)
- (iii) Mitochondrial: (mt DNA-nad1b/c exon sequence, mat R sequence)
- (iv) Ribosomal (rRNA) 5S, 16S & 23S in prokaryotes and 5S, 5.8S & 26S in eukaryotes.

Biosystematic equivalents of the conventional Infra-specific taxa

Topodeme, *Ecotype*, *Genecodeme*, *Geographical race*: equivalent to *Subspecies*. i.e. *Ecodeme* (Genecodeme of a lower order, local/ecological race): equivalent to *Variety*. *Genodeme*: (Equivalent to or minor ecological variant): equivalent to *Form*.

Biosystematics enables identification of species from

- (i) Leaves - entire or fragments even when flowers or fruits are not available.
- (ii) Plant-parts in form of dusts, pastes, juice, infusion, decoction etc.
- (iii) Herbal supplements or wood used in medicine
- (iv) Products in Commerce (pharmaceuticals and nutraceuticals)
- (v) Very old preserved or partially digested/decomposed plant parts, plant-residues in peats etc.
- (vi) Animal tissues, blood, semen, bones, teeth and other partially digested/decomposed residues.
- (vii) Plant residues in faecal matter and gut of animals.
- (vii) Conspecific elements identified as different species, presumably of the same gene-pool.

Summing up

While summing up, it may be concluded that from among the modern approaches it is biosystematics which for being the field-based experimental taxonomy, has the potential to succeed in species identification and biodiversity assessment since it has three major components, viz. morphology, genetics and ecology which are the best tools to work out in the respective manner the three components of biodiversity, viz. species-, genetic- and ecological -diversities. Perfection in assessment of biodiversity is certain to pave the pathway not only to recognition of species and infra-specific entities but also to trigger the optimum sustainable utilization of different types of bioresources including those used in traditional as well as modern systems of medicine. Undoubtedly biosystematics has the potential to establish the rapprochement between traditional taxonomy and molecular taxonomy. In view of this, greater concern of biosystematics in bioresources, especially the medicinal plants, is an absolute necessity to ensure correct identification of the species and the

products obtainable from them. Biosystematics has to come up with all its tools to use data ranging from morphology to informational homology (molecular-information packages) for augmenting success and prosperity in use of diverse medicinal plants and crude drugs obtained therefrom.

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