Molecular approach to the classification of medicinally important actinorhizal genus *Myrica*

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Actinorhizal genus *Myrica*, found in Meghalaya, India, has some dispute with respect to its classification. The present paper reviews the status of this genus. Fruits of *Myrica* sp. are commercially available in some parts of the country. It is a useful medicinal plant rich in vitamin C. Being actinorhizal, it is also useful in regeneration of nitrogen depleted soils.

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Nitrogen is required in large amounts as an important component of proteins, nucleic acids and other cellular constituents, and is frequently found limiting to the growth of the green plants. This is because of the continual loss of nitrogen from soil reserves by processes like microbial denitrification, soil erosion, leaching, chemical volatilization and removal of nitrogen-containing crop residues from the land. The replacement of soil nitrogen is brought about by either adding nitrogen containing chemical fertilizers directly in the soil or by the activity of biological nitrogen fixation (BNF) systems. The conversion of atmospheric nitrogen into nitrate, a form of nitrogen that can be metabolized into amino acids and proteins by terrestrial plants, is called nitrogen fixation. In the living world, major conversion of N\textsubscript{2} into ammonia is brought about by certain prokaryotes by a process called as BNF. The BNF involves two types of microorganisms: Non-symbiotic or free living microorganisms like *Clostridium*, *Klebsiella*, *Azotobacter*, etc., and symbiotic microorganisms like *Frankia* and *Rhizobium*. In symbiotic fixation, the nitrogen fixed by the microorganisms is made available to the host plant, whereas the microsymbionts, in turn, use organic compounds supplied by the plant as an energy source\textsuperscript{1}. Nitrogen fixing plants are key constituents in many natural ecosystems in the world.

Many nitrogen fixing plants are woody perennials or trees (NFTs), most of these being found in the tropics. *Frankia*, the actinomycete involved in symbiotic BNF, nodulates the roots of dicotyledonous plants belonging to 8 plant families and 25 genera, and they are called actinorhizal genera. The plant families belonging to such genera are Betulaceae, Casuarinaceae, Coriariaceae, Datiscaceae, Elaeagnaceae, Myricaceae, Rhamnaceae and Rosaceae. Although taxonomically diverse, the actinorhizal plants have some common features. All are perennial dicots and all except *Datisca*, which has herbaceous shoots, are woody shrubs or trees\textsuperscript{2}. Actinorhizal plants are widely distributed and are found in every continent, except Antarctica. They are mostly found in the temperate zone, while some species of Casuarinaceae and Myricaceae are considered tropical. In the Indian subcontinent, they are confined to higher altitudes in Himachal Pradesh, Jammu and Kashmir, Arunachal Pradesh, Sikkim, West Bengal hills, Meghalaya and to some extent in the coastal regions and plains. Some of the genera commonly found in India are *Alnus*, *Casuarina*, *Coriaria*, *Elaeagnus*, *Hippophae* and *Myrica*. Actinorhizal plants are pioneers on poor nitrogen soils including sandy and gravelly sites, shores of streams and lakes, wetlands and exposed raw mineral soils. Actinorhizal plants find economical use as timber, fuel wood, in land reclamation, biomass production and in forestry\textsuperscript{3}. In western Europe, actinorhizal plants are used primarily for reclamation of industrial wastelands and for land stabilization.

About 97 species of *Myrica* Linn. (Family: Myricaceae) are reported globally and this genus is widely distributed in both the temperate and sub-tropical regions with maximum species diversity in Africa and Boreal America (Index Kewensis, 1977-1985), and only one representative has been reported in Australia (*Myrica australiasica* F. Muell). Its geographical distribution in India can be seen in sub-tropical Indian Himalaya from Ravi eastwards to Assam and in Khasi, Jaintia, Naga and Lushai Hills at altitudes between 900-1200 m above mean sea level\textsuperscript{4}. However, they have also been seen in higher reaches of Himachal Pradesh. Trees of *Myrica* grow well in nitrogen depleted soils and are common associates of pine (*Pinus* sp.) and oak (*Quercus leucotrichophora*).
They are also found in mixed forests and in agricultural and marginal lands.

*Myrica* has been used variably. Fruits of this tree are used in making jams, syrups, and juices and can also be consumed raw. Bark is used in making paper and rope. Mention of *M. esculenta* in the medicinal system of *ayurveda* is noteworthy. The extract from the root and bark is known to be astringent, carminative and antiseptic, and especially a decoction of the bark is considered useful in asthma, toothache, diarrhea, fever, lung infection, cough, chronic bronchitis and dysentery. Bark is chewed to relieve toothache and headache, and is also used as fish poison. Tannin extracted from the bark is used as a tanning and dyeing agent. The oil obtained from the flowers of *Myrica* is also reported to have medicinal effect, especially in ear-ache, inflammation and paralysis. Myriconol isolated from stem bark is reported to have lesser toxicity than related rotenone.

Smith gave the following classification for genus *Myrica*: Division- Magnoliophyta; Class- Magnoliopsida; Subclass- Hamamelidae; Family- Myricaceae; Order- Myricales. The subclass Hamamelidae comprises of Myricales, Fagales, Juglandales, Hamamelidales, Urticales, Leitneriales and Casuarinales. This is a phylogenetic grouping of orders, which is characterized by strongly reduced, often unisexual flowers, which either lacks or produces a poorly developed perianth. Phylogenetically, the Myricales are thought to be most closely related to the Juglandales and the Fagales. The Myricaceae is considered to be an ancient family by taxonomists, dating to the Tertiary Epoch of the Cretaceous Period with the living members representing relics of once extensive tracts of subtropical forest that spread across the territory that is now central and southern Europe. Plants of the family Myricaceae are considered to be promiscuous hosts because several species are effectively nodulated by most isolated strains of *Frankia*. The base chromosome number throughout the family is eight, with various levels of ploidy present.

*Myrica esculenta* Buch.-Ham. ex D. Don (Syn. *M. farquhariana* Wall., *M. sapida* Wall., *M. nagi* Thunb., *M. integrifolia* Roxb.) belongs to the family Myricaceae and is commonly known as ‘Soh-Phi’ in Khasi, ‘Nagatenga’ in Assamese and ‘Kaiphal’ in Hindi. Common name in English is Box myrtle. This is the only species of the genus *Myrica* reported to be found in India. This tree is distributed in the Chinese-Japanese region including the Sub-Himalayan tract, Khasi hills, Sylhet and Southwards up to Singapore and in the Malayan islands at an altitude of 1600-2000 m above mean sea level.

*M. esculenta* is a small to moderate sized, evergreen, dioecious tree with height ranging from 3-15 m. Leaves are crowded and aromatic and are lanceolate to oblanceolate or obovate, nearly entire or sharply serrate, obtuse or acute, almost glabrous with resinous dots beneath. New leaves usually start sprouting in February-March. Flowers are minute, unisexual, male and female flowers are borne on different trees. Male flowers occur in catkin and the female flowers in axillary erect spikes. Fruits are edible and ellipsoid or ovoid drupes of the size of cherry or bigger, tubercled, reddish or cheese coloured when ripe with rugose nut. They are covered with a crust of white waxy material, permeated with brown and black spots. They ripen during summer and possess a pleasant sourish sweet taste. Fruiting starts at the beginning of April and lasts till the month of June.

Interestingly, morphological diversity within *M. esculenta* exists with regard to fruit size, fruit colour, leaf serration, leaf size, tree height, etc. This has led to confusion among various workers. Some workers claimed that different morphotypes of the plant belong to different species. One of the morphotypes was referred to as *M. nagi* and the other one as *M. esculenta*. Others also claimed that *M. nagi* is synonym of the species, *M. esculenta*. These claims were based on morphological descriptors. Therefore, in an effort to resolve this dispute, a need was felt to study molecular phylogeny of this genus using nucleotide sequence data of different morphotypes. The first molecular phylogenetic study on the family Myricaceae was done by Huguet and others where the molecular phylogeny of 13 species of the family Myricaceae was established based on *rbcL* gene and the 18S-26S ITS. Their results showed that some species of the genus *Myrica*, such as, *M. gale* and *M. hartwegii*, and genus *Comptonia* belong to a distinct phylogenetic cluster distinct from some other *Myrica* species. They transferred the latter *Myrica* species to a new genus, *Morella*. However, the taxonomy within this family is highly controversial because of morphological variation exhibited by many species.

Approach to both fundamental and applied biological problems has been transformed by the emergence of many new techniques. Amplification of
specific regions of DNA to a million fold has been made possible by the polymerase chain reaction (PCR) technique. This method was first invented by Kary Mullis in 1983\textsuperscript{19}. PCR has revolutionized approaches to molecular study in many fields. Restriction site analysis of amplified DNA is a valuable method for detecting genetic variation in some cases\textsuperscript{20}.

The ribosomal RNA genes family comprises of very conserved regions (18S and 26S gene) that can be used to infer phylogeny at higher taxonomic levels, as well as more rapid evolving segments (ITS, IGS) that may be useful at the generic, specific and even (in case of IGS) at the population level. Ribosomal DNA cistrons typically are located in the nucleolar organizing region (NOR) and may be present on several different chromosomes. The internal transcribed spacer (ITS) region of the 18S-5.8S-26S nuclear ribosomal cistron is one of the most popular sequences for phylogenetic inference at the generic and infrageneric levels in plants\textsuperscript{20}. The ITS-1 and ITS-2 regions are part of the nuclear rDNA transcript, but are not incorporated into ribosomes, and appear to play a role in the maturation of nuclear rRNAs, bringing the large and small subunits into close proximity within a processing domain\textsuperscript{20,28}. The need for sequence data from nuclear genome at lower taxonomic levels makes the ITS region a popular site for this study. Since the ITS region is G+C rich and prone to secondary structure, sequencing can be difficult\textsuperscript{21}. Different protocols have been used to amplify and sequence the ITS regions\textsuperscript{22,24}. Since ITS sequences are present in-between highly conserved regions of the ribosomal RNA genes, it is possible to design primers that are complementary to the conserved sequence for amplification purpose.

Three different morphotypes of *Myrica* sp. are found in Meghalaya. Morphotype 1 trees are considered as *M. nagi* by some workers. Morphotype 2 trees are considered as *M. esculenta*. Morphotype 3 trees have not been described separately and may have been considered as variants of morphotype 2 trees. As stated above, some workers consider all these three morphotypes as members of the species *M. esculenta*. Recently, Yanthan and others\textsuperscript{25} used ribosomal operon to develop a system for ascertaining the boundaries of species and genera. Based on a scale developed by them, they have proposed that morphotype 1 trees indeed should be classified as *M. nagi*, while morphotype 2 trees be classified as *M. esculenta*. They considered morphotype 3 to be intermediate between these two species. They found supporting evidence in the form of projected secondary folding of the 5.8S rRNA, which was similar for morphotypes 2 and 3 and different for morphotype 1.

References


