Chapter 4:

Results

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The sampling of Bamboo was carried out at many places. The process of collection, enumeration, sample data and taxonomic identification of the collected Bamboo specimen has been given in **Section 4.1: Morphological identification of Bamboo species from Southern Assam.** Also the photographs and GPS data collected during sampling has also been included in this section.

The process of Bamboo DNA extraction, PCR amplification of targeted DNA sequences, Sequencing of DNA, Editing of Sequences and Submission has been in **Section 4.2: Genomic diversity of Bamboo.** The list of Bamboo DNA Sequences submitted to NCBI database has also been tabulated in this Section.

The *in-silico* analysis of sequences to generate secondary data and information like GC% content, Codon usage pattern, Nucleotide and Amino acid composition; Pairwise distance and pattern of Nucleotide Substitution is given in **Section 4.3: *In-silico* analysis of targeted Bamboo sequences.** The generated information has also been depicted by charts and graphs in this section.

The in-depth analysis of the phylogenetic relationship of the targeted DNA sequences, which were created by Neighbour Joining method, has been given in **Section 4.4: Phylogenetic Relationship of targeted Bamboo Sequences.**

4.1 Morphological identification of Bamboo species from Southern Assam

4.1.1 Bamboo species collected

4.1.1.1 *Bambusa cacharensis* R. B. Majumdar

Local name: Betua

Uses: it is used in fencing boundaries of an area; it is used in making bamboo floor.

Location:	Sonai	Lat 24° 51 '42" N	Long 92° 47' 57" E
	Singari	Lat 24° 48 '31" N	Long 92° 45' 53" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths usually large; imperfect blades more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades whitish or glaucescent. Culm sheaths are hairy on outside. Culm sheaths have chocolate-brown hairs on outside; imperfect blades orange yellow, spreading at right angle to the axis.

Bambusa cacharensis

Description of the species:

Culms are bright green in colour and have a shiny surface. Culms have variable length and can reach upto 30m. Culms have a maximum diameter of 18cm at chest height. Branching occurs from base upwards and lower branches are spreading with 2-3 recurved spines located on the nodes. No. of culms: 55 – 70.

Culm sheaths are variable in shape and size, 15-35cm long and 18-35cm broad at base. They are coriaceous with the lower sheaths (often developing ones) are striped and upper culm sheaths orange-yellow. Culm sheaths are glabrous to pubescent with dark brown hairs, deciduous, top striate and somewhat rounded, margins plaited. The ligule is continuous with sheath top, margin fringed with whitish cilia; auricle are inconspicuous; blades are imperfect and triangular, concave with involute,

ciliate margins upto 10cm long. The outer surface are covered with dense dark-brown to black hairs, the inner surface is glabrous, shining and greenish-yellow when young, base is cordate, tip is acute and margins decurrent on the sheath.

Leaves are linear - lanceolate or linear, very variable in size, usually 6-22cm in length and 1- 3cm in breadth; rounded at the base into a short, often swollen, petiole-like base ±2 mm long. The leaf apex is acute, glabrous above except for a few long hairs near the base. Leaf is glabrous or puberulous beneath, margins are scabrous and ciliate towards base, main vein narrow and pale, secondary veins 4-6, intermediate 7-9, transverse veinlets are absent but regular pellucid glands are present at intervals. Leaf-sheaths glabrous or slightly pubescent with silvery hairs, ending in a thick, often ciliate callus and a short auricle furnished with a few stiff, curved, silvery, deciduous bristles, edges ciliate; ligule is short.

Flowers were not seen.

4.1.1.2 *Bambusa nutans* Wall. ex Munro

Local name: Makal

Uses: it is used for making fence, making handicraft etc.

Location:	Sonai	Lat 24° 51 '42" N	Long 92° 47' 57" E
	Singari	Lat 24° 48 '31" N	Long 92° 45' 53" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm sheaths usually large; imperfect blades more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades are green or without white crusts. Young culms dark green, glabrous; imperfect blades erect, leaves dark green, Culm-sheaths covered with appressed, scattered, black hairs on outside; auricles unequal, wavy.

Bambusa nutans

Description of the species:

Culms are variable in size upto10-15m tall and 5-10cm in diameter at chest height. Culms are loosely clumped, much-branched above, usually unbranched or thinly branched below. Culms are striated, green in colour, smooth surfaced but not shining and white-ringed below the nodes. The nodes are slightly raised, often it is hairy, lower nodes usually bears rootlets; internodes are usually 25-45cm long and thick-walled. No. of culms: 43 – 65.

Culm sheaths are variable in size usually 10-23cm long and upto 30cm wide at base and covered on the back with appressed, scattered, black hairs. The base, bearing a ring of soft deciduous hairs, is rounded and truncate at top. Blades are imperfect particularly of the lower nodes, usually 15-23cm long and very broad. Those located at middle nodes are shorter, acute; margins recurved, clothed within with appressed black hairs, rounded at the base and decurrent on the top of the sheath. The auricles are large, wavy, one usually erect, the other decurrent, both densely furnished with long, curved bristles. The ligule is 2-2.5mm in size, smooth and dentate.

Leaves usually have a size of 15-25cm in length and 2-3.5cm in breadth. Leaves are linear-lanceolate, acuminate at apex, rounded and usually oblique or attenuated at the base, ending in a twisted, scabrous point. Leaves are green in colour on both surfaces when young, upper surface becoming dull-green on maturity, glabrous except the scabrid hairs on the midrib and marginal veins, lower surface glaucous, glabrous or slightly hairy, scabrous on the edges. The main vein rather narrow, prominent, pale beneath, secondary veins are 7-10 in number, intermediate veins number 8-9, pellucid glands are frequent. The petiole-like base is 3-5mm long; leaf-sheaths striated, hairy when young, ending in a smooth callus and produced in a falcate auricle with a few bristles. The ligule is elongated, obtuse and hairy.

Flowers were not seen.

4.1.1.3 *Dendrocalamus hamiltonii* Nees & Arn. ex Munro

Local name: Pecha

Uses: it is used for making flute.

Location:	Santinagar	Lat 24° 26 '79" N	Long 92° 19' 84" E
	Singari	Lat 24° 48 '31" N	Long 92° 45' 53" E

Taxonomic key

Culms form a thick rhizome, not creeping; culm-sheaths rather large; imperfect blades much broader, almost occupying top of the sheath.

Dendrocalamus

Culms are not giant, without white crusts; culm-sheaths hairy on outside. Culms arborescent, straight, cavity large, culm-sheaths covered with brown-black or black hairs on outside; imperfect blades not continuous on the sheath. Ligule of the culm sheaths entire; culm sheaths with scanty patches of black, stiff, deciduous, appressed hairs on outside.

Dendrocalamus hamiltonii

Description of the species:

Culms are large usually 12-20m tall and may grow upto 25m, usually naked below, more often armed or branching from the lower nodes, much branched above, 10-15 cm in diameter at chest height. Culms are grey in colour when young with dense appressed pubescence and becoming dull-green on maturity. The nodes are prominently thickened and some lower nodes have 1-3 cm long downwardly curved rootlets. The internodes are 30-50 cm long and the wall thickness of lower internodes are 1.5cm thick and of the upper ones 0.5-0.8 cm thick. No. of culms: 20 – 37.

Culm-sheaths are long and stiff, they are variable in size. Those of lower part of larger culms 35-45cm long and upto 20cm broad at base. Culm sheaths are glabrous and shiny on inner surface, rough and glabrous or with scanty patches of brown-black, stiff, deciduous, appressed hairs on the outer surface. Culm sheaths are truncated at the top and furnished on either side with a small glabrous, triangular

point; Blades are imperfect and upto 30cm long, 5-10cm broad, ovate-lanceolate, sides incurved, glabrous on the outer surface, closely covered with black, sharp hairs at the base of the inner surface. The ligule is 5mm high, smooth, entire.

Leaves are variable in size; those present in older culms are smaller than those in new culms. Leaves are usually 36cm long and 8cm broad, the sides are unequal and rounded at the base. Leaves broadly lanceolate above, cuspidate, ending in an acuminate scabrous, twisted point, dorsal surface smooth, ventral surface rough, finely serrate in the margins. The main vein is narrow and raised, secondary veins 6-17 pairs, fairly prominent, intermediates 5-7. The petiole-like base thick, 4-5mm long; leaf-sheaths sparsely puberulous when young becoming glabrous above on maturity, furnished below with white appressed stiff hairs on outer surface, glabrous and shiny inside, its is somewhat keeled below the shining callus. The ligule is broad, usually elongate, obliquely truncated or jagged.

Flowers were not seen.

4.1.1.4 *Bambusa balcooa* Roxb.

Local name: Sil Borua

Uses: It is used in house construction; scaffolding, paper industry and the shoot are edible.

Location:	Santinagar	Lat 24° 26 '79" N	Long 92° 19' 84" E
	Sonai	Lat 24° 51 '42" N	Long 92° 47' 57" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually large; imperfect blades more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades are green or without white crusts. Young culms are dark green, glabrous; imperfect blades erect, leaves dark green. Culms stout; culm-sheaths covered with dark brown hairs on outside; auricles sub equal. Culms strong, walls thick; culm-sheaths large, longer than broad, covered with some patches of appressed hairs on outside; auricles usually small.

Bambusa balcooa

Description of the species:

Culms are 15-25m in height and 8-16cm in diameter. Culms are green in colour when young and becoming dull grayish green on maturity. The walls are about 3.5cm thick at base, branching occurs from the base; nodes are swollen with a whitish ring above, hairy below. The lower internodes are 10-12cm long and the diameter of the cavity about 1/3rd of the diameter of the culm, upper internodes are upto 45cm in length and cavities are larger than the lower internodes. No. of culms: 27 – 42.

Culm sheaths are of two kinds; those on the lower nodes are shorter and broader, with densely appressed, dark hairs on outer surface inner surface glabrous and shiny. The margins have cilia along one edge and on the other edge cilia are present only at the top, it is rounded on the top. The culm sheaths of the upper nodes are 30-45cm long and 20-35cm broad at the base, more or less glabrous, truncated; imperfect blades of the culm-sheaths of lower nodes are short, triangular, decurrent to a short fringed auricle. The culm-sheaths of upper nodes are 12-20cm long and 5-12cm broad, triangular, acute to acuminate, densely hairy on adaxial surface. They striated above, rounded at base, and then deccurrent on the sheath in a narrow band bearing a few long ciliate hairs. The ligule is 4-9mm high, denticulate, membranous; auricle is very short or sometimes absent, ciliated.

Leaves are oblong-lanceolate in shape, l5-38cm long and 2.5-5cm broad, rounded or sub-cordate at the base into a short petiole-like base. The apex has a twisted, scabrous, setaceous point, glabrous above, pale and puberulous beneath, margins scabrous-ciliated. The main vein is prominent, shiny beneath, secondary veins 7-12; intermediate veins are 6-7 in number. The leaf-sheaths are striated with appressed-white-hair, they are truncated above with a narrow callus, sometimes furnished with a very few stiff, wavy, deciduous bristles; ligule is membranous and broadly triangular.

Flowers were not seen.

4.1.1.5 *Bambusa arundinacea* Willd.

Local name: Kata Barua

Uses: Predominantly used as pillar, paper industry. The leaves are eaten in curry.

Location:	Santinagar	Lat 24° 26 '79" N	Long 92° 19' 84" E
	Sonai	Lat 24° 51 '42" N	Long 92° 47' 57" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually large; imperfect blades are more or less triangular.

Bambusa

Culms thorny, Culms and branches very densely tufted forming impenetrable look; auricles of the culm-sheaths small or inconspicuous; nodes of the branches with 2-3 recurved, short spines.

Bambusa arundinacea

Description of species:

Culms are bright green in colour and have a shiny surface. Culms have variable length and can reach upto 30m. Culms have a maximum diameter of 18cm at chest height. Branching occurs from base upwards and lower branches are spreading with 2-3 recurved spines located on the nodes. The nodes are slightly swollen, lower nodes are rooting; internodes are variable in length upto 45cm long. The lower internodes are slightly grooved and flattened on one side, the walls are 3-4cm thick and cavity is small. No. of culms: 40 – 70.

Culm sheaths are variable in shape and size, 15-35cm long and 18-35cm broad at base. They are coriaceous with the lower sheaths (often developing ones) are striped and upper culm sheaths orange-yellow. Culm sheaths are glabrous to pubescent with dark brown hairs, deciduous, top striate and somewhat rounded, margins plaited. The ligule is continuous with sheath top, margin fringed with whitish cilia; auricle are inconspicuous; blades are imperfect and triangular, concave with involute, ciliate margins upto 10cm long. The outer surface are covered with dense dark-

brown to black hairs, the inner surface is glabrous, shining and greenish-yellow when young, base is cordate, tip is acute and margins decurrent on the sheath.

Leaves are linear - lanceolate or linear, very variable in size, usually 6-22cm in length and 1- 3cm in breadth; rounded at the base into a short, often swollen, petiole-like base ±2 mm long. The leaf apex is acute, glabrous above except for a few long hairs near the base. Leaf is glabrous or puberulous beneath, margins are scabrous and ciliate towards base, main vein narrow and pale, secondary veins 4-6, intermediate 7-9, transverse veinlets are absent but regular pellucid glands are present at intervals. Leaf-sheaths glabrous or slightly pubescent with silvery hairs, ending in a thick, often ciliate callus and a short auricle furnished with a few stiff, curved, silvery, deciduous bristles, edges ciliate; ligule is short.

Flowers were not seen.

4.1.1.6 *Bambusa pallida* Munro

Local name: Bakal

Uses: This species is used for house building, baskets, mats, toys, wall plates, screens and wall hangers.

Location: Santinagar Lat 24° 26 '79" N Long 92° 19' 84" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually large; imperfect blades are more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades are whitish or glaucescent. Culm sheaths are hairy on outside. Auricles of the culm sheaths are small; imperfect blades wavy, sparsely hairy. Culms are arborescent; more than 8m high and 4cm in diameter**.**

Bambusa pallida

Description of the species:

Culms are 13-20m high and 5-8cm in diameter; they are smooth and olive-green in colour. The young shoots covered with white powder; the nodes are not very prominent and the lower nodes have root and not many branches. The internodes are 45-70cm long with thin walls. No. of culms: 70 – 81.

Culm sheaths are 18-30cm long and 20-25cm broad; slightly attenuated upwards and very straight truncated at top, only when young somewhat rounded truncated. Sheaths are glabrous or covered on the back with appressed white hairs when young, turning black on maturity. The imperfect blades are long, usually longer than the sheath, wavy along the middle, often upto 35cm long, triangular-acuminate from a broad base which covers nearly the whole top of the sheath. The blades have appressed black hairy within, glabrous or sparsely hairy on the back; blades are slightly rounded at the lower edges. The auricles are small, rounded and furnished with bristles. The ligule is very narrow.

Leaves have a size of about 10-22cm in length and 1-2.5cm in breadth which are linear- lanceolate, rounded or sub-cuneate at the base. Leaves are furnished above with a subulate, twisted scabrous point, glabrous above, except for the scabrous marginal veins which are whitish and hirsute beneath. The margins are scabrous, main veins are conspicuous, shining, secondary veins 4-6, rarely more, intermediate veins are 7-9 in number. The petiole like base is very short, about 2mm long and leaf-sheaths glabrous with prominent, smooth callus. The auricles are rounded, erect and fringed with a few stiff, long, white bristles. The ligule is very short.

Flowers were not seen.

4.1.1.7 *Bambusa assamica* Barooah & Borthakur

Local name: Mrittinga

Uses: This species is commonly used for pulp and paper industries, constructions, scaffoldings, fencing, handicrafts, shoots used as vegetable etc.

Location:	Santinagar	Lat 24° 26 '79" N	Long 92° 19' 84" E
	Singari	Lat 24° 48 '31" N	Long 92° 45' 53" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm sheaths are usually large; imperfect blades more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades are green or without white crusts. Culm sheath are glabrous; Auricles of the culm-sheaths are large, unequal. Imperfect blade of the culm sheaths is shorter than the sheath; culms upto 12m high and 3cm in diameter.

Bambusa assamica

Description of the species:

Culms are caespitose, erect, 5-12m high and 1.5-3cm in diameter, not very straight. Culms are white powdery when young turning glabrous and green on maturity. New culms are branched from the lower nodes upwards; often the few lower nodes are without branches, when present branches not very long. The nodes are prominently thickened; the internodes are 15-40 cm long with 3-4mm thick walls. No. of culms: 40 – 50.

Culm sheaths are 8-16cm long and 5-12cm broad at base. Sheaths are glossy green when young, outer surface glabrous or white powdery, often shiny; attenuated and rounded at the top. The imperfect blades are 6-15cm long and 5-8cm broad, ovate, acuminate, bases rounded, glabrous on the outer surface, appressed pale hairy within with smooth margins. The ligule is upto 1 mm high, narrow, entire; auricles 2, rounded, ±lcm high, unequal with wavy, ± 1cm long bristles, one upwardly conspicuous, narrow, the other downwardly broad, decurrent on the sheath.

Leaves are 10-24cm long and 2.5-4cm broad which are oblong-lanceolate, unequally rounded at the base. Leaves have scabrid margin, ending above in a twisted, subulate, scabrous point, glabrous above, whitish or glaucous beneath, main vein conspicuous, scabrous, secondary veins 8-10, intermediate 6-7. The leaf-sheaths sparsely pubescent when young becoming glabrous striated and polished on maturity. Auricles are 2 in number, long, rounded upto 1.5cm, pale white bristles. The ligule is short, minutely dentade.

Flowers were not seen.

4.1.1.8 *Bambusa tulda* Roxb.

Local name: Jama betua

Uses: It is used for scaffolding, handy craft, furniture, cooking utensils, etc. Shoots is used as vegetable and leaves as fodder.

Location: Singari Lat 24° 48 '31" N Long 92° 45' 53" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually large; imperfect blades are more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades are whitish or glaucescent. Culm sheaths are hairy on outside. Auricles of the culm-sheaths are large; imperfect blades not wavy; culm-sheaths densely hairy. Auricles of the culm-sheaths are wavy and fringed with cilia. Culms uniformly very straight with a white ring just above the nodes; nodes rooting only at ground level; culm-sheaths usually as broad as long.

Bambusa tulda

Description of the species:

Culms are 10-23m high and 5-10cm in diameter, glabrous, green when young turning grayish green on maturity. Culms are almost unbranched below; nodes are not swollen, lower ones have fibrous root. The internodes are 40-70cm long, with white ring below the internodes with 7-12mm thin walls, 7-12mm. There are many branches from nearly all the nodes, lower branches are slender, horizontal with few leaves. No. of culms: 37 – 45.

Culm sheaths are 15-25cm long and 15-27cm broad, deciduous, inner surface smooth, shining, often whitish powdered. The outer surface is covered with appressed black hairs, attenuate upwards and rounded or truncated at top. The imperfect blades are broadly triangular, reniform or cordate, cuspidate, erect and hairy within. The ligule is continuous with the sheath at the top, narrow, entire.

There are 2 auricles which are unequal, large and wavy; one continuous with the blade and the other rounded, upward, long-fringed.

Leaves are linear oblong or linear lanceolate in shape, 15-31cm long and 2-4cm broad, equally rounded at the base. Leaves are acuminate above in a subulate twisted point, glabrous above, except for the scabrous veins near the margin on one side, glaucescent and puberulous beneath, scabrous on the edges. The main vein is rather narrow, secondary veins number 6-10; intermediate veins are 7-8 in number. The pellucid glands are faint, scanty; petiole like base is short about 2.5mm long. The leaf sheaths are striated, glabrous and ligule very small. Auricles rounded and fringed with white hairs.

Flowers were not seen.

4.1.1.9 *Bambusa vulgaris* Schrad. ex Wendl.

Local name: Jai

Uses: It is used for making handicraft, used for making fence, used as a rope etc.

Location: Sonai Lat 24° 51 '42" N Long 92° 47' 57" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually large; imperfect blades are more or less triangular.

Bambusa

Culms are without spines. Ventral surface of the leaf blades are green or without white crusts. Young culms are dark green, glabrous; imperfect blades erect, leaves dark green. Culms stout; culm-sheaths covered with dark brown hairs on outside; auricles sub equal. Culms are not very strong, walls thin; internodes green or striped with yellow or pitcher-shaped. Culm-sheaths broader than long, covered with thick, appressed hairs on outside; auricles large with pale, wavy stiff bristles.

Bambusa vulgaris

Description of the species:

Culms erect or sub-erect which are 8-20m high and 5-15cm in diameter. Culms are bright green or striped green and yellow, matured culms are more yellowish than green, surface looks polished and shiny. The branching starts usually from mid culm to top. The nodes are prominent, the lower ones often with a narrow ring of brown roots. The internodes are upto 45cm long with 7-15mm thick walls. No. of culms: 35 – 58.

Culm sheaths are 15-25cm long and 20-35cm broad, often streaked when young with green and yellow, they are rounded at top and concavely truncated, striated and the outer surface densely covered with thick appressed brown-black hairs, edges ciliate. Auricles 2, sub equal, falcate, conspicuous, continuous with the blade, ± 1.5 cm high, with pale, wavy, 6-8mm long, stiff bristles. The ligule is 5-7.5mm broad, dentate, sometimes long fimbriate; imperfect blades are triangular, acute, 5-15cm long and upto 12cm broad at base. Sheaths are greenish yellow with appressed hairy within, margins bristly, revolute, rounded at the base and decurrent on the sheath.

Leaves are narrowly or broadly lanceolate, 15-32cm long and 2-4cm broad which are rounded or attenuate at the base, ending above in a long: twisted, scabrous point. Leaves are pale green, glabrous on both surfaces, sparsely hairy beneath when young, scabrous on the margin and on the adjacent nerves. The main vein narrow, pale, secondary veins 6-8, intermediate veins are 8-9 in number. The petiole like base is ± 5 mm long; leaf-sheaths are striated, ending in a smooth ciliate callus. Auricles are smooth and rounded, with a few deciduous bristles; ligule is short and ciliated.

Flowers were not seen.

4.1.1.10 *Melocanna baccifera* (Roxb.) Kurz.

Local name: Muli

Uses: It is used for making fence, handicraft etc

Location:	Sonai	Lat 24° 51 '42" N	Long 92° 47' 57" E
	Singari	Lat 24° 48 '31" N	Long 92° 45' 53" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually small or absent. Culms distant, culm nodes without spine; culm sheaths wavy at top; imperfect blades linear or sickle-shaped.

Melocanna

Culms are upto 20m high and 7cm in diameter; nodes with a circular band of white bloom little below; culm-sheaths appressed hairy on outside, broader than long; imperfect blades broadly lanceolate, sickle-shaped, recurved.

Melocanna baccifera

Description of the species:

Culms are 10-20m high and 3-7 cm in diameter, green when young turning pale greenish-yellow when old, very straight and erect. Culms are mostly unbranched, shortly branched at the proximate end, branches short and slender; nodes not prominent, with a circular band of white bloom little below. The internodes are upto 50 cm long and, 2-4 mm thin walls, lower internodes are covered by persistent culm-sheaths. No. of culms: 65 – 90.

Culm sheaths are 10-15 cm long and 12-25 cm broad at base, undulated above, yellowish green when young turning yellowish-brown on maturity. Sheaths are brittle, striated, truncated or concave at top, glabrous or sparsely with whitish appressed hairs on the back. Ligule is very short with undulated or toothed margin; auricles small, sub equal, membranous, fringed with silvery bristles. The imperfect blades are deciduous, 15-30 cm long and 2-3 cm broad at base which gradually tapers, linear-lanceolate, sickle-shaped. Shape of lower sheaths is subulate and base is decurrent along the top of the sheath.

Leaves are 15-38cm long and 2.5-5 cm broad, lanceolate to oblong lanceolate. The apex is acuminate with long, scabrous, penicillate, hairs, base rounded, often oblique, glabrous above, glaucescent or sparsely pubescent beneath. Leaf margin is finely ciliate, one margin scabrous, not only on the margin but on 2 or 3 adjoining veins. The main vein is prominent, secondary veins 8- 2, intermediate veins are 5-6 in number, inconspicuous, no regular transverse veinlets. Petiole like base is 5-12

mm long and leaf-sheaths thick, glabrous, smooth, margins ciliate. Auricles 1-1.5 cm long and pointed, 10-12 or more conspicuous, whitish, stiff, deciduous, bristles and Ligule is very short.

Flowers were not seen.

4.1.1.11 *Schizostachyum dullooa* (Gamble) R. B. Majumdar

Local name: Dolu

Uses: It is used for making fence, handicraft etc

Location:	Santinagar	Lat 24° 26 '79" N	Long 92° 19' 84" E
	Singari	Lat 24° 48 '31" N	Long 92° 45' 53" E

Taxonomic key

Culm sheaths are coriaceous to crustaceous; culms cylindrical. Auricles of the culm-sheaths are usually small or absent. Culms are tufted and arborescent, culm sheaths persistent (usually on lower part); imperfect blades narrow, subulate, median on the top of the sheath; internodes thin-walled.

Schizostachyum

Culms are upto l0cm in diameter, arborescent, tufted. Internodes long, upto 90cm long; culm-sheaths straw coloured, deciduous, covered with white or golden, appressed hairs on outside; imperfect blades narrow, subulate, recurved, densely golden hairy within; auricles inconspicuous; ligule long and fimbriate.

Schizostachyum dullooa

Description of the species:

Culms are variable in size 10-20 m high, 3-10cm in diameter at chest height. Culms are dark-green with a few white hairs, whitish below the nodes, glossy when dry. The nodes are slightly prominent; internodes are 40-90 cm, sometimes upto 1 m long with 3-8mm thin walls. No. of culms: 60 – 75

Culm sheaths are also variable in size, 12-30 cm long and 10-30cm broad at base, striated. The young sheaths are with whitish and older ones with golden, appressed hairs on outside, rounded at the top and then concavely truncated and loosely fringed

with bristles. The imperfect blades are narrow 7-15 cm long and 1-2.5 cm broad at base. The culm sheaths are subulate, recurved, base rounded, densely hairy within with golden brown hairs, edges convoluted. Ligule is prominent, long-fimbriate.

Leaves are variable in size ranging from10-39cm in length and 2-6 cm in breadth. Leaf shape is oblong-lanceolate, acuminate, base equally rounded, subulately acuminate above. The leaf point is scabrous, twisted, dorsal surface rough, almost glabrous beneath, edges scabrous. The main vein is pale, not very prominent, secondary veins 6-10, intermediate veins usually 7, no transverse veinlets. Petiole like base is 5-10mm long; leaf sheaths striated and ciliate on the edges, callus is ciliate with a few long, deciduous bristles and glabrous on maturity. Ligule is broad, long and fimbriate.

Flowers were not seen.

Figure 4.1: ***Bambusa chacharensis*** with different characteristics


Fig: 4.1.a


Fig: 4.1.b


Fig: 4.1.c


Fig: 4.1.d


Fig: 4.1.e


Fig: 4.1.f


Fig: 4.1.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.2: ***Bambusa nutans*** with different characteristics


Fig: 4.2.a


Fig: 4.2.b


Fig: 4.2.c


Fig: 4.2.d


Fig: 4.2.e


Fig: 4.2.f


Fig: 4.2.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.3: ***Dendrocalamus hamiltonii*** with different characteristics

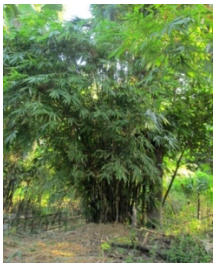


Fig: 4.3.a


Fig: 4.3.b


Fig: 4.3.c

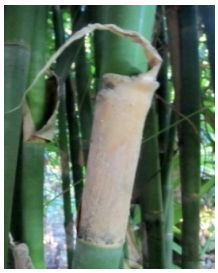


Fig: 4.3.d


Fig: 4.3.e


Fig: 4.3.f


Fig: 4.3.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.4: ***Bambusa balcooa*** with different characteristics


Fig: 4.4.a


Fig: 4.4.b


Fig: 4.4.c


Fig: 4.4.d


Fig: 4.4.e


Fig: 4.4.f


Fig: 4.4.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.5: ***Bambusa arundinacea*** with different characteristics

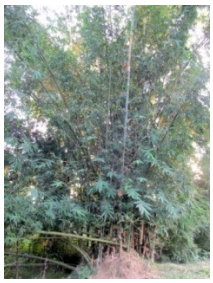


Fig: 4.5.a


Fig: 4.5.b

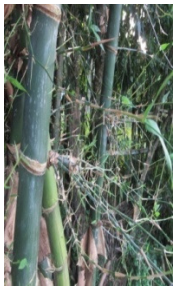


Fig: 4.5.c


Fig: 4.5.d


Fig: 4.5.e


Fig: 4.5.f

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath [f] culm sheath with scale .

Figure 4.6: ***Bambusa pallida*** with different characteristics

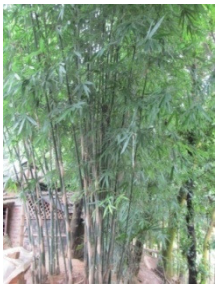


Fig: 4.6.a


Fig: 4.6.b


Fig: 4.6.c


Fig: 4.6.d


Fig: 4.6.e

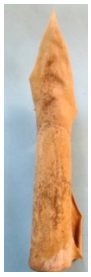


Fig: 4.6.f

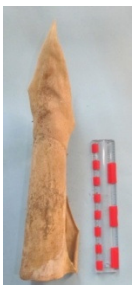


Fig: 4.6.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.7: ***Bambusa assamica*** with different characteristics


Fig: 4.7.a


Fig: 4.7.b

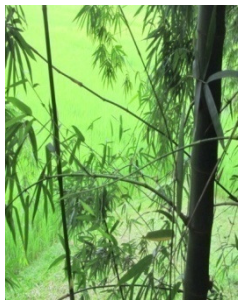


Fig: 4.7.c


Fig: 4.7.d


Fig: 4.7.e


Fig: 4.7.f


Fig: 4.7.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.8: ***Bambusa tulda*** with different characteristics


Fig: 4.8.a


Fig: 4.8.b


Fig: 4.8.c


Fig: 4.8.d

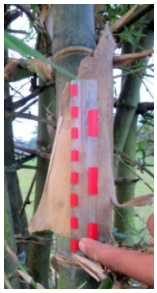


Fig: 4.8.e

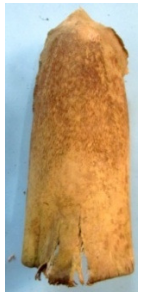


Fig: 4.8.f


Fig: 4.8.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.9: ***Bambusa vulgaris*** with different characteristics

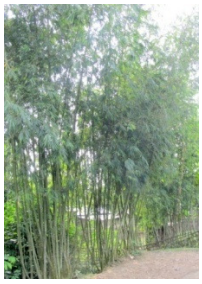


Fig: 4.9.a


Fig: 4.9.b


Fig: 4.9.c


Fig: 4.9.d


Fig: 4.9.e


Fig: 4.9.f


Fig: 4.9.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.10: ***Melocanna baccifera*** with different characteristics


Fig: 4.10.a


Fig: 4.10.b


Fig: 4.10.c


Fig: 4.10.d


Fig: 4.10.e


Fig: 4.10.f

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

Figure 4.11: ***Schizostachyum dullooa*** with different characteristics

Fig: 4.11.a

Fig: 4.11.b

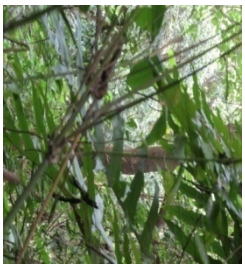


Fig: 4.11.c

Fig: 4.11.d

Fig: 4.11.e

Fig: 4.11.f

Fig: 4.11.g

[a] bamboo clump [b] leaves [c] stems, [d] culm sheath with bamboo, [e] culm sheath with scale [f] culm sheath [g] culm sheath with scale showing length in inch and centimetre.

4.2 Genomic diversity of Bamboo

4.2.1 Bamboo DNA Extraction

About 40mg of young leaves were homogenized in DNA extraction buffer (containing 50mM Tris HCl (pH 8.0), 25mM EDTA (pH 8.0) and 150mM NaCl) along with 2μL/mL β-mercaptoethanol). Genomic DNA was extracted in less than three hours using RNAse, Potassium acetate (pH 9.0), Phenol: Chloroform: Isoamyl alcohol (in the ration 25:24:1), Chloroform: Isoamyl alcohol (in the ration 24:1) to obtain high quality DNA, free of polysaccharides and other metabolites that might interfere with DNA amplification. Purified DNA concentration of each samples was estimated both fluorometrically and by comparison of Ethidium bromide-stained band intensities with λ DNA standard. Once the DNA was obtained, all possible primers were studied in silico.

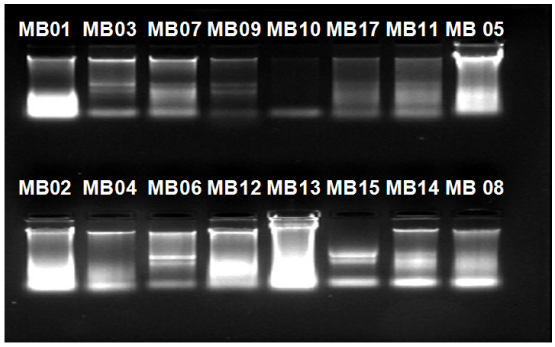


Fig 4.12: Genomic DNA isolated from plants and visualized in UV transilluminator on 1% Agarose gel.

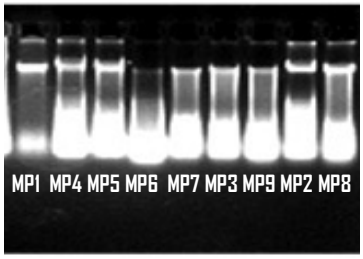


Fig 4.13: Preliminary effort to extract genomic DNA, showing mixed result.

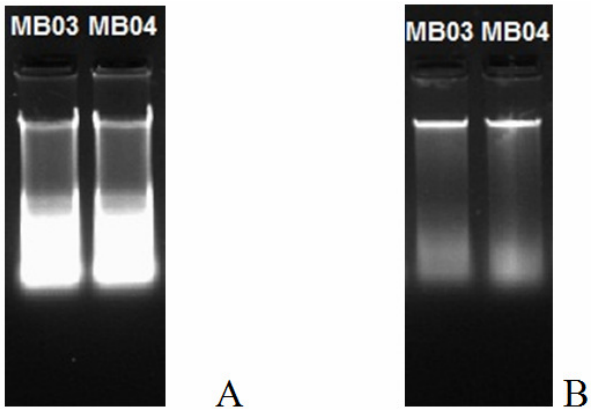


Fig 4.14: Presence of RNA along with DNA present in young leaves (A), the use of RNAse solved the problem (B).

4.2.2 PCR Amplification

PCR was performed with *matK*, *psbA-trnH* and *ITS* primers. The PCR mixture contained 20ng genomic DNA, 50pmole each primer in a reaction of 40 µL volume was prepared. The PCR thermal profile for *matK* was 94ºC for 3 min, (94ºC for 1 min; 48ºC for 45 sec; 72ºC for 45 sec) X 30 cycles and a final extension at 72ºC for 10 min. The PCR thermal profile for *psbA-trnH* was 94ºC for 3 min, (94ºC for 1 min; 51ºC for 45 sec; 72ºC for 45 sec) X 30 cycles and a final extension at 72ºC for 10 min. The PCR thermal profile for *ITS* was 94ºC for 3 min, (94ºC for 1 min; 55ºC for 45 sec; 72ºC for 45 sec) X 30 cycles and a final extension at 72ºC for 10 min. In the present study PCR was successful for all the regions. The PCR products were then subjected to Gel Electrophoresis to check whether the amplification was correct or not.

After lot of study the following primers were used for the amplification of *matK, psbA-trnH* and *ITS* intron regions. The primers were selected for amplification in both the forward and reverse direction.

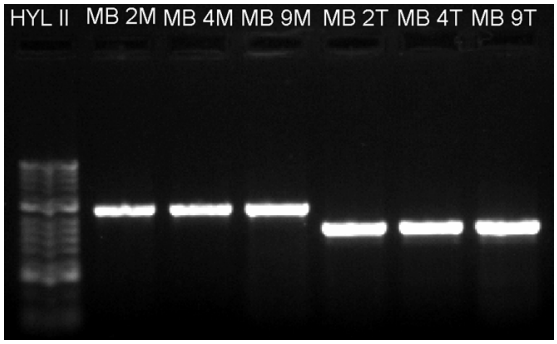


Fig 4.15: PCR Amplification products of *matK* and *psbA-trnH* of Bamboo.

4.2.3 Purification of PCR product and DNA sequencing

The PCR product of expected size was extracted using QIA quick PCR purification kit (QIAGEN, Cat. No. 28704). The PCR products were sequenced for both strands, in forward and reverse direction, using the BigDye Terminator Cycle Sequence Kit 3.1 (ABI Applied Biosystems) and was run on an ABI 3500 DNA Analyzer (ABI Applied Biosystems).

4.2.4 Sequence

Manual editing of raw traces and subsequent alignments of forward and reverse sequences is capable of assigning edited sequences for most species. The trace files were visually checked against the sequence reported by the sequencing software. Any ambiguity was then removed. The 3' and 5' terminals were clipped to generate consensus sequences. The average sequence length is about 1500bp in case of *matK* of which about 1000 bp (approx) variable region is taken up for study. The trace file of one raw DNA sequence of one of such study is given in the following page:

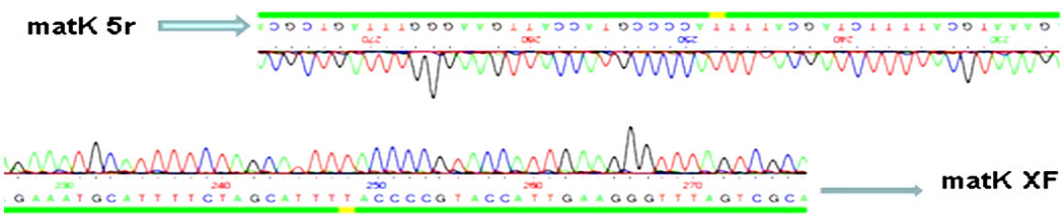


Fig 4.16: Raw tracefile of a DNA sequence of Bamboo

The raw DNA trace files were edited using Chroma or ClustalX. These powerful sequence editing softwares is available online.

A reverse sequence is given below:

>MB02-DM-*MatK*- Forward seq (663nt)
TTTGTATTAGATATACTAATACCCCACCCCGTTCATCTGGAAATCTTGGTTCAAAC
CCTTCGCTATTGGGTAAAAGATGCCCCTTCTTTGCACTTATTACGATTATTTCTCC
GCGAGTATTGTAATTGGAATAATCTTATTGCTACAAAGAACCCCCGCTTGGATTTT
TTAACAAAAAGAAATCAAAGATTATTCTTCTTTTTATATAATTTTTATGTATGTGA
ATACGAATCCATTTTCGTCTTTCTACATAAGCAATCTTCACATTTACGATCAACGT
CCTTTGGGGTCCTTCTTGAACGAATCTATTTCTATAGAAAAATAGAACCTCTTGTC
GAAGTCTTTGCTAAGGATTTTCAGGCCAACTTATGCTTTTTCAAAGATCCTTTCAT
GCATTATGTTAGGTATCAAGGAAAATCGATTCTGGTTTCAAAGGGGACGGCTCTTT
TGGTGAATAAATGGAAATCTTATCTTGTGAATTTTTGGCAATGTAATTTTGACCTG
TGGTTTCACTCGGAAAGGGTCTATATAAAACAATTATCCAATCATTCTCTTTACTT
TATGGGTTATCTTTCAATTGTGCGACGAAACCCTTCAATTGTACGGAGTCAAATGC
TAGAAAATGCATTTCTAATCAATAATGCTATTAAGAAATTCGATACC

A forward sequence is given below:

>MB02-TP-*MatK*-Reverse seq (583nt)
CCGCTATGATAATGAGAAAGGTTTCTGCATATACGCCCAAATCTGTCCATAATATC
AGAATCTGATAAATCAGTCCGAGCCGGCTTACTAATGGGATCTCCTAATAAGTTAC
AAAATTTTGCTTTAGCTAATGATCCAATCAGAGGAATAATTGGAACAAGGGTATCG
AATTTCTTAATAGCATTATTGATTAGAAATGCATTTTCTAGCATTTGACTCCGTAC
AATTGAAGGGTTTCGTCGCACAATTGAAAGATAACCCATAAAGTAAAGAGAATGAT
TGGATAATTGTTTTATATAGACCCTTTCCGAGTGAAACCACAGGTCAAAATTACAT
TGCCAAAAATTCACAAGATAAGATTTCCATTTATTCACCAAAAGAGCCGTCCCCTT
TGAAACCAGAATCGATTTTCCTTGATACCTAACATAATGCATGAAAGGATCTTTGA
AAAAGCATAAGTTGGCCTGAAAATCCTTAGCAAAGACTTCGACAAGAGGTTCTATT
TTTCTATAGAAATAGATTCGTTCAAGAAGGACCCCAAAGGACGTTGATCGTAAATG
TGAAGATTGCTTATGTAGAAAGA

The forward sequence is converted into reverse complement of forward sequence by using Sequence Manipulation Suit which is available at www.bioinformatic.org. The reverse complement of the forward sequence is given below:

>MB02-DM-*MatK*-Forward seq (663) rev_comp of forward seq
GGTATCGAATTTCTTAATAGCATTATTGATTAGAAATGCATTTTCTAGCATTTGAC
TCCGTACAATTGAAGGGTTTCGTCGCACAATTGAAAGATAACCCATAAAGTAAAGA
GAATGATTGGATAATTGTTTTATATAGACCCTTTCCGAGTGAAACCACAGGTCAAA
ATTACATTGCCAAAAATTCACAAGATAAGATTTCCATTTTTCACCAAAAGAGCCGT
CCCCTTTGAAACCAGAATCGATTTTCCTTGATACCTAACATAATGCATGAAAGGAT
CTTTGAAAAAGCATAAGTTGGCCTGAAAATCCTTAGCAAAGACTTCGACAAGAGGT
TCTATTTTTCTATAGAAATAGATTCGTTCAAGAAGGACCCCAAAGGACGTTGATCG
TAAATGTGAAGATTGCTTATGTAGAAAGACGAAAATGGATTCGTATTCACATACAT
AAAAATTATATAAAAAGAAGAATAATCTTTGATTTCTTTTTGTTAAAAAATCCAAG
CGGGGGTTCTTTGTAGCAATAAGATTATTCCAATTACAATACTCGCGGAGAAATAA
TCGTAATAAGTGCAAAGAAGGGGCATCTTTTACCCAATAGCGAAGGG(TTTGAACC
AAGATTTCCAGATGAACGGGGTGGGGTATTAGTATATCTAATACAAA

The sequences are aligned and the overlapping region of the 2 sequences was taken up for further study. In the following case we got an over lapping sequence of 422 nt, which is also the final sequence, it is depicted below:

> MB02 total seq
CCGCTATGATAATGAGAAAGGTTTCTGCATATACGCCCAAATCTGTCCATAATATCAGAAT
CTGATAAATCAGTCCGAGCCGGCTTACTAATGGGATCTCCTAATAAGTTACAAAATTTTGC
TTTAGCTAATGATCCAATCAGAGGAATAATTGGAACAAG**GGTATCGAATTTCTTAATAGCA**
TTATTGATTAGAAATGCATTTTCTAGCATTTGACTCCGTACAATTGAAGGGTTTCGTCGCA
CAATTGAAAGATAACCCATAAAGTAAAGAGAATGATTGGATAATTGTTTTATATAGACCCT
TTCCGAGTGAAACCACAGGTCAAAATTACATTGCCAAAAATTCACAAGATAAGATTTCCAT
TTATTCACCAAAAGAGCCGTCCCCTTTGAAACCAGAATCGATTTTCCTTGATACCTAACAT
AATGCATGAAAGGATCTTTGAAAAAGCATAAGTTGGCCTGAAAATCCTTAGCAAAGACTTC
GACAAGAGGTTCTATTTTTCTATAGAAATAGATTCGTTCAAGAAGGACCCCAAAGGACGTT
GATCGTAAATGTGAAGATTGCTTATGTAGAAAGACGAAAATGGATTCGTATTCACATACAT
AAAAATTATATAAAAAGAAGAATAATCTTTGATTTCTTTTTGTTAAAAAATCCAAGCGGGG

GTTCTTTGTAGCAATAAGATTATTCCAATTACAATACTCGCGGAGAAATAATCGTAATAAG
TGCA

Since *matK* is a coding gene, the ORF was checked and correct amino acid sequences are determined by online software ORF Prediction (www.ncbi.nlm.nih.gov /gorf/gorf.html). The amino acid sequences were then matched with other matK sequences from different plant.

4.2.5 Submission

The sequences were submitted using BankIt. After proper checking by NCBI, the *matK* sequences were published and an Accession Number was allotted for each sequence. The list of *matK* sequence submitted to NCBI with their Accession Number is given in the table below:

Sl	Species	Accession No.
1	*Bambusa balcooa**	JX966236
2	*Bambusa nutans**	JX966234
3	*Bambusa chacharensis**	JX966237
4	*Bambusa bambos**	JX966238
5	*Bambusa bambos**	JX966235
6	*Bambusa tulda*	JX966239

Table 4.1: List of *matK* sequences of Bamboo submitted in NCBI database. * indicates that the sequences for these species has been submitted for the 1st time for *matK* gene in NCBI database.

Sl	Species	Accession No.
1	*Bambusa balcooa* *	KC150890
2	*Bambusa vulgaris*	KC150894
3	*Bambusa chacharensis* *	KC150891
4	*Bambusa bambos*	KC150892
5	*Bambusa pallida* *	KC150896
6	*Bambusa tulda*	KC150893
7	*Bambusa tuldoides**	KC150898
8	*Melocanna baccifera*	KC150897
9	*Dendrocalamus hamiltonii* *	KC150895

Table 4.2: List of *psbA-trnH* sequences of Bamboo submitted in NCBI database. * indicates that the sequences for these species has been submitted for the 1st time for *psbA-trnH* region in NCBI database.

It must also be noted here that *Bambusa bambos* is synonym of *Bambusa arundinacea.* In the present study *Bambusa arundinacea* the synonym of *Bambusa bambos* was used. The names of the plants were checked in the website "The Plant List". ***The Plant List*** is a working list of all known plant species. It aims to be comprehensive for species of Vascular plant (flowering plants, conifers, ferns and their allies) and of *Bryophytes* (mosses and liverworts). It is collaboration between the Royal Botanic Gardens, Kew and Missouri Botanical Garden which enabled the creation of The Plant List by combining multiple checklist data sets held by these institutions and other collaborators. (`http://www.theplantlist.org/`)

4.3 *In-silico* analysis of targeted Bamboo sequences

In the present study of Genomic variation, 3 fragments of Bamboo was chosen based on the literature studies as they showed the most promise in elucidating the problem of Bamboo taxonomy and Phylogenetic relationship. Of the 3 fragments, 1 was a gene viz. *matK* encoding for the Maturase K protein and the other 2 sequences targeted was non coding regions namely *psbA-trnH* and *ITS*. It also worth pointing out that of the 3 targeted sequences, 2 were from Chloroplast DNA i.e. *matK* and *psbA-trnH* and 1 has been taken from the Nuclear DNA i.e. *ITS*.

In the in-silico study, apart from the DNA sequences that has been generated in our Laboratory, many more sequences of the 3 regions were downloaded from databases, particularly from NCBI.

4.3.1 Analysis of *matK*

A total of 152 sequence (including 6 sequences generated by our laboratory) belonging to 103 different species was downloaded from the public domain of NCBI Popset database. These sequences were subjected to analysis using various Bioinformatics tools and softwares. The list of species and their NCBI Accession Number is given in the Table below:

Sl No	Species	Accession Number
1	*Alopecurus pratensis*	gi|194021753
2	*Arrhenatherum elatius*	gi|194021751
3	*Arthrostylidium excelsum*	gi|325515828
4	*Arthrostylidium merostachyoides*	gi|194021703
5	*Aulonemia laxa*	gi|325515840
6	*Bambusa balcooa*	**gi|444361762***
7	*Bambusa bambos*	gi|194021662; **gi|444361760*; gi|444361766***
8	*Bambusa beecheyana*	gi|194021664
9	*Bambusa cacharensis*	**gi|444361764***
10	*Bambusa chungii*	gi|335355609
11	*Bambusa dolichomerithalla*	gi|335355611
12	*Bambusa emeiensis*	gi|335355637
13	*Bambusa malingensis*	gi|194021666
14	*Bambusa membranacea*	gi|194021680
15	*Bambusa nutans*	**gi|444361758***
16	*Bambusa oldhamii*	gi|194021668
17	*Bambusa oliveriana*	gi|194021670
18	*Bambusa pachinensis*	gi|335355613

19	*Bambusa tulda*	gil194021672; **gil444361768***
20	*Bambusa tuldoides*	gil335355615
21	*Bambuseae sp.*	gil194021700
22	*Borinda sp.*	gil194021646
23	*Cephalostachyum pergracile*	gil194021722
24	*Chimonobambusa quadrangularis*	gil194021652
25	*Chimonocalamus pallens*	gil194021648
26	*Chimonocalamus sp.*	gil194021650
27	*Chusquea bilimekii*	gil325515822
28	*Chusquea liebmannii*	gil325515824
29	*Chusquea patens*	gil194021654
30	*Cryptochloa strictiflora*	gil194021736
31	*Dendrocalamopsis valida*	gil194021686
32	*Dendrocalamus asper*	gil194021674
33	*Dendrocalamus asper*	gil335355617
34	*Dendrocalamus brandisii*	gil335355619
35	*Dendrocalamus copelandii*	gil335355621
36	*Dendrocalamus dumosus*	gil335355623
37	*Dendrocalamus giganteus*	gil194021676
38	*Dendrocalamus hamiltonii*	gil335355625
39	*Dendrocalamus khoonmengii*	gil335355631
40	*Dendrocalamus latiflorus*	gil194021678
41	*Dendrocalamus minor*	gil194021682
42	*Dendrocalamus pendulus*	gil335355627
43	*Dendrocalamus sinicus*	gil335355629
44	*Dendrocalamus strictus*	gil194021684
45	*Dinochloa malayana*	gil194021694
46	*Ehrharta calycina*	gil194021745
47	*Gigantochloa albociliata*	gil335355633
48	*Gigantochloa atroviolacea*	gil335355635
49	*Gigantochloa ligulata*	gil194021690
50	*Gigantochloa scortechinii*	gil194021692
51	*Guadua amplexifolia*	gil325515852
52	*Guadua angustifolia subsp.*	gil194021702
53	*Guadua paniculata*	gil325515826
54	*Leersia hexandra*	gil194021741
55	*Lithachne pauciflora*	gil194021737
56	*Lolium perenne*	gil194021750
57	*Lygeum spartum*	gil194021748
58	*Melocalamus compactiflorus*	gil194021696
59	*Menstruocalamus sichuanensis*	gil194021656
60	*Miscanthus sinensis*	gil194021759
61	*Mullerochloa moreheadiana*	gil194021716
62	*Nardus stricta*	gil194021746
63	*Neohouzeaua fimbriata*	gil194021724
64	*Neohouzeaua kerriana*	gil194021726
65	*Neololeba atra*	gil194021714
66	*Neosinocalamus affinis*	gil194021688
67	*Oligostachyum glabrescens*	gil194021644
68	*Olmeca clarkiae*	gil325515832 ; gil325515834
69	*Olmeca fulgor*	gil325515854; gil325515860; gil325515862; gil325515864; gil325515866; gil325515868; gil325515842; gil325515850; gil325515848; gil325515846; gil325515844;
70	*Olmeca recta*	gil325515950; gil325515952; gil325515954;

		gil325515836
71	*Olmeca reflexa*	gil325515856; gil325515936; gil325515938; gil325515942; gil325515944; gil325515946; gil325515830
72	*Olmeca zapotecorum*	gil325515870; gil325515872; gil325515874; gil325515876; gil325515878; gil325515880
73	*Olyra latifolia*	gil194021738
74	*Oreobambos buchwaldii*	gil194021718
75	*Oryza rufipogon*	gil194021743
76	*Oryza sativa*	gil194021744
77	*Otatea acuminata*	gil325515916; gil325515918; gil325515910; gil325515914; gil325515912
78	*Otatea carrilloi*	gil325515888; gil325515890; gil325515892
79	*Otatea fimbriata*	gil325515900; gil325515902; gil325515904; gil325515906; gil325515908
80	*Otatea glauca*	gil325515882; gil325515884; gil325515886; gil325515940
81	*Otatea reynosoana*	gil325515930; gil325515932; gil325515934
82	*Otatea transvolcanica*	gil325515920; gil325515922; gil325515924; gil325515926; gil325515928
83	*Otatea ximenae*	gil325515894; gil325515896; gil325515898
84	*Oxytenanthera abyssinica*	gil194021720
85	*Panicum virgatum*	gil194021755
86	*Phyllostachys edulis*	gil194021660
87	*Phyllostachys nigra*	gil194021658
88	*Piresia sp.*	gil194021739
89	*Pseudosasa cantorii*	gil194021642
90	*Pseudostachyum polymorphum*	gil194021728
91	*Rhipidocladum bartlettii*	gil325515838
92	*Rhipidocladum martinezii*	gil325515948
93	*Rhipidocladum racemiflorum*	gil194021704
94	*Rhipidocladum racemiflorum*	gil325515858
95	*Saccharum officinarum*	gil194021757
96	*Schizostachyum grande*	gil194021730
97	*Schizostachyum jaculans*	gil194021732
98	*Schizostachyum zollingeri*	gil194021734
99	*Temburongia simplex*	gil194021712
100	*Temochloa liliana*	gil194021710
101	*Thyrsostachys siamensis*	gil194021698
102	*Vietnamosasa ciliata*	gil194021706
103	*Vietnamosasa pusilla*	gil194021708

Table 4.3: List of *matK* sequence downloaded from database along with Species and Accession Number.

4.3.1.1 Nucleotide composition of *matK*

Analysis of *matK* gene Nucleotide composition showed in Table 4.5 and Figure 4.17 that there is a strong A and T bias compared to significantly low values for G and C. The overall average for all codon positions is: A = 30.7; T (U) = 35.7; G = 15.6 and

C = 17.9. The GC content is equal to 33.6, which indicates that the AT content (66.4) is double that of GC content. In the 1st codon position analysis of the *matK* gene Nucleotide composition again indicates that there is a strong AT bias. The average of nucleotides for 1st codon positions are: A = 31.6; T = 36.9; G = 14.9 and C = 16.7. The GC content (31.6) is half of AT content (68.4). In the 2nd codon position analysis of the *matK* gene Nucleotide composition again follows the trend of a strong AT bias. The average of nucleotides for 1st codon positions are: A = 30.9; T = 34.9; G = 14.3 and C = 19.8. The GC content (34.1) is much less than that of AT content (65.9). In the 3rd codon position analysis of the *matK* gene Nucleotide composition again indicates that there is a strong AT bias. The average of nucleotides for 1st codon positions are: A = 29.7; T = 35.3; G = 17.7 and C = 17.3. The GC content (35.0) is half of AT content (65.0).

Analysis of the figure indicates that apart from the overall nucleotide composition, A, T content of *matK* sequences are much higher than the nucleotides for G and C for all codon positions as well. This is not seen in a few sequences, but is clearly observed across all the 152 *matK* sequences that were used. Figure 4.18, 4.19, 4.20 & 4.21 are given in an effort to understand the low GC content and the nucleotide composition in each codon position, separate line graphs for overall (Fig 4.18), 1st codon (Fig 4.19), 2nd codon (Fig 4.20) and 3rd codon (Fig 4.21) has been included. This again clearly shows that there is a strong AT bias for *matK* sequences across all codon positions.

Figure 4.22: In this graph an analysis was done be comparing the GC content for overall and each codon positions. It shows that the GC content for the 3rd codon has least value while the overall GC content shows the highest GC content. There is a tussle throughout the array of sequence for the 2nd highest rank between the 1st and the 2nd codon.

Based with the conventional wisdom that higher %GC content is an indicator higher complexity, it shows that *matK* sequences with higher %AT content most probably will not have enough complexity to successfully serve to identify at species level nor it can give a clear picture about the phylogenetic relationship amongst them.

***matK* sequence**	**T(U)**	**C**	**A**	**G**	**Total**	**T-1**	**C-1**	**A-1**	**G-1**	**Pos #1**
Avg.	35.7	17.9	30.7	15.6	1313.2	37	16.7	31.6	14.9	436.8
	T-2	**C-2**	**A-2**	**G-2**	**Pos #2**	**T-3**	**C-3**	**A-3**	**G-3**	**Pos #3**
Avg	35	19.8	30.9	14.3	438.2	35	17.3	29.7	17.7	438.2

Table 4.4: The nucleotide composition of *matK* sequences.

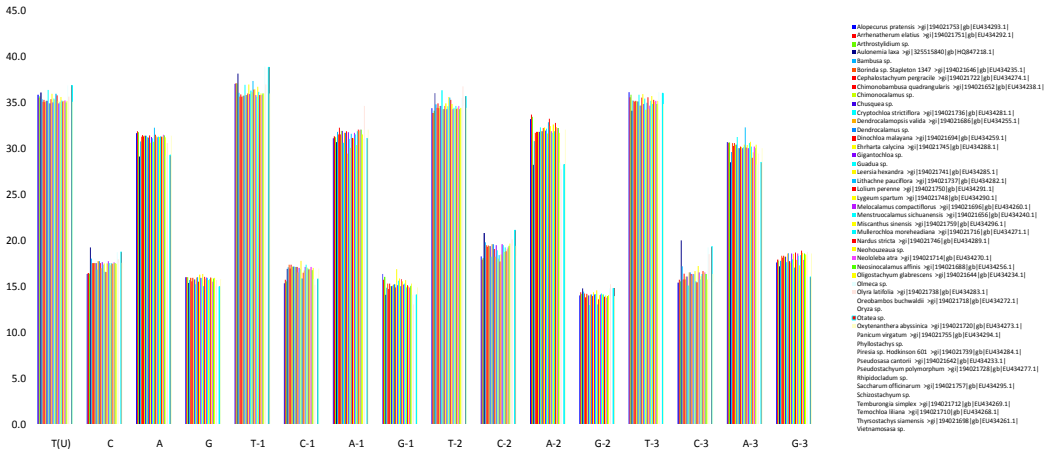


Fig 4.17: Graph showing the nucleotide composition of *matK* at all codon positions.

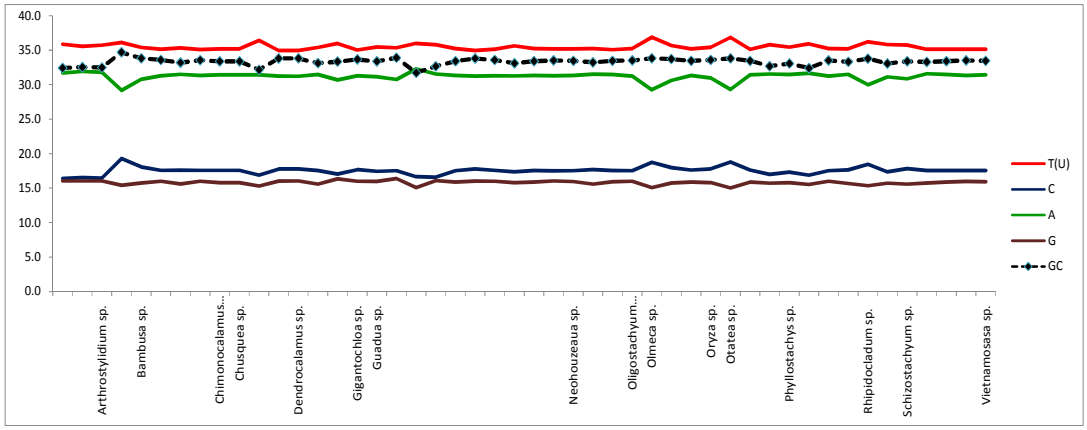


Figure 4.18: Overall nucleotide composition of *matK* with GC content

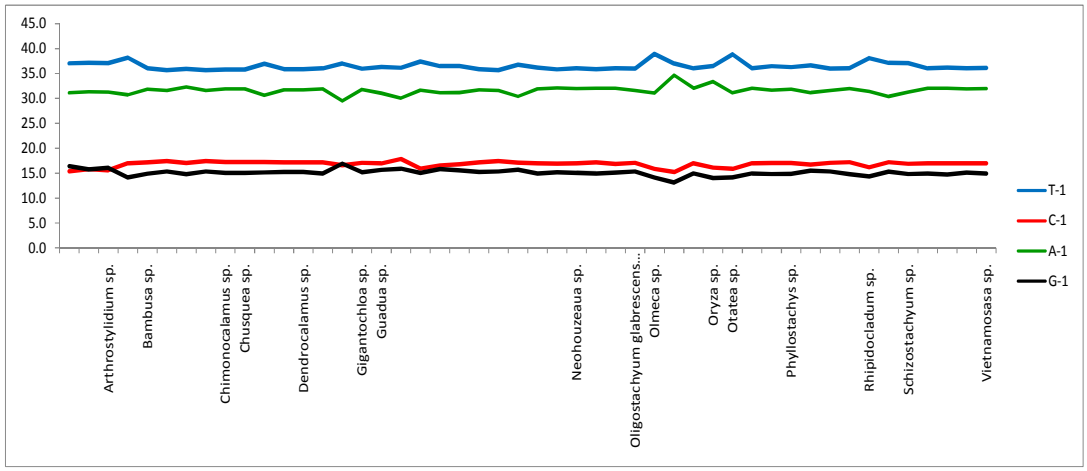


Figure 4.19: Nucleotide composition at 1st codon of *matK* sequence

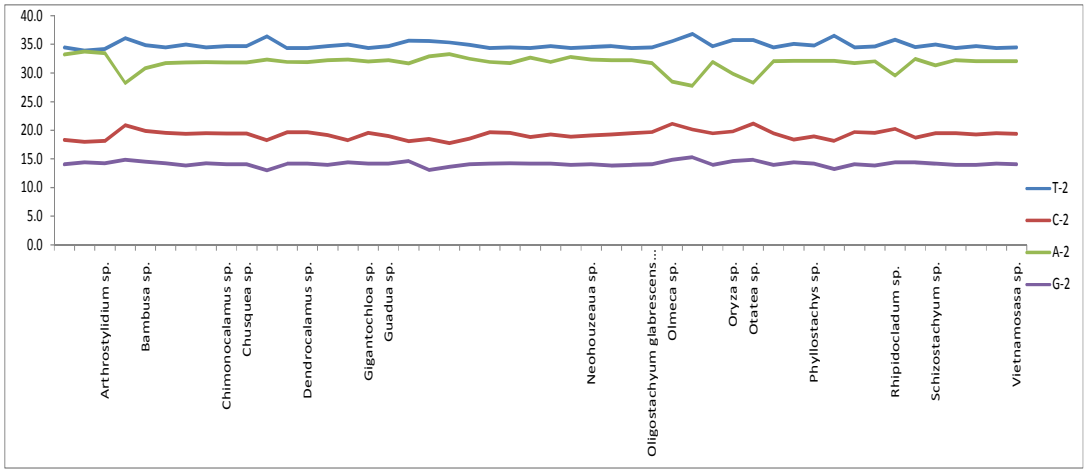


Figure 4.20: Nucleotide composition at 2nd codon of *matK* sequence

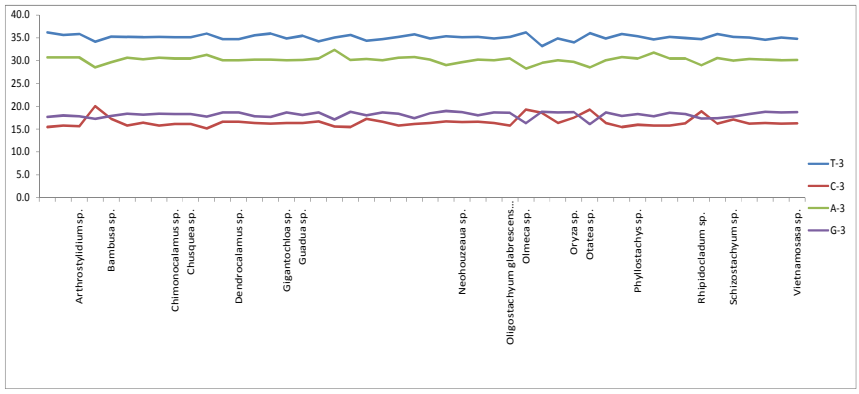


Figure 4.21: Nucleotide composition at 3rd codon of *matK* sequence

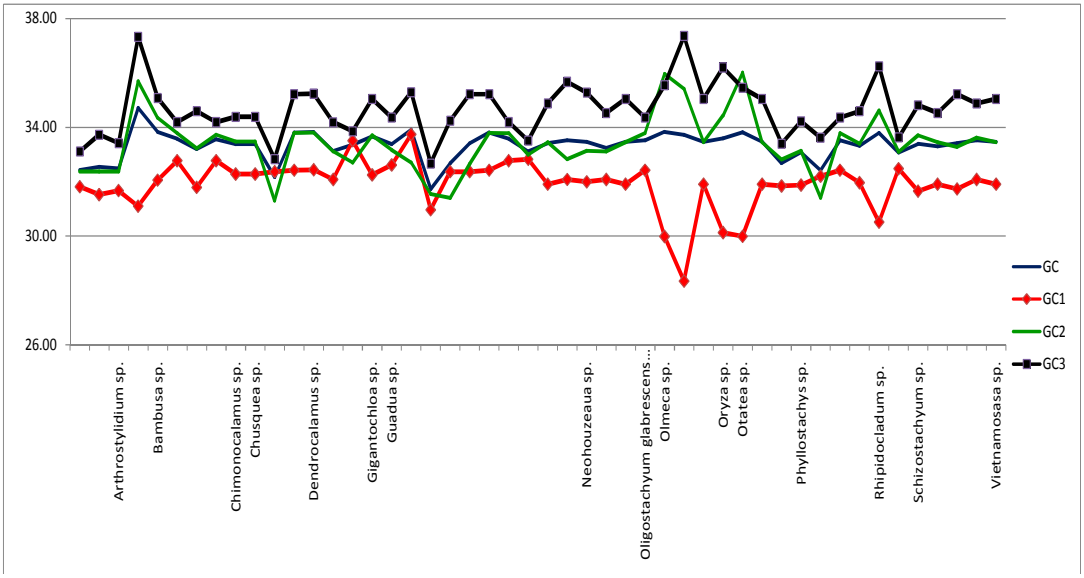


Figure 4.22: Comparison of GC content at 1st, 2nd and 3rd codon with overall GC content of *matK* sequences.

4.3.1.2 Nucleotide pair frequencies of *matK*

Analysis of Nucleotide pair frequencies of *matK* gene for all codon positions indicates that the number of identical pairs (ii) is in much greater number than the transitional pairs (si) and Transversional pairs (sv). The number of si and sv pairs doesn't show much variation. The si value for *matK* gene is 1049, while that of sv is 15 showed in the Table 4.6. This again clearly indicates with its extreme high number of identical pairs that there is low level of complexity in the sequences. For proper species level identification through DNA sequences, a higher level of complexity is always desired.

Domain	ii	si	sv	R	TT	TC	TA	TG	CT	CC	CA
1	Avg	1049.00	15.00	15.00	1.01	380.00	5.00	2.00	2.00	4.00	190.00
1st	349.00	6.00	4.00	1.48	131.00	2.00	1.00	0.00	2.00	56.00	1.00
2nd	351.00	4.00	5.00	0.77	124.00	1.00	1.00	0.00	1.00	71.00	0.00
3rd	349.00	5.00	6.00	0.87	124.00	1.00	1.00	1.00	1.00	63.00	0.00

Domain	CG	AT	AC	AA	AG	GT	GC	GA	GG	Total
1	1.00	1.00	2.00	2.00	319.00	3.00	3.00	1.00	3.00	161.00
1st	0.00	1.00	1.00	110.00	1.00	1.00	0.00	1.00	51.00	359.01
2nd	0.00	1.00	1.00	106.00	1.00	1.00	0.00	1.00	51.00	359.83
3rd	0.00	1.00	1.00	103.00	1.00	2.00	0.00	1.00	60.00	360.21

Table 4.5: Nucleotide pair frequencies of *matK* sequences

4.3.1.3 Codon usage of *matK*

The analysis of Codon usage of *matK* gene shows that some of the codons are much more frequently used compared to others. The codon UUU and AUU show count of over 20 while CCG and GUC showed the least count is an average of 431 codons. The RSCU value is also given in the Table 4.6. Analysis of the Amino acid composition of *matK* sequence indicates that Serine, Leucine, Isoleucine, Phenylalanine and Lysine are much more abundant than other amino acid. Methionine and Alanine shows up least in the *matK* protein shown in Figure 4.23.

Analysis of the codon usage bias is self evident and shows that UUU and AUU show count of over 20 while CCG and GUC about 0.5 shows the least count is an average of 431 codons. So we see that *matK* shows significant bias towards some codon or else we would have seen a flat and average graph.

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	27.9	1.42	UCU(S)	15.2	1.79	UAU(Y)	11.4	1.21	UGU(C)	6.9	1.53
UUC(F)	11.4	0.58	UCC(S)	5.4	0.64	UAC(Y)	7.5	0.79	UGC(C)	2.1	0.47
UUA(L)	11.1	1.38	UCA(S)	11.1	1.31	UAA(*)	5.3	0.95	UGA(*)	4.4	0.8
UUG(L)	13.1	1.63	UCG(S)	10.9	1.29	UAG(*)	7	1.26	UGG(W)	8.7	1
CUU(L)	12.7	1.58	CCU(P)	5.2	1.24	CAU(H)	9	1.77	CGU(R)	1.5	0.46
CUC(L)	6.1	0.75	CCC(P)	4.5	1.09	CAC(H)	1.2	0.23	CGC(R)	1	0.32
CUA(L)	3.7	0.46	CCA(P)	6.4	1.55	CAA(Q)	6.8	1.38	CGA(R)	5.9	1.83
CUG(L)	1.5	0.19	CCG(P)	0.6	0.13	CAG(Q)	3.1	0.62	CGG(R)	2.8	0.88
AUU(I)	17.9	1.31	ACU(T)	4.6	1.04	AAU(N)	15	1.32	AGU(S)	4.8	0.57
AUC(I)	13	0.95	ACC(T)	5.9	1.34	AAC(N)	7.8	0.68	AGC(S)	3.5	0.41
AUA(I)	10.1	0.74	ACA(T)	4.7	1.07	AAA(K)	21.5	1.43	AGA(R)	5.5	1.71
AUG(M)	7.1	1	ACG(T)	2.4	0.55	AAG(K)	8.7	0.57	AGG(R)	2.6	0.8
GUU(V)	7.6	1.87	GCU(A)	1.1	0.61	GAU(D)	6.9	1.38	GGU(G)	3.3	1.05
GUC(V)	0.5	0.13	GCC(A)	0.7	0.36	GAC(D)	3.1	0.62	GGC(G)	1	0.33
GUA(V)	6	1.48	GCA(A)	4.1	2.23	GAA(E)	16.8	1.81	GGA(G)	5.2	1.65
GUG(V)	2.1	0.52	GCG(A)	1.5	0.81	GAG(E)	1.8	0.19	GGG(G)	3	0.97

Table 4.6: The Codon usage table for *matK* gene in Bamboo species.

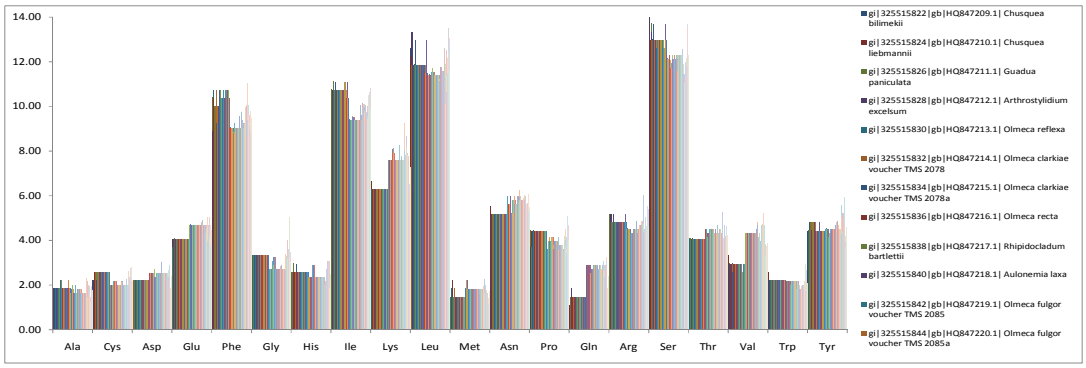


Figure 4.23: Amino acid composition of *matK* gene.

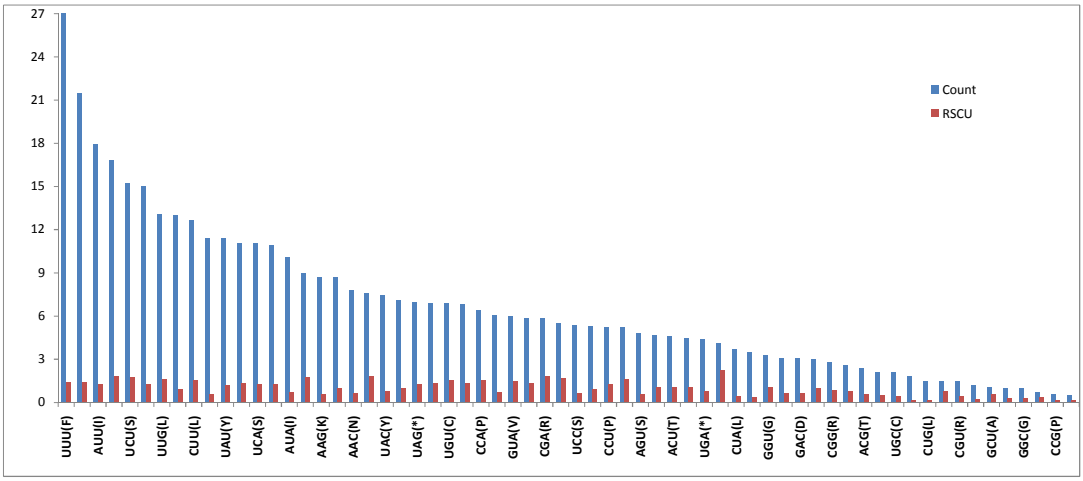


Figure 4.24: Codon usage bias of *matK* gene.

4.3.1.4 Mean divergence in *matK*

Analysis of Mean divergence in *matK* sequences using Kimura-2-parameter (K2P) within 152 sequences of Bamboo group. The distance matrix shows the level of divergence/ difference among the species. This included the 6 sequence of Southern Assam generated by us. As the data shows, there are no variations among many species. It indicates there is no or very low level of divergence on a molecular basis/level among the bamboo species. The sequences marked with "AUS" have been generated by our Laboratory.

The analysis indicates that although the species that has been taken for analysis are different, but the *matK* sequences among those species does not show much divergence. The *matK* region seems to be a conserved sequence among the Bamboo

group. It is observed that even where the genus is different, we see that a value of "0.000" is given, indicating that there is no divergence amongst the genus and species.

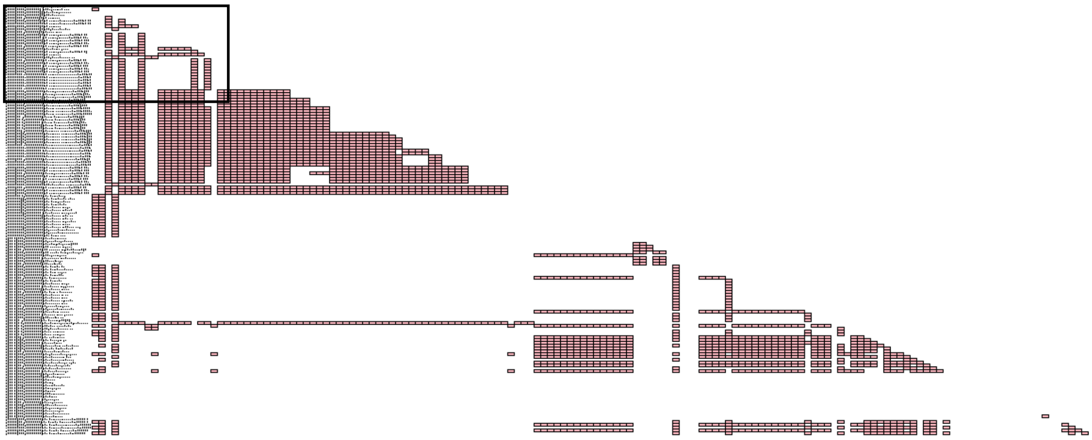


Table 4.7 A: Pairwise divergence table of *matK* with cells conditioned to become red with a value of 0.000

gi|325515822|gb|HQ847209.1|_Chusquea_bilimekii																	
gi|325515824|gb|HQ847210.1|_Chusquea_liebmannii	0.002																
gi|325515826|gb|HQ847211.1|_Guadua_paniculata	0.014	0.012															
gi|325515828|gb|HQ847212.1|_Arthrostylidium_excelsum	0.014	0.012	0.014														
gi|325515830|gb|HQ847213.1|_Olmeca_reflexa	0.017	0.015	0.006	0.017													
gi|325515832|gb|HQ847214.1|_Olmeca_clarkiae_voucher_TMS_207	0.017	0.015	0.006	0.017	0.006												
gi|325515834|gb|HQ847215.1|_Olmeca_clarkiae_voucher_TMS_207	0.017	0.015	0.006	0.017	0.006	0.000											
gi|325515836|gb|HQ847216.1|_Olmeca_recta	0.018	0.017	0.008	0.018	0.008	0.006	0.006										
gi|325515838|gb|HQ847217.1|_Rhipidocladum_bartlettii	0.014	0.012	0.015	0.009	0.018	0.018	0.018	0.020									
gi|325515840|gb|HQ847218.1|_Aulonemia_laxa	0.012	0.011	0.014	0.011	0.017	0.017	0.017	0.018	0.011								
gi|325515842|gb|HQ847219.1|_Olmeca_fulgor_voucher_TMS_2085	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017							
gi|325515844|gb|HQ847220.1|_Olmeca_fulgor_voucher_TMS_2085a	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000						
gi|325515846|gb|HQ847221.1|_Olmeca_fulgor_voucher_TMS_2085b	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000					
gi|325515848|gb|HQ847222.1|_Olmeca_fulgor_voucher_TMS_2085c	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000				
gi|325515850|gb|HQ847223.1|_Olmeca_fulgor_voucher_TMS_2085c	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000			
gi|325515852|gb|HQ847224.1|_Guadua_amplexifolia	0.014	0.012	0.000	0.014	0.006	0.006	0.006	0.008	0.015	0.014	0.006	0.006	0.006	0.006	0.006		
gi|325515854|gb|HQ847225.1|_Olmeca_fulgor_voucher_TMS_2031	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	
gi|325515856|gb|HQ847226.1|_Olmeca_reflexa	0.020	0.018	0.009	0.020	0.003	0.008	0.008	0.008	0.022	0.020	0.008	0.008	0.008	0.008	0.008	0.009	0.0
gi|325515858|gb|HQ847227.1|_Rhipidocladum_racemiflorum	0.012	0.011	0.014	0.008	0.017	0.017	0.017	0.019	0.002	0.009	0.017	0.017	0.017	0.017	0.017	0.014	0.01
gi|325515860|gb|HQ847228.1|_Olmeca_fulgor_voucher_TMS_2054	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515862|gb|HQ847229.1|_Olmeca_fulgor_voucher_TMS_2054a	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515864|gb|HQ847230.1|_Olmeca_fulgor_voucher_TMS_2054b	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515866|gb|HQ847231.1|_Olmeca_fulgor_voucher_TMS_2054c	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515868|gb|HQ847232.1|_Olmeca_fulgor_voucher_TMS_2054c	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515870|gb|HQ847233.1|_Olmeca_zapotecorum_voucher_ERS	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515872|gb|HQ847234.1|_Olmeca_zapotecorum_voucher_ERS	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515874|gb|HQ847235.1|_Olmeca_zapotecorum_voucher_ERS	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0
gi|325515876|gb|HQ847236.1|_Olmeca_zapotecorum_voucher_ERS	0.017	0.015	0.006	0.017	0.006	0.000	0.000	0.006	0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.006	0.0

Table 4.7 B: Close up of the black rectangle of Pairwise divergence table of *matK* with cells with value of 0.000 highlighted in red, of the table above.

4.3.1.5 Pattern of Nucleotide Substitution in *matK*

The Pattern of Nucleotide Substitution in *matK* region using the Maximum Composite Likelihood Estimate method by MEGA 6 shows that the transitional rate is significantly higher than the transversional rates. The nucleotide frequencies are 30.03% (A), 36.54% (T/U), 18.70% (C), and 14.74% (G). The transition/transversion rate ratios are k_1 = 1.936 (purines) and k_2 = 2.442 (pyrimidines). The overall transition/transversion bias is R = 1.021, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]. The analysis involved 152 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 658 positions in the final dataset.

	A	**T**	**C**	**G**
A	-	*8.67*	*4.44*	**6.77**
T	*7.12*	-	**10.83**	*3.5*
C	*7.12*	**21.16**	-	*3.5*
G	**13.79**	*8.67*	*4.44*	-

Table 4.8: Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution in *matK* sequences.

4.3.2 Analysis of *psbA-trnH*

A total of 120 sequence (including 9 sequences generated by our laboratory) belonging to 109 different species was downloaded from the public domain of NCBI Popset database. These sequences were subjected to analysis using various Bioinformatics tools and softwares. The list of species and their NCBI Accession Number is given in the Table below:

Sl. No.	*Species*	Accession Number
1	*Aulonemia laxa*	gi|156633467
2	*Bambusa bambos*	gi|305694150 ; gi|289552130
3	*Bambusa beecheyana*	gi|305694189
4	*Bambusa blumeana*	gi|305694153; gi|289552132
5	*Bambusa cerosissima*	gi|305694204
6	*Bambusa chungii*	gi|305694222
7	*Bambusa cornigera*	gi|305694144
8	*Bambusa distegia*	gi|305694168
9	*Bambusa distegia*	gi|289552134
10	*Bambusa farinacea*	gi|289552136
11	*Bambusa flexuosa*	gi|289552138
12	*Bambusa gibba*	gi|289552140
13	*Bambusa grandis*	gi|305694219
14	*Bambusa intermedia*	gi|305694225
15	*Bambusa membranacea*	gi|305694282
16	*Bambusa multiplex*	gi|305694180
17	*Bambusa multiplex*	gi|289552142
18	*Bambusa oldhamii*	gi|305694192
19	*Bambusa pervariabilis*	gi|305694177
20	*Bambusa polymorpha*	gi|305694159
21	*Bambusa remotiflora*	gi|305694171
22	*Bambusa sinospinosa*	gi|305694156
23	*Bambusa sinospinosa*	gi|289552144
24	*Bambusa surrecta*	gi|305694207
25	*Bambusa textilis*	gi|305694162
26	*Bambusa tulda*	gi|305694183
27	*Bambusa tuldoides*	gi|305694174
28	*Bambusa tuldoides*	gi|289552146
29	*Bambusa valida*	gi|289552148
30	*Bambusa ventricosa*	gi|305694147
31	*Bambusa vulgaris*	gi|305694216
32	*Bambusa vulgaris*	gi|156633468
33	*Bambusa yunnanensis*	gi|305694210
34	*Chusquea bilimekii*	gi|156633469
35	*Dendrocalamopsis bicicatricata*	gi|305694213
36	*Dendrocalamopsis stenoaurita*	gi|305694288
37	*Dendrocalamopsis variostriata*	gi|305694186

38	*Dendrocalamus asper*	gi|305694246
39	*Dendrocalamus bambusoides*	gi|305694327
40	*Dendrocalamus barbatus*	gi|305694240
41	*Dendrocalamus birmanicus*	gi|305694267
42	*Dendrocalamus brandisii*	gi|305694276
43	*Dendrocalamus elegans*	gi|289552150
44	*Dendrocalamus farinosus*	gi|305694318
45	*Dendrocalamus fugongensis*	gi|305694294
46	*Dendrocalamus giganteus*	gi|305694279
47	*Dendrocalamus hamiltonii*	gi|305694255
48	*Dendrocalamus jianshuiensis*	gi|305694243
49	*Dendrocalamus latiflorus*	gi|305694324
50	*Dendrocalamus ovatus*	gi|305694195
51	*Dendrocalamus pachystachys*	gi|305694315
52	*Dendrocalamus peculiaris*	gi|305694249
53	*Dendrocalamus rongchengensis*	gi|305694165
54	*Dendrocalamus semiscandens*	gi|305694252
55	*Dendrocalamus strictus*	gi|305694285
56	*Dendrocalamus strictus*	gi|289552152
57	*Dendrocalamus tibeticus*	gi|305694291
58	*Dendrocalamus tomentosus*	gi|305694270
59	*Dendrocalamus tsiangii*	gi|305694261
60	*Dendrocalamus xishuangbannaensis*	gi|305694258
61	*Dinochloa malayana*	gi|289552154
62	*Dinochloa scabrida*	gi|289552158
63	*Dinochloa sp.*	gi|289552156
64	*Eremocaulon asymmetricum*	gi|156633470
65	*Eremocaulon aureofimbriatum*	gi|156633471
66	*Gigantochloa albociliata*	gi|305694306
67	*Gigantochloa atter*	gi|305694297
68	*Gigantochloa balui*	gi|305694300
69	*Gigantochloa balui*	gi|289552160
70	*Gigantochloa latifolia*	gi|289552164
71	*Gigantochloa levis*	gi|305694264
72	*Gigantochloa ligulata*	gi|289552162
73	*Gigantochloa parviflora*	gi|305694273
74	*Gigantochloa scortechinii*	gi|305694312
75	*Gigantochloa sp. daluoensis*	gi|305694303
76	*Gigantochloa verticillata*	gi|305694309
77	*Gigantochloa wrayi*	gi|289552166
78	*Guadua aculeata*	gi|156633472
79	*Guadua amplexifolia*	gi|156633473
80	*Guadua angustifolia*	gi|156633474
81	*Guadua longifolia*	gi|156633475
82	*Guadua paniculata*	gi|156633476
83	*Guadua velutina*	gi|156633477
84	*Holttumochloa magica*	gi|289552168
85	*Kinabaluchloa nebulosa*	gi|289552170
86	*Maclurochloa montana*	gi|289552172
87	*Melocalamus arrectus*	gi|305694228

88	*Melocalamus compactiflorus var. fimbriatus*	gi|305694231
89	*Melocalamus scandens*	gi|305694234
90	*Melocanna baccifera*	gi|305694138
91	*Melocanna baccifera*	gi|289552174
92	*Neosinocalamus affinis*	gi|305694198
93	*Olmeca clarkiae*	gi|156633465
94	*Olmeca fulgor*	gi|156633466
95	*Olmeca recta*	gi|156633480
96	*Olmeca reflexa*	gi|156633481
97	*Otatea acuminata*	gi|156633482
98	*Otatea carrilloi*	gi|156633483
99	*Otatea fimbriata*	gi|156633478
100	*Otatea glauca*	gi|156633479
101	*Oxytenanthera abyssinica*	gi|305694321
102	*Rhipidocladum racemiflorum*	gi|156633484
103	*Schizostachyum blumei*	gi|305694141
104	*Schizostachyum gracile*	gi|289552180
105	*Schizostachyum zollingeri*	gi|289552182
106	*Soejatmia ridleyi*	gi|289552178
107	*Sphaerobambos hirsuta*	gi|289552176
108	*Thyrsostachys oliveri*	gi|305694237
109	*Thyrsostachys siamensis*	gi|305694201

Table 4.9: List of *psbA-trnH* sequence downloaded from database along with Species and Accession Number.

4.3.2.1 Nucleotide composition of *psbA-trnH*

Analysis of *psbA-trnH* non-coding region Nucleotide composition indicates that there is a strong A and T bias compared to significantly low values for G and C. The average for all codon positions is: A = 30.8; T (U) = 32.7; G = 19.0 and C = 17.5. The AT content is equal to 63.5 while the GC content is 36.5 indicating AT content is much greater than that of GC content. Analysis of the Table 4.10 and Fig 4.25 indicates that the overall nucleotide composition, A, T content of *psbA-trnH* sequences are much higher than the nucleotides for G and C. This is not seen in a few sequences, but is clearly observed across all the 120 *psbA-trnH* sequences that were used. Fig 4.26 has been given in an effort to understand the low GC content and the nucleotide composition in each codon position, separate line graphs for overall nucleotide composition and GC content is given. It shows a similar trend with *matK* sequences as *psbA-trnH* sequences also show a strong AT bias with low GC content. Again the scenario in case of *psbA-trnH* is similar to that of *matK*

sequences. As discussed earlier for proper species level identification and clear phylogenetic relationship, a higher level of complexity in the sequence is always desired. But as we know that lower %GC content is indicative of low complexity, which is exactly the case here.

	T(U)	C	A	G	Total
Avg.	32.7	17.5	30.8	19.0	579.8

Table 4.10: Nucleotide composition of *psbA-trnH*.

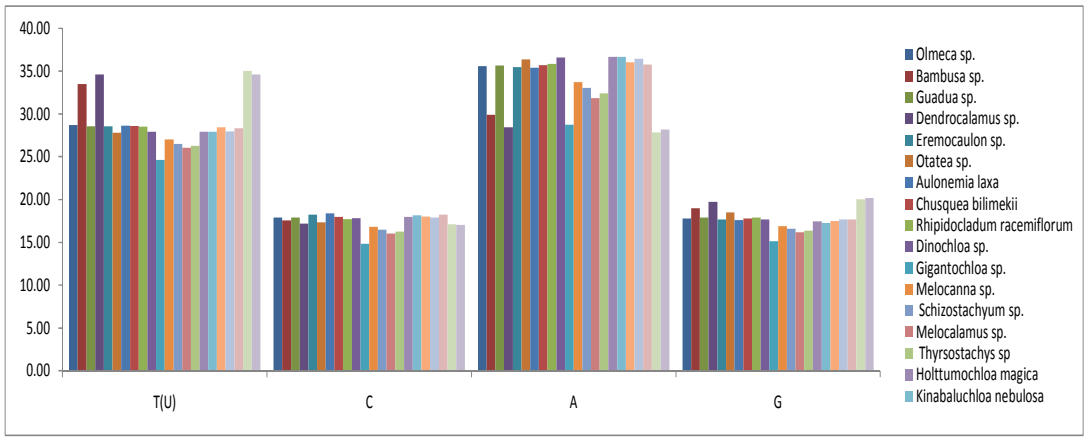


Figure 4.25: Graph showing the nucleotide composition of *psbA-trnH*.

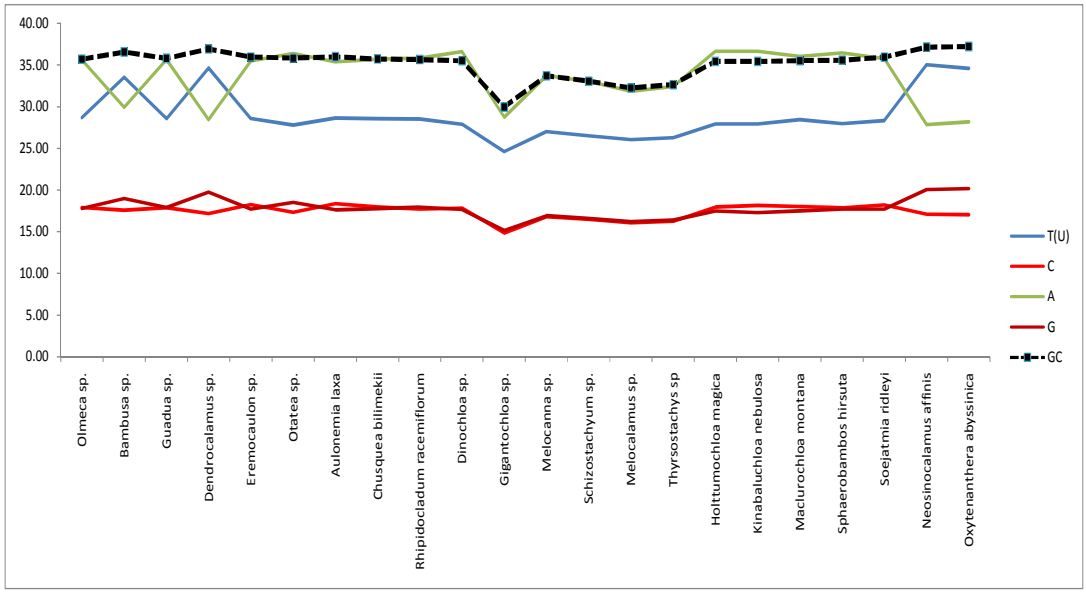


Figure 4.26: Overall nucleotide composition along with GC content of *psbA-trnH*.

4.3.2.2 Nucleotide pair frequencies of *psbA-trnH*

Analysis of Nucleotide pair frequencies of *psbA-trnH* non coding region indicates that the number of transitional pairs (si) and transversional pairs (sv) are 399 and 57 respectively indicating that the transitional pairs outnumber that of transversional pairs by a multiple of 7.

Domain	ii	si	sv	R	TT	TC	TA	TG	CT	CC	CA
1	Avg	399.00	57.00	95.00	0.60	137.00	15.00	28.00	7.00	13.00	64.00

CG	AT	AC	AA	AG	GT	GC	GA	GG	Total	Domain Info
11.00	9.00	13.00	8.00	127.00	13.00	9.00	11.00	15.00	72.00	550.92

Table 4.11: Nucleotide pair frequencies of *psbA-trnH*.

4.3.2.3 Mean divergence in *psbA-trnH*

Analysis of Mean divergence in *psbA-trnH* sequences using Kimura-2-parameter (K2P) within 120 sequences of Bamboo group. The distance matrix shows the level of divergence/ difference among the species. This included the 9 sequence of Southern Assam generated by us. As the data shows, there are no variations among many species particularly among the first 45 species in the table. It indicates there is no or very low level of divergence on a molecular basis/level among the bamboo species. The sequences marked with "AUS" have been generated by our Laboratory.

The analysis of mean divergence shows a similar trend to *matK* sequences. The *psbA-trnH* sequences also indicated that although the species that has been taken for analysis are different, but the *psbA-trnH* sequences among those species does not show much divergence. The *psbA-trnH* although being an intronic fragment region seems to be conserved among the Bamboo group. It is observed that even where the genus is different, we see that a value of "0.000" is given, indicating that there is no divergence amongst the genus and species.

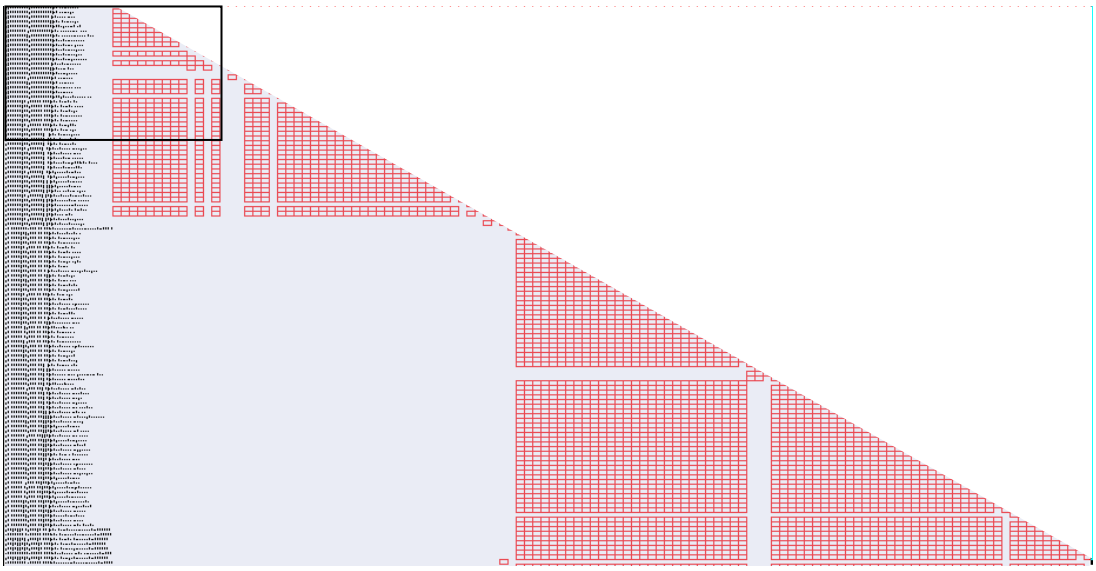


Table 4.12 A: Pairwise divergence table of *psbA-trnH* with cells conditioned to become red with a value of 0.000.

gi|156633465|gb|EF589628.1|_Olmeca_clarkiae									
gi|156633466|gb|EF589629.1|_Olmeca_fulgor	0.000								
gi|156633467|gb|EF589630.1|_Aulonemia_laxa	0.000	0.000							
gi|156633468|gb|EF589631.1|_Bambusa_vulgaris	0.000	0.000	0.000						
gi|156633469|gb|EF589632.1|_Chusquea_bilimekii	0.000	0.000	0.000	0.000					
gi|156633470|gb|EF589633.1|_Eremocaulon_asymmetricum	0.000	0.000	0.000	0.000	0.000				
gi|156633471|gb|EF589634.1|_Eremocaulon_aureofimbriatum	0.000	0.000	0.000	0.000	0.000	0.000			
gi|156633472|gb|EF589635.1|_Guadua_aculeata	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
gi|156633473|gb|EF589636.1|_Guadua_amplexifolia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
gi|156633474|gb|EF589637.1|_Guadua_angustifolia	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
gi|156633475|gb|EF589638.1|_Guadua_longifolia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|156633476|gb|EF589639.1|_Guadua_paniculata	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
gi|156633477|gb|EF589640.1|_Guadua_velutina	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|156633478|gb|EF589641.1|_Otatea_fimbriata	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
gi|156633479|gb|EF589642.1|_Otatea_glauca	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
gi|156633480|gb|EF589643.1|_Olmeca_recta	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
gi|156633481|gb|EF589644.1|_Olmeca_reflexa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|156633482|gb|EF589645.1|_Otatea_acuminata	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|156633483|gb|EF589646.1|_Otatea_carrilloi	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|156633484|gb|EF589647.1|_Rhipidocladum_racemiflorum	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
gi|289552130|gb|GU390993.1|_Bambusa_bambos	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552132|gb|GU390994.1|_Bambusa_blumeana	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552134|gb|GU390995.1|_Bambusa_distegia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552136|gb|GU390996.1|_Bambusa_farinacea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552138|gb|GU390997.1|_Bambusa_flexuosa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552140|gb|GU390998.1|_Bambusa_gibba	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552142|gb|GU390999.1|_Bambusa_multiplex	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552144|gb|GU391000.1|_Bambusa_sinospinosa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
gi|289552146|gb|GU391001.1|_Bambusa_tuldoides	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.12 B: Close up of the black rectangle of Pairwise divergence table of *matK* with cells with value of 0.000 highlighted in red, of the table above.

4.3.2.4 Pattern of Nucleotide Substitution

The Pattern of Nucleotide Substitution in *psbA-trnH* region using the Maximum Composite Likelihood Estimate method by MEGA 6 shows that the transitional rate is significantly higher than the transversional rates. The nucleotide frequencies are 30.68% (A), 30.96% (T/U), 19.41% (C), and 18.95% (G). The transition/transversion rate ratios are k_1 = 2.041 (purines) and k_2 = 2.009 (pyrimidines). The overall transition/transversion bias is R = 0.958, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]. The analysis involved 120 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 334 positions in the final dataset.

	A	**T**	**C**	**G**
A	-	*7.69*	*4.82*	**9.61**
T	*7.62*	-	**9.69**	*4.71*
C	*7.62*	**15.46**	-	*4.71*
G	**15.55**	*7.69*	*4.82*	-

Table 4.13: Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution in *psbA-trnH*.

4.3.3 Analysis of *ITS* (Internal Transcribed Spacer)

A total of 166 sequences belonging to 124 different species were downloaded from the public domain of NCBI Popset database. These sequences were subjected to analysis using various Bioinformatics tools and softwares. The list of species and their NCBI Accession Number is given in the Table below:

Sl No	Species	Accession No
1	*Acidosasa chinensis*	gi|194359740
2	*Ampelocalamus scandens*	gi|331035183
3	*Arundinaria faberi*	gi|194359741
4	*Arundinaria tecta*	gi|331035185
5	*Bambusa bambos*	gi|72256981
6	*Bambusa blumeana*	gi|82570024
7	*Bambusa chungii*	gi|82570028
8	*Bambusa intermedia*	gi|82570026
9	*Bambusa membranacea*	gi|82570037
10	*Bambusa multiplex*	gi|82570025
11	*Bambusa sinospinosa*	gi|72256982
12	*Bambusa surrecta*	gi|82570027
13	*Bashania fargesii*	gi|331035186
14	*Bashania qingchengshanensis*	gi|331035188
15	*Brachystachyum densiflorum*	gi|331035191 ; gi|194359726
16	*Cephalostachyum fuchsianum*	gi|72256992
17	*Cephalostachyum mannii*	gi|72256987
18	*Cephalostachyum pallidum*	gi|72256993
19	*Cephalostachyum pergracile*	gi|72256990
20	*Cephalostachyum scandens*	gi|72256988
21	*Cephalostachyum virgatum*	gi|72256991
22	*Chimonobambusa grandifolia*	gi|194359720
23	*Chimonobambusa marmorea*	gi|331035193 ; gi|194359721
24	*Chimonobambusa ningnanica*	gi|194359724
25	*Chimonobambusa quadrangularis*	gi|331035192
26	*Chimonobambusa szechuanensis*	gi|331035194 ; gi|194359722
27	*Chimonobambusa tumidissinoda*	gi|331035195 ; gi|194359725
28	*Chimonobambusa utilis*	gi|194359723
29	*Chusquea delicatula*	gi|331035196
30	*Dendrocalamopsis oldhamii*	gi|82570023
31	*Dendrocalamus bambusoides*	gi|82570035
32	*Dendrocalamus brandisii*	gi|82570031
33	*Dendrocalamus giganteus*	gi|82570032
34	*Dendrocalamus latiflorus*	gi|82570033
35	*Dendrocalamus sinicus*	gi|82570034
36	*Dendrocalamus strictus*	gi|82570036
37	*Dinochloa malayana*	gi|72256972
38	*Dinochloa scandens*	gi|72256973
39	*Drepanostachyum falcatum*	gi|331035197
40	*Fargesia dracocephala*	gi|331035198
41	*Fargesia macclureana*	gi|331035189
42	*Fargesia murielae*	gi|331035199
43	*Fargesia nitida*	gi|194359718

44	*Fargesia perlonga*	gil331035190
45	*Fargesia utilis*	gil331035200
46	*Ferrocalamus strictus*	gil194359735
47	*Gaoligongshania megalothyrsa*	gil331035201
48	*Gelidocalamus stellatus*	gil194359742
49	*Gigantochloa albociliata*	gil82570029
50	*Gigantochloa verticillata*	gil82570030
51	*Hibanobambusa tranquillans*	gil331035202 ; gil331035217
52	*Himalayacalamus cupreus*	gil331035203
53	*Himalayacalamus falconeri*	gil331035204
54	*Indocalamus latifolius*	gil194359736
55	*Indocalamus longiauritus*	gil194359737
56	*Indocalamus tessellatus*	gil331035207 ; gil331035208
57	*Indosasa hispida*	gil194359728
58	*Indosasa sinica*	gil194359729
59	*Leptocanna chinensis*	gil72256986
60	*Melocalamus arrectus*	gil72256985
61	*Melocalamus compactiflorus*	gil72256984
62	*Melocalamus scandens*	gil72256983
63	*Melocanna baccifera*	gil72256994
64	*Menstruocalamus sichuanensis*	gil194359734
65	*Monocladus amplexicaulis*	gil72256976
66	*Monocladus levigatus*	gil72256977
67	*Monocladus saxatilis*	gil72256975 ; gil331035206 ; gil72256974
68	*Neomicrocalamus andropogonifolius*	gil331035209
69	*Neomicrocalamus microphyllus*	gil82570019
70	*Neomicrocalamus prainii*	gil194359717
71	*Neosinocalamus affinis*	gil82570020
72	*Neurolepis elata*	gil331035210
73	*Oligostachyum sulcatum*	gil194359743
74	*Olmeca recta*	gil283558303
75	*Otatea acuminata*	gil283558304 ; gil283558305 ; gil283558306; gil283558307 ; gil283558308; gil283558309; gil283558310; gil283558311; gil283558312; gil283558313; gil283558314; gil283558315; gil283558316; gil283558317; gil283558318; gil283558319
76	*Otatea carrilloia*	gil283558336
77	*Otatea fimbriata*	gil283558320; gil283558332; gil283558333
78	*Otatea glauca*	gil283558334 ; gil283558335
79	*Otatea reynosoana*	gil283558321
80	*Otatea transvolcanica*	gil283558322; gil283558323; gil283558324; gil283558325; gil283558326; gil283558327; gil283558328; gil283558329; gil283558330
81	*Otatea ximenae*	gil283558331
82	*Oxytenanthera abyssinica*	gil72256980
83	*Phyllostachys dulcis*	gil72256969
84	*Phyllostachys flexuosa*	gil331035211
85	*Phyllostachys heteroclada*	gil194359730
86	*Phyllostachys nidularia*	gil72256970
87	*Pleioblastus chino*	gil194359745
88	*Pleioblastus fortunei*	gil194359738
89	*Pleioblastus gramineus*	gil72256971
90	*Pleioblastus oleosus*	gil194359746
91	*Pleioblastus pygmaeus*	gil331035212

92	*Pleioblastus simonii*	gi|194359747
93	*Pseudosasa amabilis*	gi|194359744
94	*Pseudosasa japonica*	gi|194359748
95	*Pseudostachyum polymorphum*	gi|72256989
96	*Racemobambos hepburnii*	gi|331035213
97	*Racemobambos prainii*	gi|82570021
98	*Racemobambos yunnanensis*	gi|82570022
99	*Sarocalamus faberi*	gi|331035187
100	*Sasa palmata*	gi|194359739; gi|331035214
101	*Schizostachyum blumei*	gi|72257003
102	*Schizostachyum brachycladum*	gi|72257002
103	*Schizostachyum caudatum*	gi|331035215
104	*Schizostachyum dumetorum*	gi|72256997
105	*Schizostachyum funghomii*	gi|72256995
106	*Schizostachyum gracile*	gi|72257005
107	*Schizostachyum hainanense*	gi|72257004
108	*Schizostachyum jaculans*	gi|72256998
109	*Schizostachyum pseudolima*	gi|72256996
110	*Schizostachyum sanguineum*	gi|72257000
111	*Schizostachyum xinwuense*	gi|72256999
112	*Schizostachyum zollingeri*	gi|331035216; gi|72257001
113	*Semiarundinaria fastuosa*	gi|194359727
114	*Shibataea hispida*	gi|194359719
115	*Shibataea kumasaca*	gi|331035218; gi|72256968
116	*Sinobambusa intermedia*	gi|194359731
117	*Sinobambusa rubroligula*	gi|194359733
118	*Sinobambusa tootsik*	gi|194359732
119	*Thamnocalamus spathiflorus*	gi|331035219
120	*Thyrsostachys oliveri*	gi|72256979
121	*Thyrsostachys siamensis*	gi|72256978
122	*Yushania alpina*	gi|331035184
123	*Yushania boliana*	gi|331035205
124	*Yushania brevipaniculata*	gi|331035220

Table 4.14: List of *ITS* sequence downloaded from database along with Species and Accession Number.

4.3.3.1 Nucleotide Composition of *ITS* Sequences

Analysis of the *ITS* sequences show that the overall nucleotide composition shows a strong bias towards the nucleotides G and C bias across all the sequences. And not only the nucleotide composition leaning towards G and C content is high; it's high by significant amount. Graphs in Fig 4.27 and 4.28 showed the overall nucleotide composition as well as the GC content and it shows clearly a high GC content along with significantly low AT content. Also it is seen that the GC bias is not only confined to few sequences but all the sequences shows a bias towards high G and C content.

As the conventional wisdom goes, higher %GC content is indicative of higher complexity. This high level of complexity is exactly what is required for discrimation upto the species level and proper phylogenetic tree formation.

	T(U)	C	A	G	Total
Avg.	10.7	37.8	17.9	33.6	571.8

Table 4.15: Nucleotide composition of *ITS* sequence.

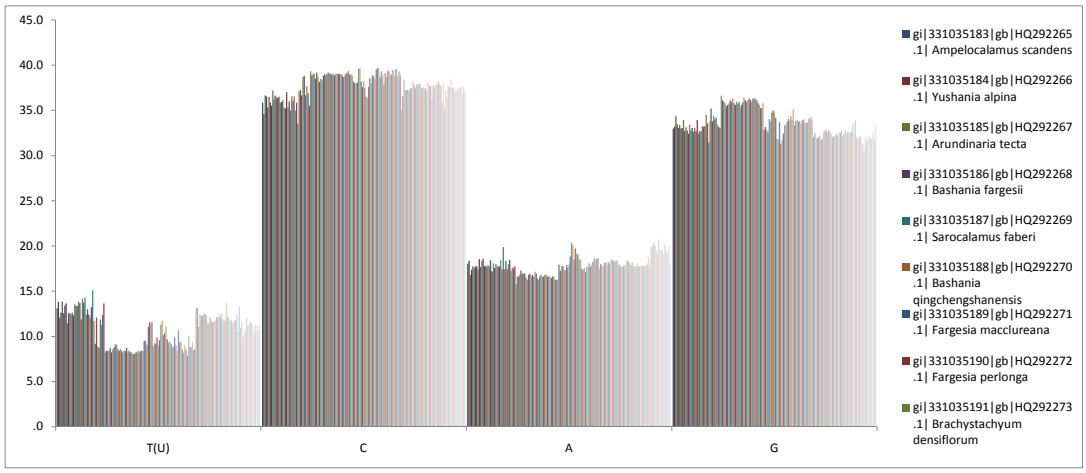


Figure 4.27: Graph showing the Nucleotide composition of *ITS*.

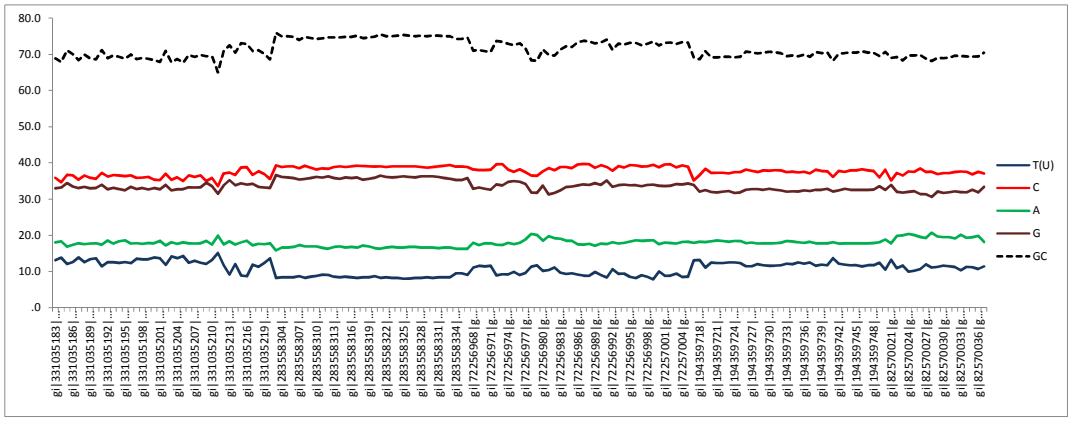


Figure 4.28: Overall nucleotide composition along with GC content of *ITS*.

4.3.3.2 Nucleotide pair frequencies of *ITS*

Analysis of Nucleotide pair frequencies of *ITS* sequence for all codon positions indicates that the number of identical pairs (ii) is in much greater number than the transitional pairs (si) and Transversional pairs (sv). The number of si and sv pairs doesn't show much variation. The average ii value for *matK* gene is 469, while that of si and sv is 26 each showed in the Table 4.17.

Domain		ii	si	sv	R	TT	TC	TA	TG	CT	CC
1.00	Avg	469.00	26.00	26.00	1.01	46.00	7.00	1.00	2.00	7.00	178.00

CA	CG	AT	AC	AA	AG	GT	GC	GA	GG	Total
3.00	6.00	2.00	4.00	82.00	7.00	1.00	6.00	5.00	163.00	520.47

Table 4.16: Nucleotide pair frequencies of *ITS*

4.3.3.3 Mean divergence in *ITS*

Analysis of Mean divergence in *ITS* sequences using Kimura-2-parameter (K2P) with sequences of Bamboo group showed the level of divergence/ difference among the species. As the data shows, there is significant level of variations among many species. It indicates there is divergence on a molecular basis/level among the bamboo species.

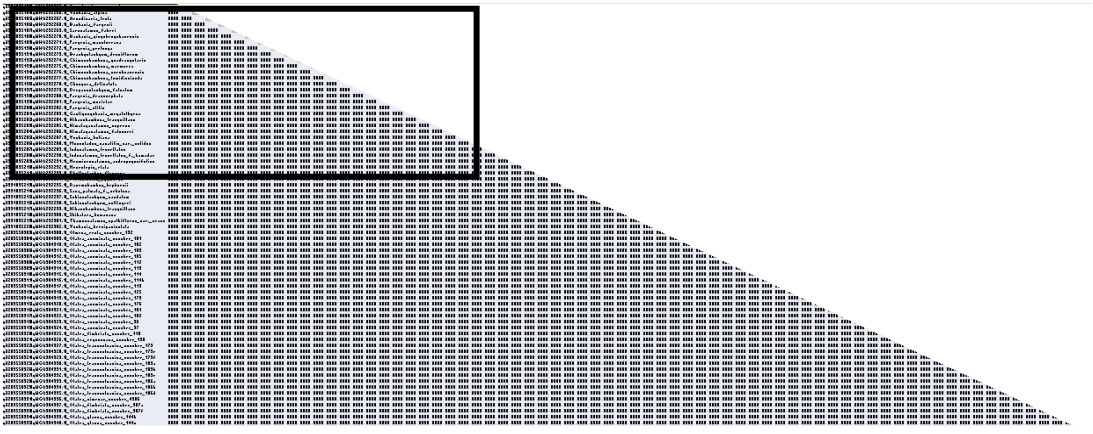


Table 4.17 A: Pairwise divergence table of *ITS* with cells conditioned to become red with a value of 0.000.

gi|283558334|gb|GQ384339.1|_Otatea_glauca_voucher_144b	0.070	0.077	0.073	0.066	0.066	0.066	0.066	0.066	0.066	0.070	0.066	0.066	0.077	0.070	0.070
gi|283558335|gb|GQ384340.1|_Otatea_glauca_voucher_144c	0.077	0.084	0.080	0.073	0.073	0.073	0.073	0.073	0.073	0.073	0.070	0.073	0.080	0.077	0.077
gi|283558336|gb|GQ384341.1|_Otatea_carrilloi_voucher_147a	0.070	0.077	0.073	0.066	0.066	0.066	0.066	0.066	0.066	0.070	0.066	0.066	0.077	0.070	0.070
gi|72256968|gb|DQ131501.1|_Shibataea_kumasaca	0.003	0.013	0.006	0.000	0.003	0.000	0.010	0.003	0.000	0.003	0.006	0.000	0.010	0.032	0.006
gi|72256969|gb|DQ131502.1|_Phyllostachys_dulcis	0.010	0.019	0.013	0.006	0.010	0.006	0.016	0.010	0.006	0.010	0.013	0.006	0.016	0.039	0.013
gi|72256970|gb|DQ131503.1|_Phyllostachys_nidularia	0.003	0.013	0.006	0.000	0.003	0.000	0.010	0.003	0.000	0.003	0.006	0.000	0.010	0.032	0.006
gi|72256971|gb|DQ131504.1|_Pleioblastus_gramineus	0.010	0.019	0.013	0.006	0.010	0.006	0.016	0.010	0.006	0.010	0.013	0.006	0.016	0.039	0.013
gi|72256972|gb|DQ131505.1|_Dinochloa_malayana	0.056	0.056	0.059	0.053	0.053	0.053	0.053	0.053	0.053	0.056	0.053	0.053	0.063	0.056	0.056
gi|72256973|gb|DQ131506.1|_Dinochloa_scandens	0.053	0.053	0.056	0.049	0.049	0.049	0.049	0.049	0.049	0.053	0.049	0.049	0.060	0.053	0.053
gi|72256974|gb|DQ131507.1|_Monocladus_saxatilis_var._solidus	0.042	0.042	0.046	0.039	0.039	0.039	0.039	0.039	0.039	0.042	0.039	0.039	0.049	0.036	0.042
gi|72256975|gb|DQ131508.1|_Monocladus_saxatilis	0.056	0.056	0.059	0.053	0.053	0.053	0.053	0.053	0.053	0.056	0.053	0.053	0.063	0.049	0.056
gi|72256976|gb|DQ131509.1|_Monocladus_amplexicaulis	0.042	0.042	0.046	0.039	0.039	0.039	0.039	0.039	0.039	0.042	0.039	0.039	0.049	0.036	0.042
gi|72256977|gb|DQ131510.1|_Monocladus_levigatus	0.066	0.066	0.070	0.063	0.063	0.063	0.063	0.063	0.063	0.066	0.063	0.063	0.074	0.053	0.066
gi|72256978|gb|DQ131511.1|_Thyrsostachys_siamensis	0.063	0.063	0.066	0.059	0.059	0.059	0.059	0.059	0.059	0.063	0.059	0.059	0.070	0.053	0.063
gi|72256979|gb|DQ131512.1|_Thyrsostachys_oliveri	0.070	0.070	0.073	0.066	0.066	0.066	0.066	0.066	0.066	0.070	0.066	0.066	0.077	0.059	0.070
gi|72256980|gb|DQ131513.1|_Oxytenanthera_abyssinica	0.070	0.070	0.073	0.066	0.066	0.066	0.066	0.066	0.066	0.070	0.066	0.066	0.077	0.059	0.070
gi|72256981|gb|DQ131514.1|_Bambusa_bambos	0.053	0.053	0.056	0.049	0.049	0.049	0.049	0.049	0.049	0.053	0.049	0.049	0.059	0.042	0.053
gi|72256982|gb|DQ131515.1|_Bambusa_sinospinosa	0.066	0.066	0.070	0.063	0.063	0.063	0.063	0.063	0.063	0.066	0.063	0.063	0.073	0.056	0.066
gi|72256983|gb|DQ131516.1|_Melocalamus_scandens	0.056	0.059	0.059	0.053	0.053	0.053	0.053	0.053	0.053	0.056	0.053	0.053	0.063	0.042	0.056
gi|72256984|gb|DQ131517.1|_Melocalamus_compactiflorus_ar._fimbriatus	0.056	0.056	0.059	0.053	0.053	0.053	0.053	0.053	0.053	0.056	0.053	0.053	0.063	0.039	0.056
gi|72256985|gb|DQ131518.1|_Melocalamus_arrectus	0.056	0.056	0.059	0.053	0.053	0.053	0.053	0.053	0.053	0.056	0.053	0.053	0.063	0.039	0.056
gi|72256986|gb|DQ131519.1|_Leptocanna_chinensis	0.039	0.039	0.043	0.036	0.036	0.036	0.036	0.036	0.036	0.039	0.036	0.036	0.046	0.029	0.039
gi|72256987|gb|DQ131520.1|_Cephalostachyum_mannii	0.049	0.049	0.053	0.046	0.046	0.046	0.046	0.046	0.046	0.049	0.046	0.046	0.056	0.036	0.049
gi|72256988|gb|DQ131521.1|_Cephalostachyum_scandens	0.049	0.049	0.053	0.046	0.046	0.046	0.046	0.046	0.046	0.049	0.046	0.046	0.056	0.036	0.049
gi|72256989|gb|DQ131522.1|_Pseudostachyum_polymorphum	0.043	0.039	0.046	0.039	0.036	0.039	0.036	0.036	0.039	0.043	0.039	0.039	0.049	0.032	0.039
gi|72256990|gb|DQ131523.1|_Cephalostachyum_pergracile	0.042	0.042	0.046	0.039	0.039	0.039	0.039	0.039	0.039	0.042	0.039	0.039	0.049	0.029	0.042

Table 4.17 B: Close up of the black rectangle area of Pairwise divergence table of *ITS*.

4.3.3.4 Pattern of Nucleotide Substitution

The Pattern of Nucleotide Substitution in *psbA-trnH* region using the Maximum Composite Likelihood Estimate method shows that the transitional rate is significantly higher than the transversional rates. Rates of different transitional substitutions are shown in **bold** and those of transversional substitutions are shown in *italics*. The nucleotide frequencies are 19.55% (A), 11.31% (T/U), 36.78% (C), and 32.36% (G). The transition/transversion rate ratios are k_1 = 2.915 (purines) and k_2 = 1.231 (pyrimidines). The overall transition/transversion bias is R = 0.944, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]. The analysis involved 166 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 315 positions in the final dataset.

	A	**T**	**C**	**G**
A	-	*2.76*	*8.96*	**22.98**
T	*4.76*	-	**11.03**	*7.88*
C	*4.76*	**3.39**	-	*7.88*
G	**13.88**	*2.76*	*8.96*	-

Table 4.18: Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution in *ITS* sequences.

4.4 Phylogenetic Relationship of targeted Bamboo Sequences

4.4.1 Phylogenetic analysis of *matK* sequences

4.4.1.1 Analysis of contribution of Subtribes to the dataset

The *matK* sequences downloaded from the database showed that a total 9 subtribes of the tribe Bambuseae were represented in the set of sequence. The 9 subtribes are: Arthrostylidiinae, Arundinariinae, Bambusinae, Chusqueinae, Guaduinae, Hickelinae, Melocanninae, Racemobambosinae and Shibataeinae. Apart from the tribe Bambuseae, a few sequences from other tribes and subtribes were also taken to test the robustness of the MEGA 6 program. For example, the subtribes Alopecurinae, Aveninae, Loliinae and Neurachninae of the tribe Pooideae were included in the phylogenetic study.

The analysis of (Table 4.20) and (Figure 4.29 and 4.30) also shows that the maximum contribution was by the subtribe Bambusinae, which constituted 42 species and a total of 45 sequences. It is also notable that although the subtribe Guaduinae has only 16 species but it has 62 sequences in the data set, making it the highest contributor in terms of sequence at 41%. The contributors with least number of Species and sequences are in ascending order Hickelinae (1 Species; 1 Sequence); Racemobambosinae (2 Species; 2 Sequences) and Shibataeinae (3 Species; 3 Sequences)

Sl	Tribe	Subtribe	No. of Species	No. of Sequence
1	Bambuseae	Arthrostylidiinae	8	8
2		Arundinariinae	6	6
3		Bambusinae	42	45
4		Chusqueinae	3	3
5		Guaduinae	16	62
6		Hickelinae	1	1
7		Melocanninae	7	7
8		Racemobambosinae	2	2
9		Shibataeinae	3	3
10	Various	Others	15	15

Table 4.19: Contribution of Species diversity and No of Sequence of each Subtribes in *matK* Phylogenetic analysis.

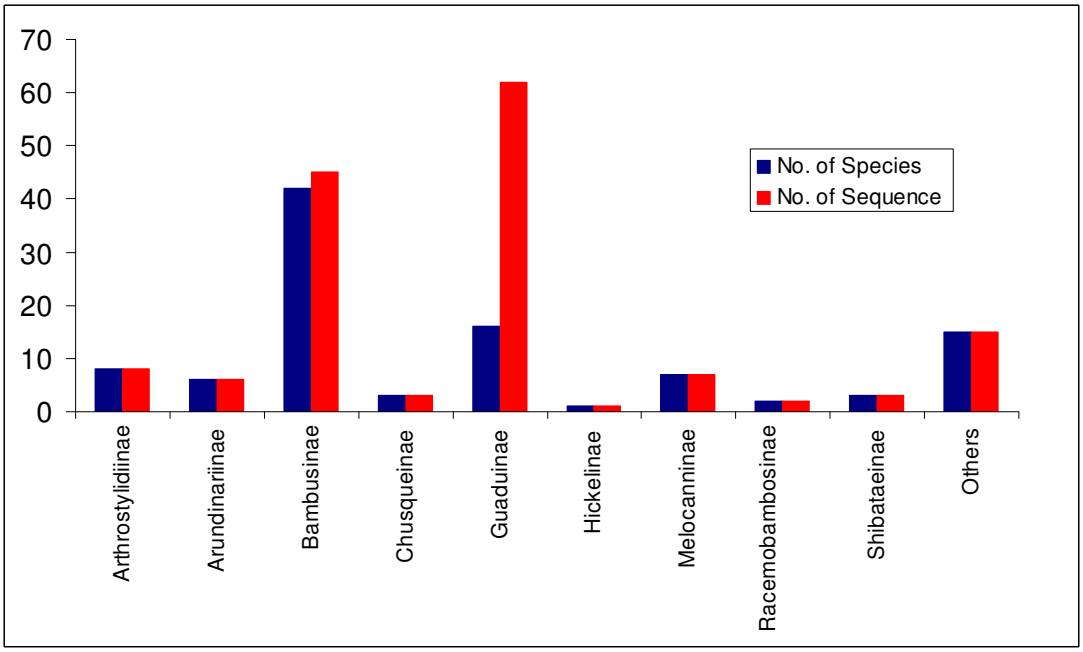


Fig 4.29: Graph showing the No. of Species and Sequence contributed by each Subtribe to the total *matK* sequences used in Phylogenetic analysis.

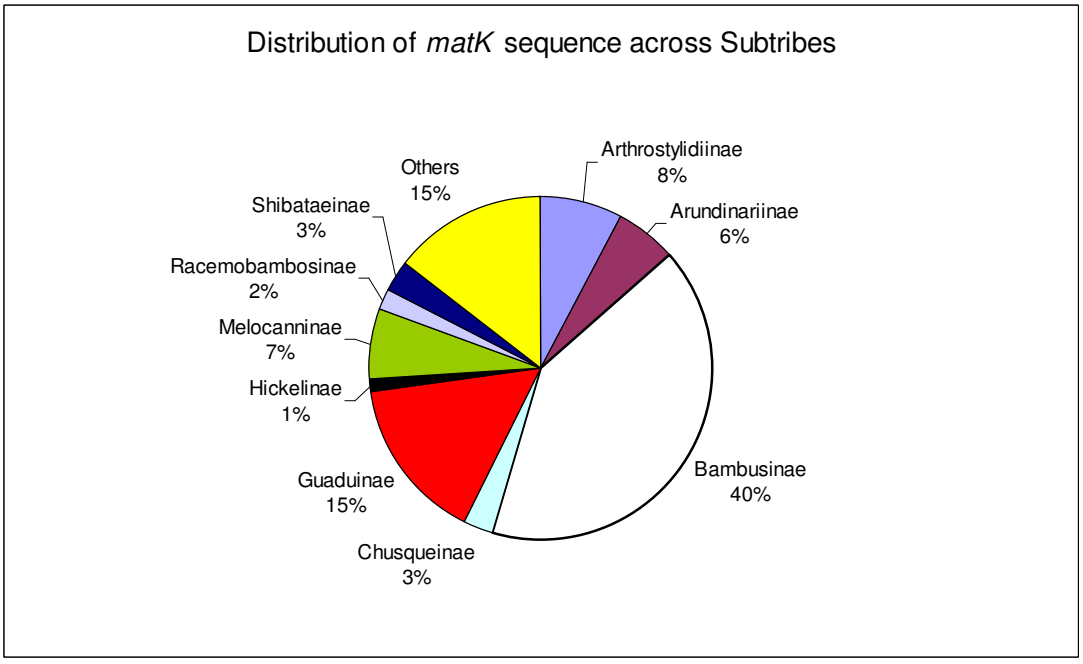


Fig 4.30: Graph showing percentage contribution by each Subtribe to the total *matK* sequences used in Phylogenetic analysis.

4.4.1.2 Analysis of *matK* Phylogenetic tree

Analysis of this portion of the *matK* phylogenetic tree shown in Fig 4.32 indicates fascinating trends. All the sequences are of the Genus *Otatea* and it should have formed a single clade with the same species together. But we can see the formation of 2 distinct clades (marked with rectangle) (Fig 4.33) and the non formation of any clade in the middle (marked by oval) in Fig 4.32.

But things are not all bad in *matK* Phylogenetic tree, analysis of Fig 4.34 shows that *matK* has correctly placed all genus and species of the Subtribe Guaduinae together and forms a super clade. Not only that, *matK* is capable of elucidating the correct hierarchy and ancestry as it has formed the correct phylogenetic tree at the Genus and Subtribe level.

Analysis of this portion of the *matK* phylogenetic tree (Fig 4.34) shows that the Genus *Olmeca* has formed 7 clades (marked with rectangle). What is interesting is the fact there seems to be no clear logic for these clades formation. It is not on the basis of species level diversity as the same species e.g. *Olmeca fulgor* is interspersed in a number of clades (marked with an arrow) as indicated in Fig 4.34.

It is seen in Fig 4.36, that the *matK* sequence is capable of elucidating the phylogenetic relationship in Bamboo with moderate competence upto genus level. In the Fig 4.35, it is clearly seen that the genus *Otatea* has been clearly demarcated and grouped together in a distinct clade, giving a very clear idea about its phylogenetic aspect. But it is seen that which trying to distinguish at species level, it shows poor resolution and mix things up. It is seen in the Fig 4.36 that the species *Otatea acuminata, O. transvolcanica* and *O. ximenae* are poorly resolved at the species level. It is seen that within the genus *Otatea*, the above mentioned species occurs haphazardly. Instead of the 3 species being grouped together in a clade in the phylogenetic tree, they are interspersed in between other species.

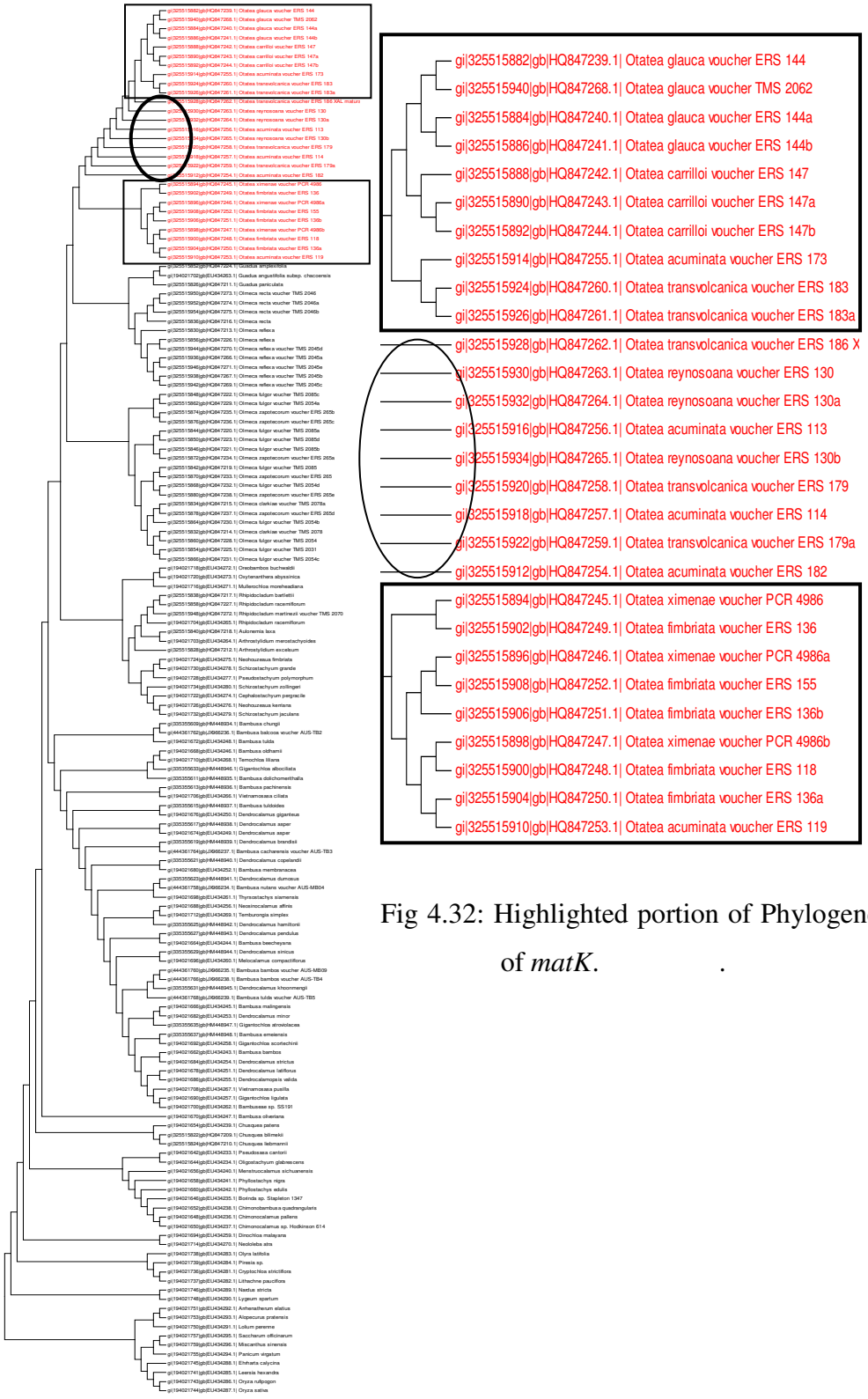


Fig 4.32: Highlighted portion of Phylogenetic tree of *matK*.

Fig 4.31: Phylogenetic tree of *matK*

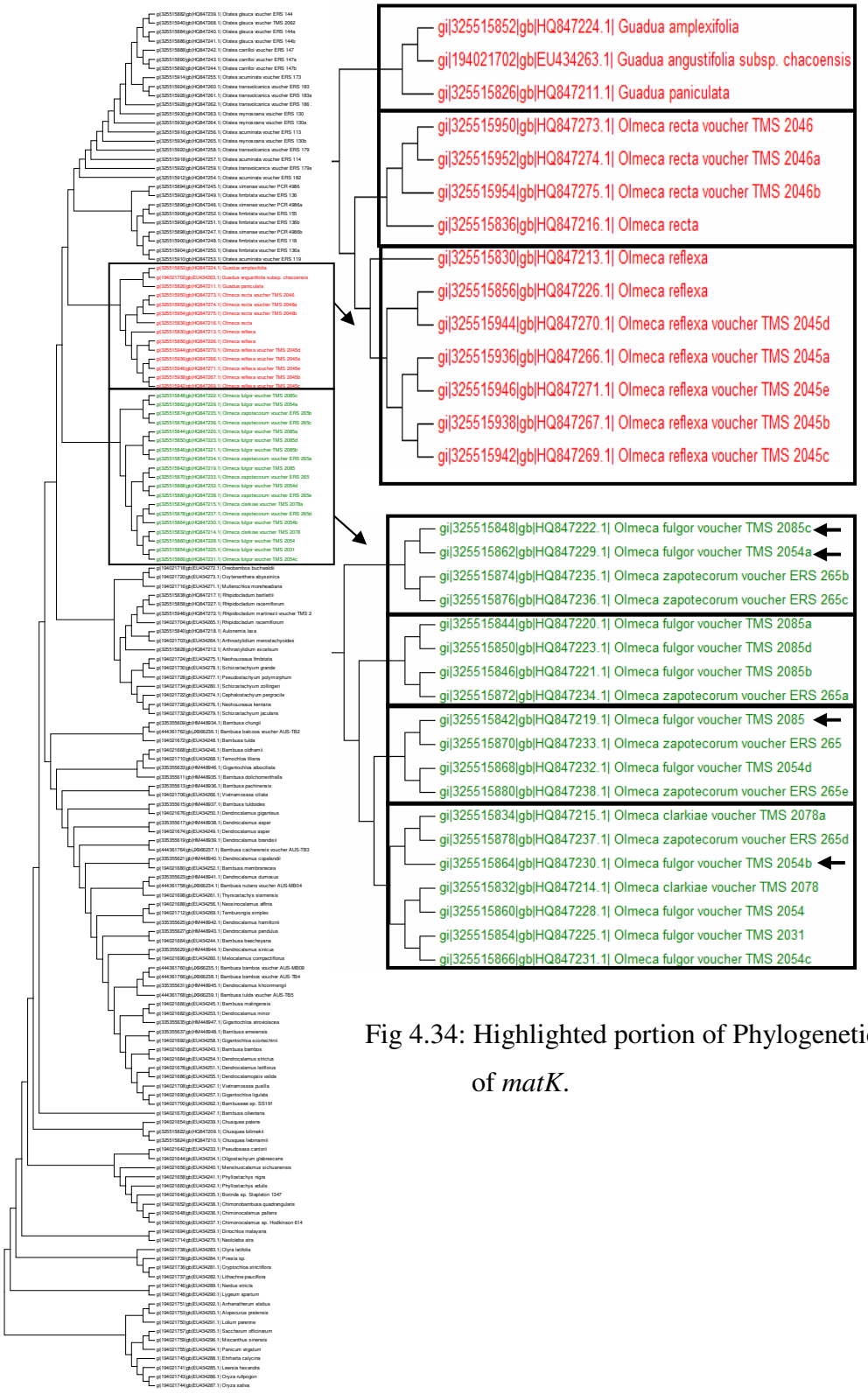


Fig 4.34: Highlighted portion of Phylogenetic tree of *matK*.

Fig 4.33: Phylogenetic tree of *matK*

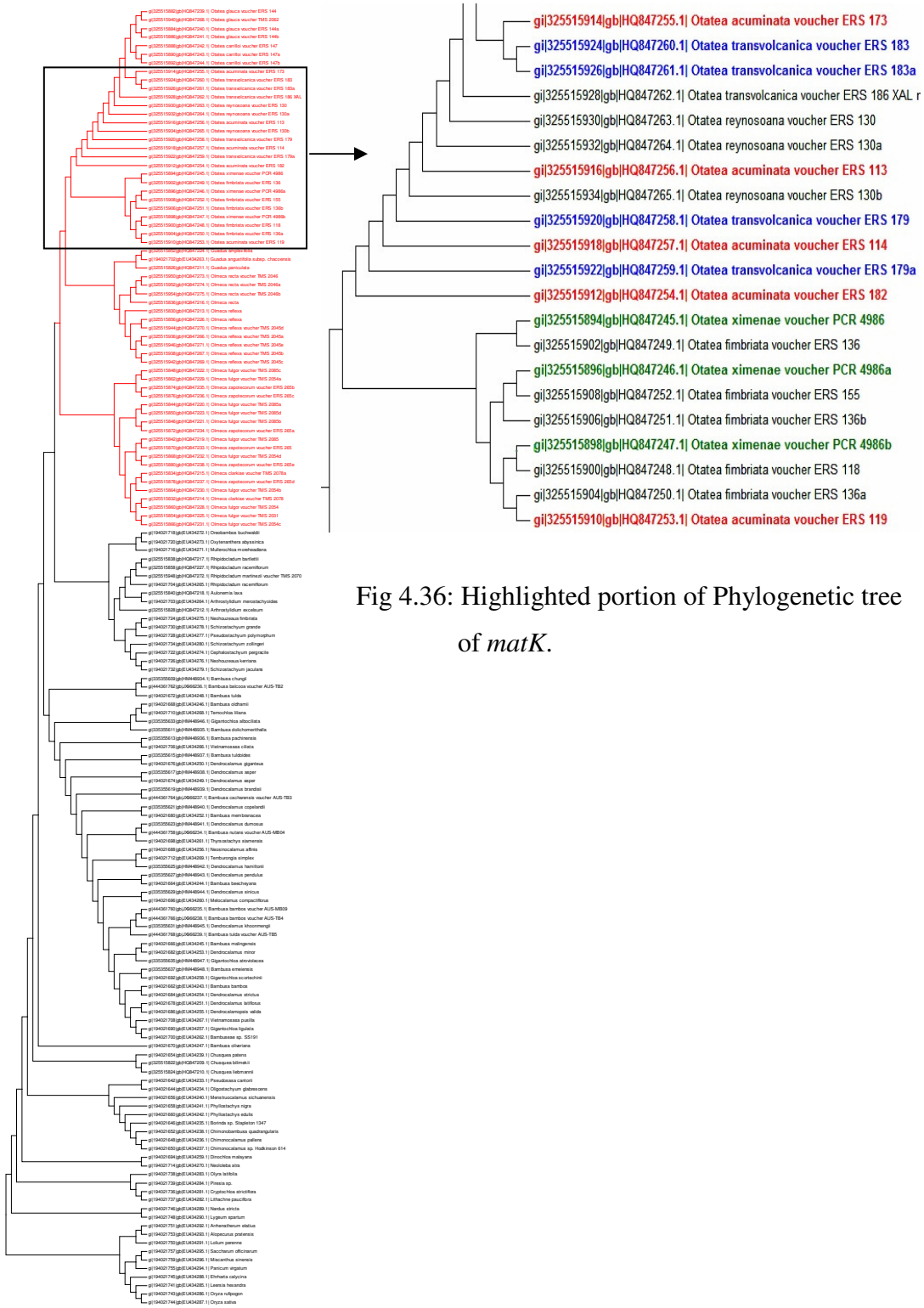


Fig 4.36: Highlighted portion of Phylogenetic tree of *matK*.

Fig 4.35: Phylogenetic tree of *matK*

Analysis of the red portion of the *matK* Phylogenetic NJ tree (Fig 4.38) shows formation of 3 distinct clades but it is also noticeable that the members allocated in the clades are unrelated and forms wrong clades. This clearly indicated the inability of *matK* sequences of Bamboo to resolve the phylogenetic relationship upto the species level.

Analysis of the blue portion of the *matK* Phylogenetic NJ tree shows very weak clade formation or no formation at all. The different species are interspersed haphazardly without showing any distinct phylogenetic patterns. But a positive aspect that can be inferred from analyzing the blue portion indicates that it has grouped the sequences correctly into the Subtribe Bambusinae. So it is apparent that *matK* sequences of Bamboo can resolve upto the Subtribe level correctly.

Analysis of the green portion in Fig 4.40, shows clear failure of *matK* sequences as it is clearly seen the various genus and species have been badly mixed up not only at the Genus level but also at the Sub tribe level. Whereas Nardus strictus belongs to the Subtribe Arundinariinae; Lygeum spartum belongs to the Subtribe Neurachninae. But as indicated by arrows, they have been brought together to form a clade which is absolutely wrong.

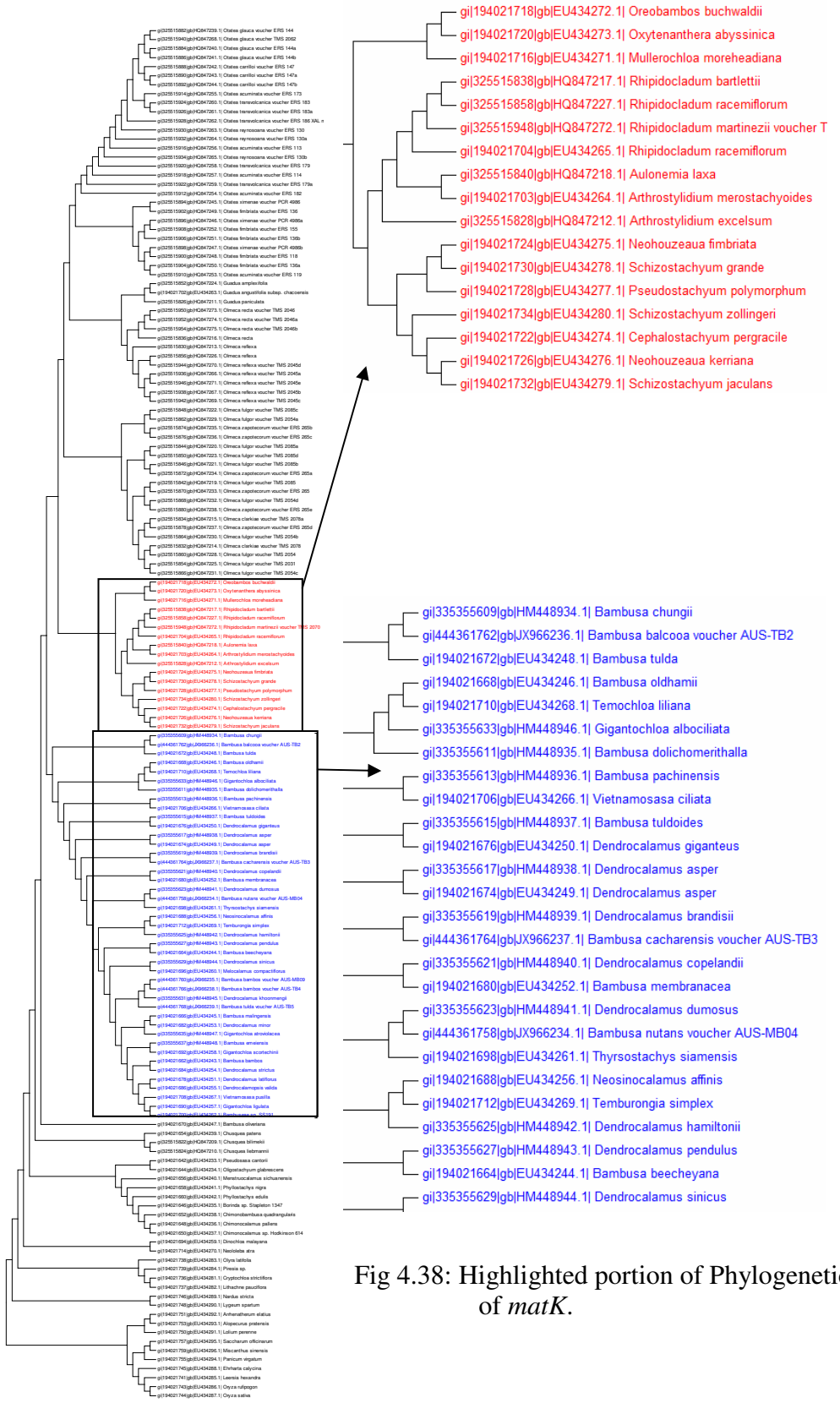
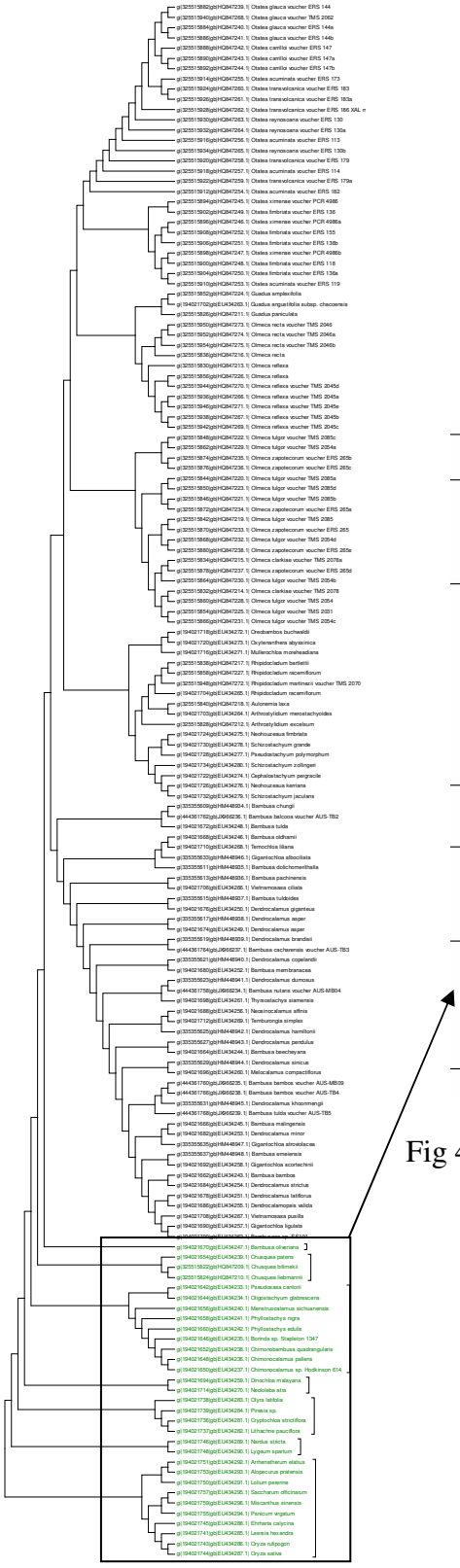


Fig 4.38: Highlighted portion of Phylogenetic tree of *matK*.

Fig 4.37: Phylogenetic tree of *matK*



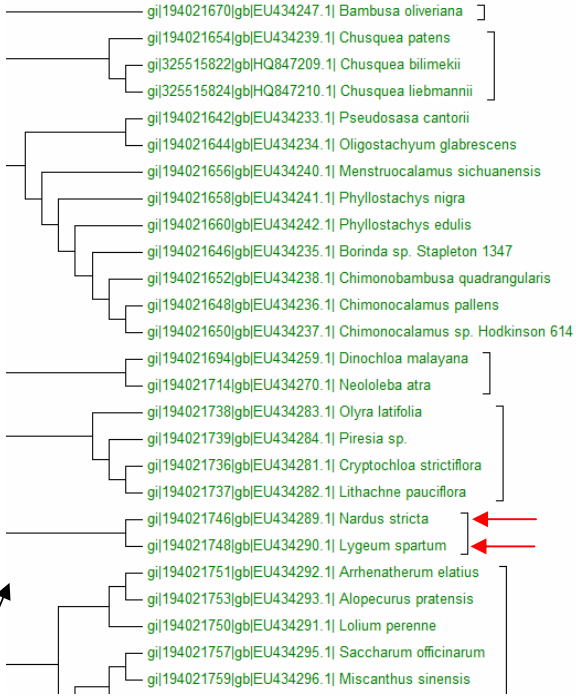


Fig 4.40: Highlighted portion of Phylogenetic tree of *matK*.

Fig 4.39: Phylogenetic tree of *matK*

4.4.2 Phylogenetic analysis of *psbA-trnH* sequences

4.4.2.1 Analysis of contribution of Subtribes to the dataset

The *psbA-trnH* sequences that were downloaded from the database shows that a total 5 subtribes of the tribe Bambuseae were represented in the set of sequence. The 5 subtribes are: Arthrostylidiinae, Bambusinae, Chusqueinae, Guaduinae, and Melocanninae.

The analysis of (Table 4.20) and (Figure 4.41 and 4.42) also shows that the maximum contribution was by the subtribe Bambusinae, which constituted 81 species and a total of 90 sequences. It is also notable that the subtribe Bambusinae has 90 sequences in the data set, thus making it the highest contributor in terms of sequence at 79%.

The contributors with least number of Species and sequences are in ascending order Chusqueinae (1 Species; 1 Sequence); Arthrostylidiinae (2 Species; 2 Sequences) and Melocanninae (3 Species; 3 Sequences)

Sl	Tribe	Subtribe	No. of Species	No. of Sequence
1	Bambuseae	Arthrostylidiinae	2	2
2		Bambusinae	81	90
3		Chusqueinae	1	1
4		Guaduinae	16	16
5		Melocanninae	3	3

Table 4.20: Contribution of Species diversity and No of Sequence of each Subtribes in *psbA-trnH* Phylogenetic analysis.

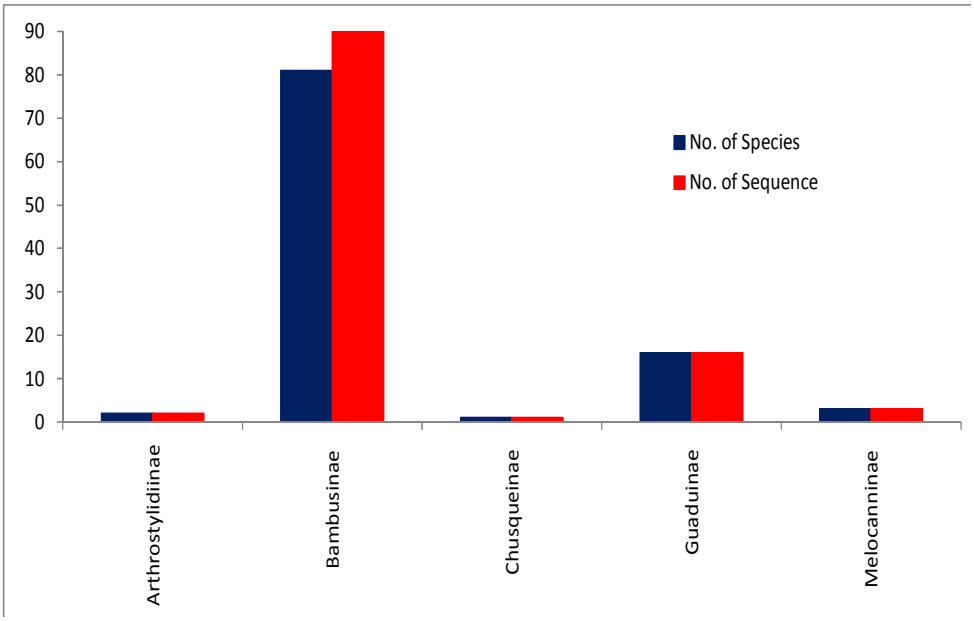


Fig 4.41: Graph showing the No. of Species and Sequence contributed by each Subtribe to the total *psbA-trnH* sequences used in Phylogenetic analysis.

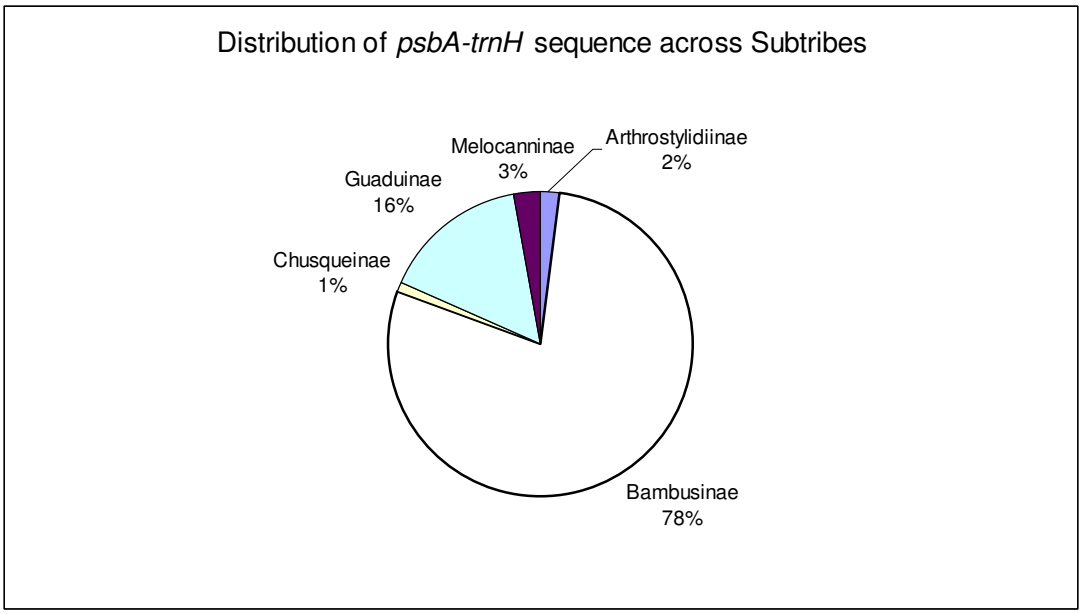


Fig 4.42: Graph showing percentage contribution by each Subtribe to the total *psbA-trnH* sequences used in Phylogenetic analysis.

4.4.2.2 Analysis of *psbA-trnH* Phylogenetic tree

Analysis of the Neighbour-Joining Phylogenetic tree created with MEGA6 using Kimura 2 parameters shows a few startling facts.

Analysis of the *psbA-trnH* phylogenetic tree brings forth a number of glaring short coming in the capacity of the DNA sequence to elucidate the phylogenetic relationship in Bamboo. Looking at Fig 4.44, where 2 genuses, *Melocanna* and *Schizostachyum* are considered, it is observed *psbA-trnH* sequences failed to resolve at genus level. The phylogenetic tree shows that the sequences do not have enough resolving characters even for genus level. Looking further, it appears that *psbA-trnH* has brought the Subtribe Melocanninae together. But looking closer at the red lines it's clear that the clades are different, showing different ancestors for the same genus. The genus indicates a level which shares a common ancestry, but it is observed that *psbA-trnH* sequences fails even to elucidate the common ancestry in Bamboo.

Analysis of Fig 4.45 shows another great short coming of *psbA-trnH* to resolve the phylogenetic relationship in Bamboo. In this case, Dinochloa and Kinabaluchloa belong to the Subtribe Bambusinae. Chusquea belongs to the Subtribe Chusqueinae and Olmeca belongs to the Subtribe Guaduinae. But analysis of the Fig 4.45 clearly shows that *psbA-trnH* sequences have placed 3 different Subtribes under a single clade. It is a well known fact that Subtribes have different ancestry and as such they tend to form separate clades in a phylogenetic tree. So *psbA-trnH* fails not in resolving species and genus diversity, it also fails at a higher hierarchy level i.e. Subtribe. As such *psbA-trnH* is best not used alone in elucidating the phylogenetic relationship of Bamboo.

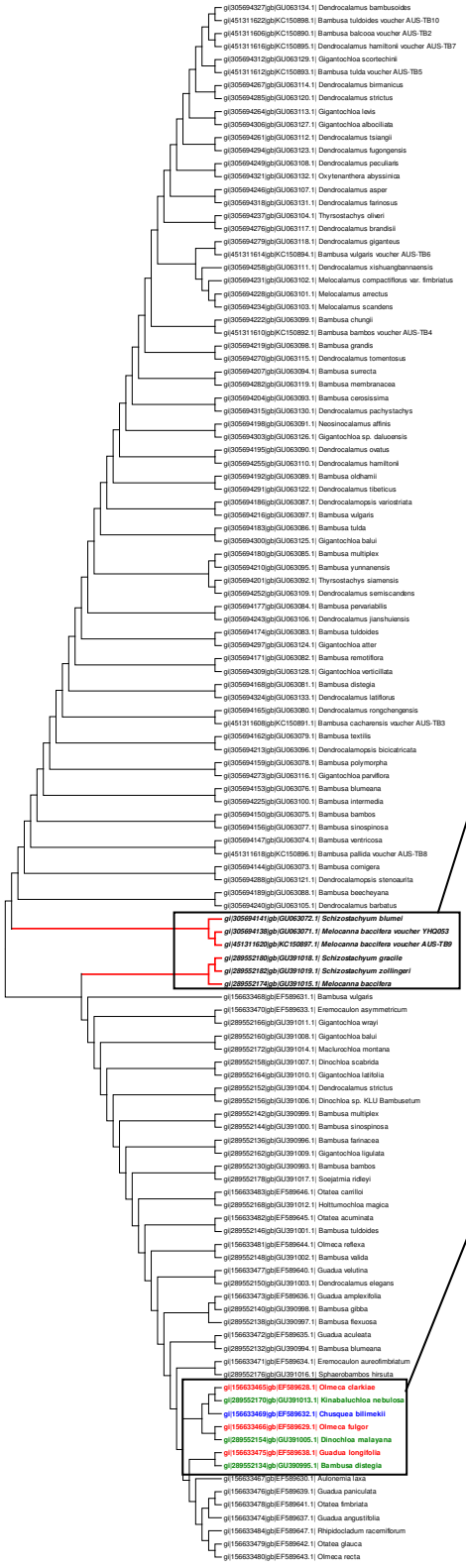


Fig 4.43: Phylogenetic tree of *psbA-trnH*

Fig 4.44: Highlighted portion of Phylogenetic tree of *psbA-trnH*

Fig 4.45: Highlighted portion of Phylogenetic tree of *psbA-trnH*

Analyzing the overall *psbA-trnH* phylogenetic tree and specially Figures 4.47, 4.49, 4.50 and 4.52 a general trend can be clearly seen.

In Fig 4.47 some weak clade formation is seen but then the Genus and species included in them are haphazardly spread out. The portion of the *psbA-trnH* marked in red showed a positive aspect as it could cluster all the Genuses into the correct sub tribe Bambusinae.

In Fig 4.49 and 4.50 the part of the *psbA-trnH* highlighted in blue and red showed no clade formation. Also there is no clustering of the sequences into the correct subtribes. The tree clearly showed that the phylogenetic relationship whether the hierarchy or ancestry is not formed and *psbA-trnH* sequences of Bamboo is incapable of resolving the phylogenetic relationship.

Fig 4.52 shows that the top portion of the phylogenetic tree, which is highlighted in green showed no clade formation. But the lower half of the portion indicated formation of clade and the Subtribe Guadinae has been correctly resolved.

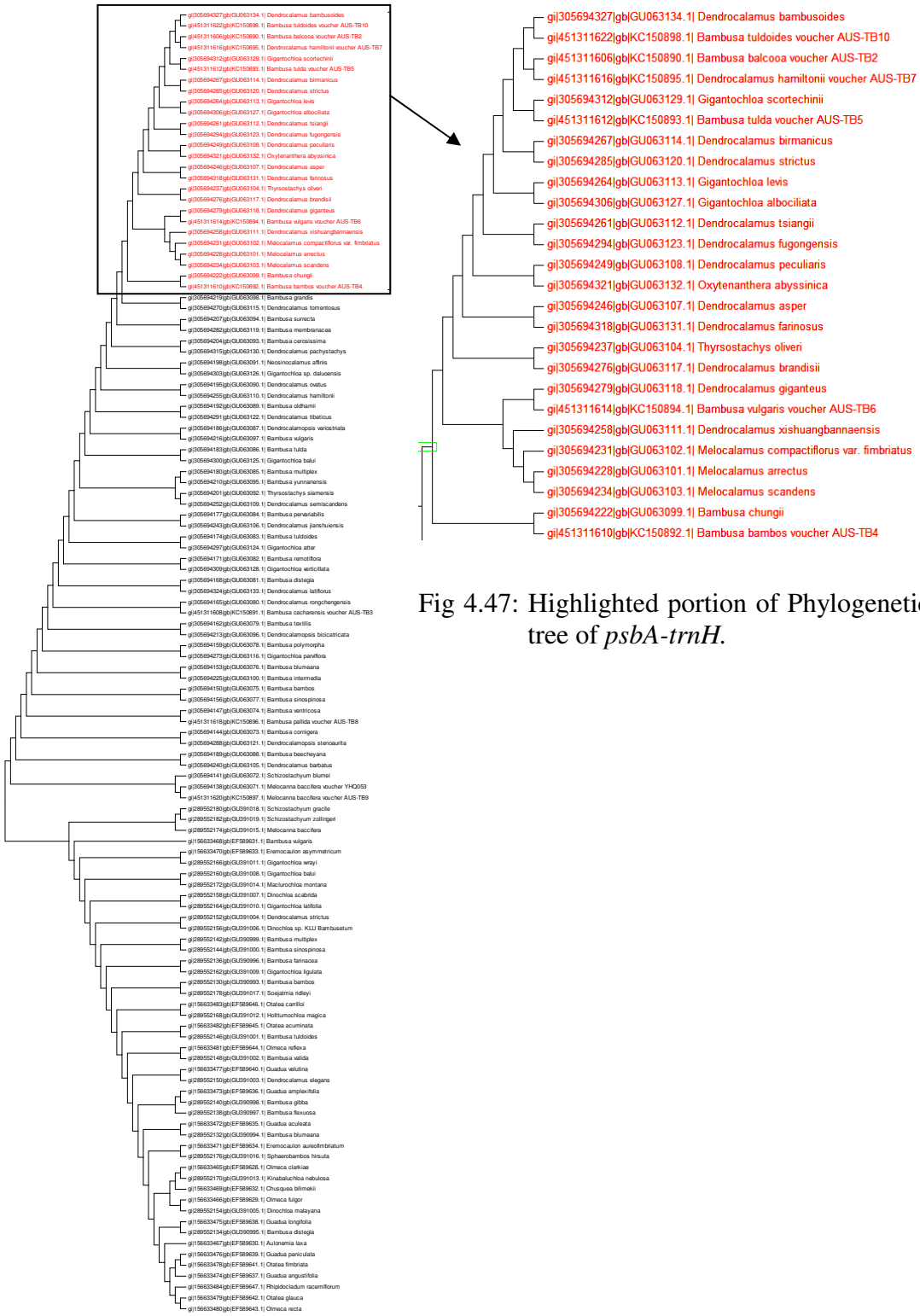


Fig 4.47: Highlighted portion of Phylogenetic tree of *psbA-trnH*.

Fig 4.46: Phylogenetic tree of *psbA-trnH*

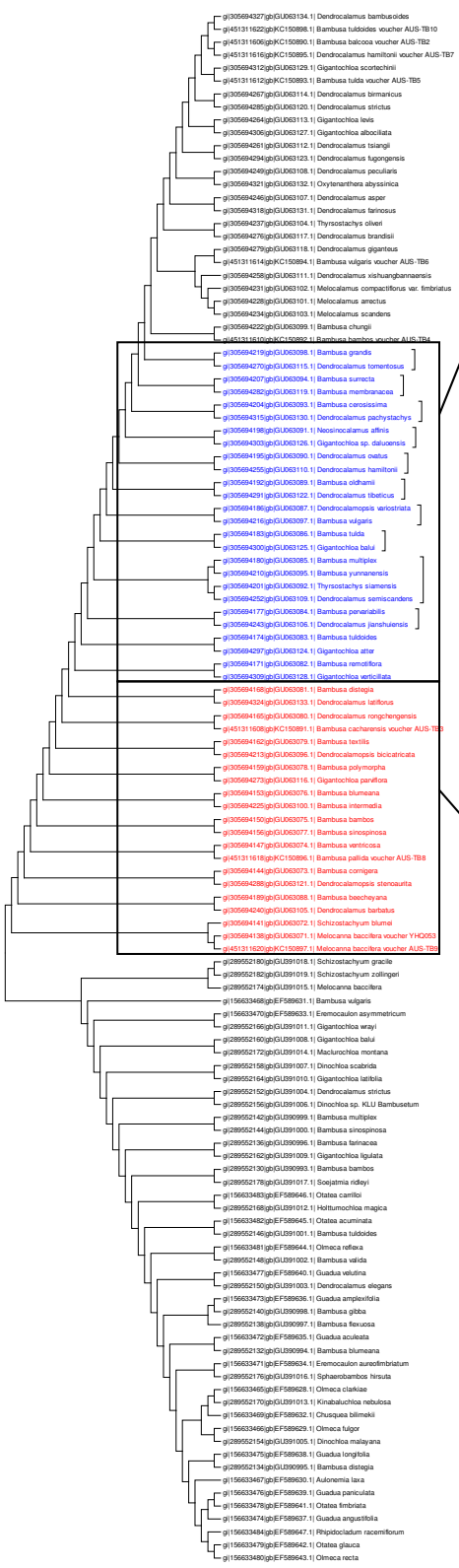


Fig 4.48: Phylogenetic tree of *psbA-trnH*

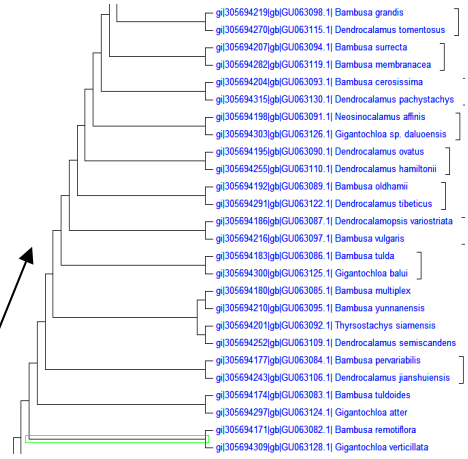


Fig 4.49: Highlighted portion of Phylogenetic tree of *psbA-trnH*.

Fig 4.50: Highlighted portion of Phylogenetic tree of *psbA-trnH*.

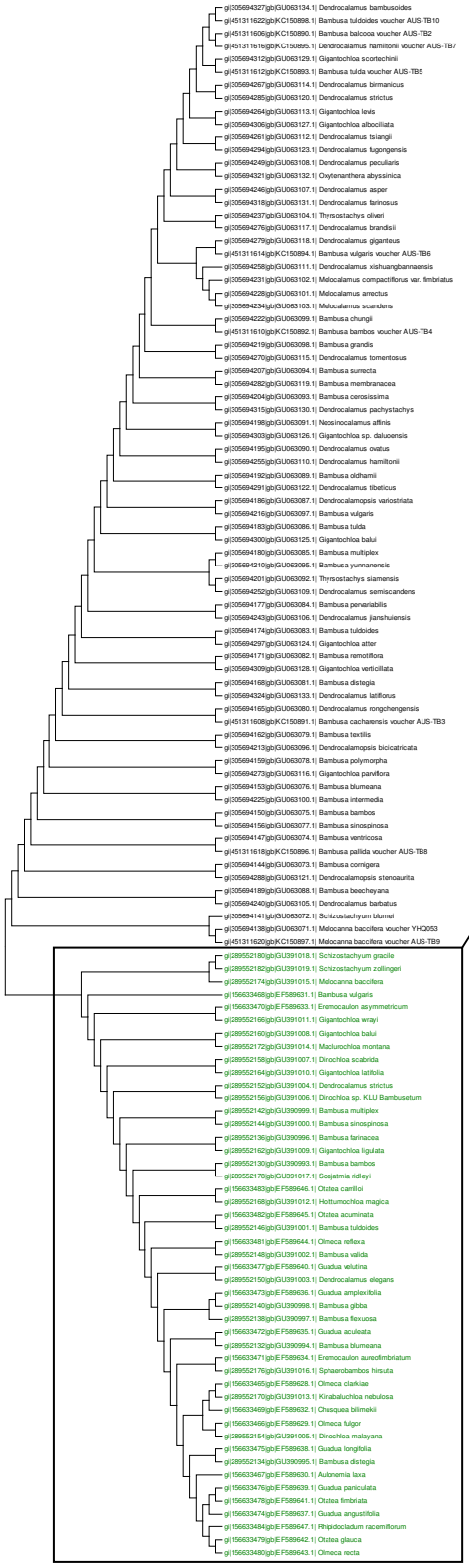


Fig 4.51: Phylogenetic tree of *psbA-trnH*

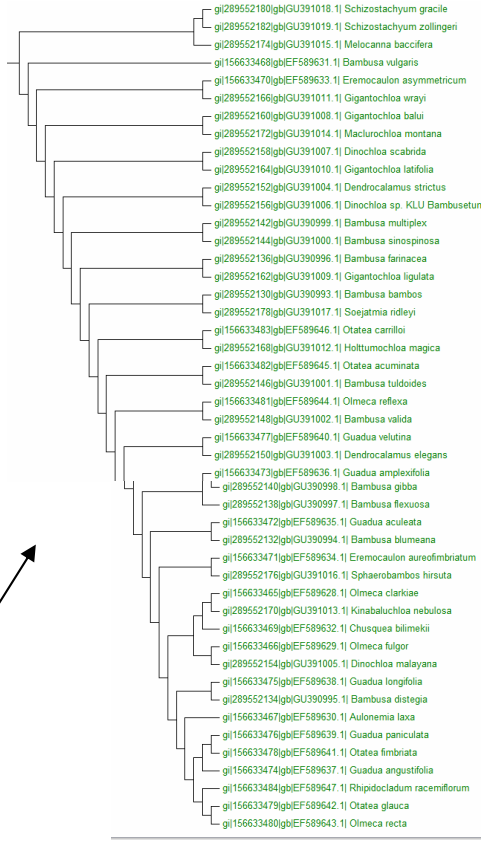


Fig 4.52: Highlighted portion of Phylogenetic tree of *psbA-trnH*.

4.4.3 Phylogenetic analysis of Internal transcribed spacer (*ITS*) sequences

4.4.3.1 Analysis of contribution of Subtribes to the dataset

Analysis of *ITS* sequences that were downloaded from the database shows that a total 8 subtribes of the tribe Bambuseae were represented in the set of sequence. The 8 subtribes are: Arundinariinae, Bambusinae, Chusqueinae, Guaduinae, Melocanninae, Racemobambosinae, Shibataeinae and Thamnocalaminae.

The analysis of (Table 4.21) and (Figure 4.53 and 4.54) also shows almost equal contribution was made by three subtribes Bambusinae, Arundinariinae and Melocanninae which contributed about 23 species and more than 23 sequences each. But first among the equals the subtribe Bambusinae which contributed 27 sequences in the data set, thus makes it the highest contributor in terms of sequence at 22%.

The contributor with least number of Species and sequences is Chusqueinae (2 Species; 2 Sequence).

Sl	Tribe	Subtribe	No. of Species	Number of Sequence
1	Bambuseae	Arundinariinae	23	25
2		Bambusinae	27	27
3		Chusqueinae	2	2
4		Guaduinae	8	34
5		Melocanninae	23	24
6		Racemobambosinae	6	6
7		Shibataeinae	21	27
8		Thamnocalaminae	14	14

Table 4.21: Contribution of Species diversity and No of Sequence of each Subtribe in *ITS* Phylogenetic analysis.

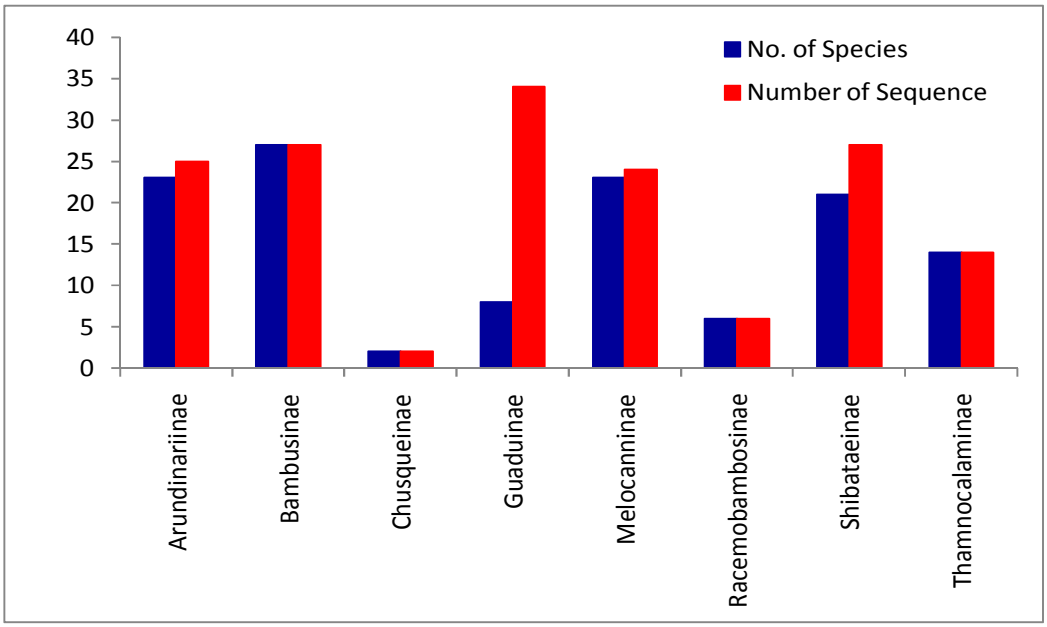


Fig 4.53: Graph showing the No. of Species and Sequence contributed by each Subtribe to the total *ITS* sequences used in Phylogenetic analysis.

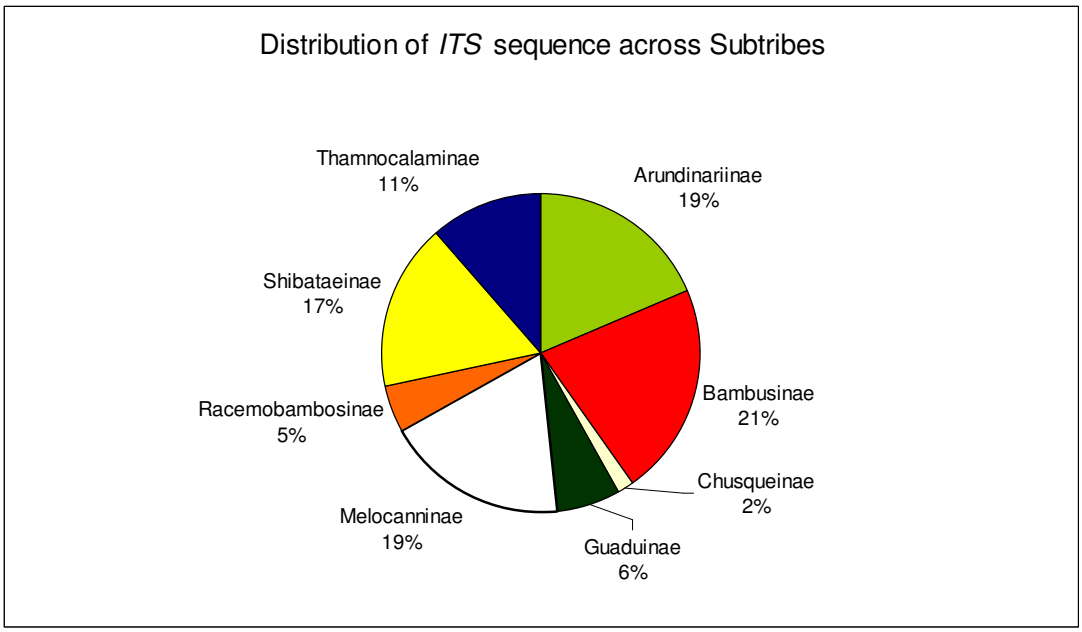


Fig 4.54: Graph showing percentage contribution by each Subtribe to the total *ITS* sequences used in Phylogenetic analysis.

4.4.3.2 Analysis of *ITS* Phylogenetic tree

Analysis of the NJ Phylogenetic Tree of *ITS* sequences shows a remarkable resolution power to elucidate the phylogenetic relationship of Bamboo. The resolution power of *ITS* works at every hierarchical level and it is efficiency is stated below:

Analysis of Fig 4.56 shows Species marked in red are from Subtribe Bambusinae; species marked in Teal/Green are from Subtribe Racemobambosinae and species marked in Purple are from Subtribe Chusqueinae. As can be clearly seen, the resolution of *ITS* works correctly at Subtribe, Genus and Species level.

Analysis of Fig 4.58 of the NJ Phylogenetic tree of *ITS* sequences of Bamboo clearly demarcates at Subtribe level. Species marked in blue are from Subtribe Melocanninae; species marked in Green are from Subtribe Guaduinae and the single species marked in red appears to be an error. Apart from the correct genus level discrimination, it is clearly indicated within the red box that the various species under the genus *Otatea* has been clearly demarcated and correct clade formation has occurred. The phylogenetic tree clearly shows the evolutionary relationship and the ancestry of the various specimen sequences.

Analysis of Fig 4.60 of NJ Phylogenetic tree of *ITS* again clearly shows the demarcation at Subtribe, Genus and Species level. Species marked in blue are from Subtribe Bambusinae; species marked in Green are from Subtribe Shibataeinae. Also it is seen just like in Fig 4.58 that all the species under the genus *Chimonobambusa* (indicated in green) has been clearly demarcated and correct clade formation has occurred. The phylogenetic tree clearly shows the evolutionary relationship and the ancestry of the various specimen sequences.

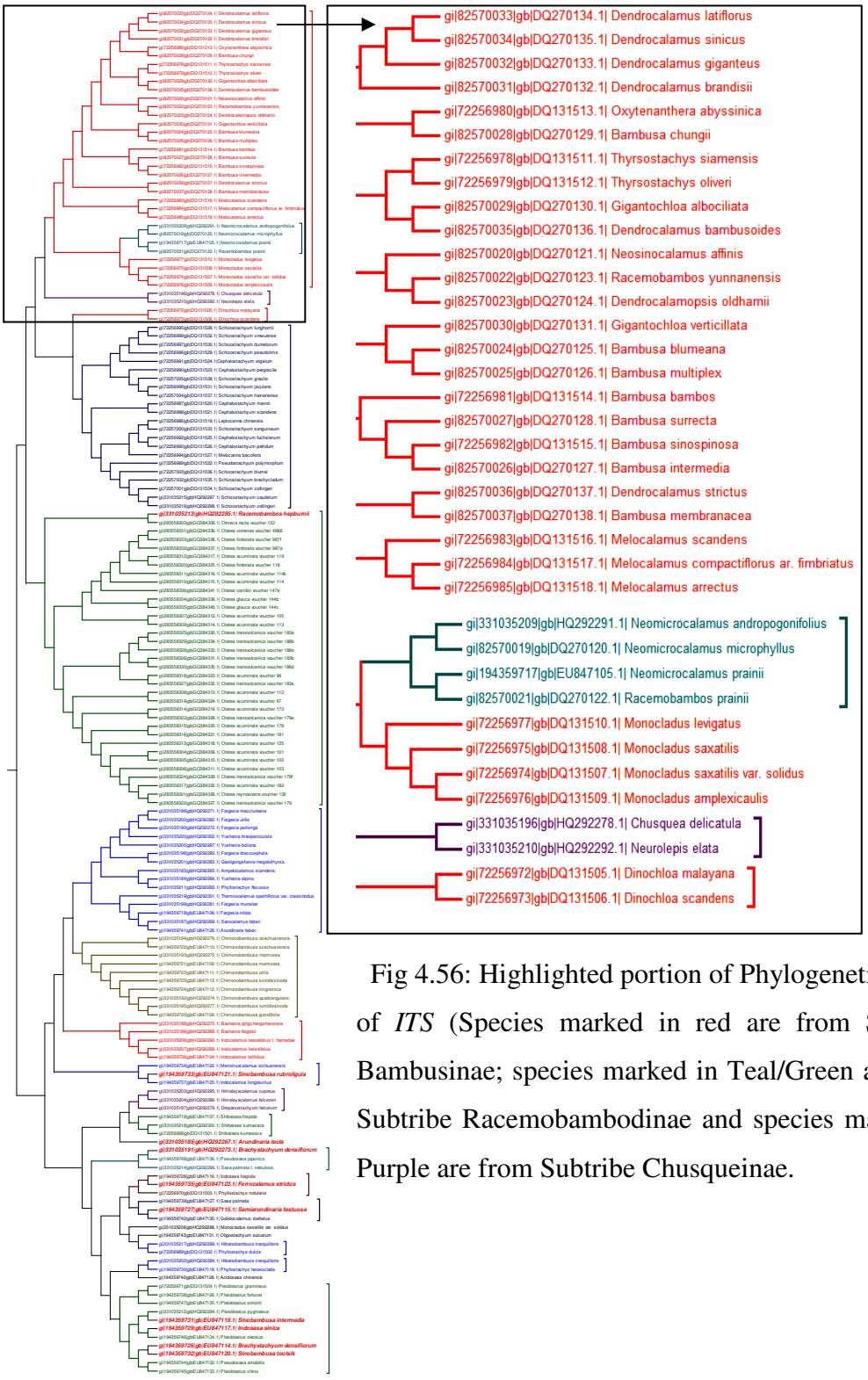


Fig 4.56: Highlighted portion of Phylogenetic tree of *ITS* (Species marked in red are from Subtribe Bambusinae; species marked in Teal/Green are from Subtribe Racemobambodinae and species marked in Purple are from Subtribe Chusqueinae.

Fig 4.55: Phylogenetic tree of *ITS*

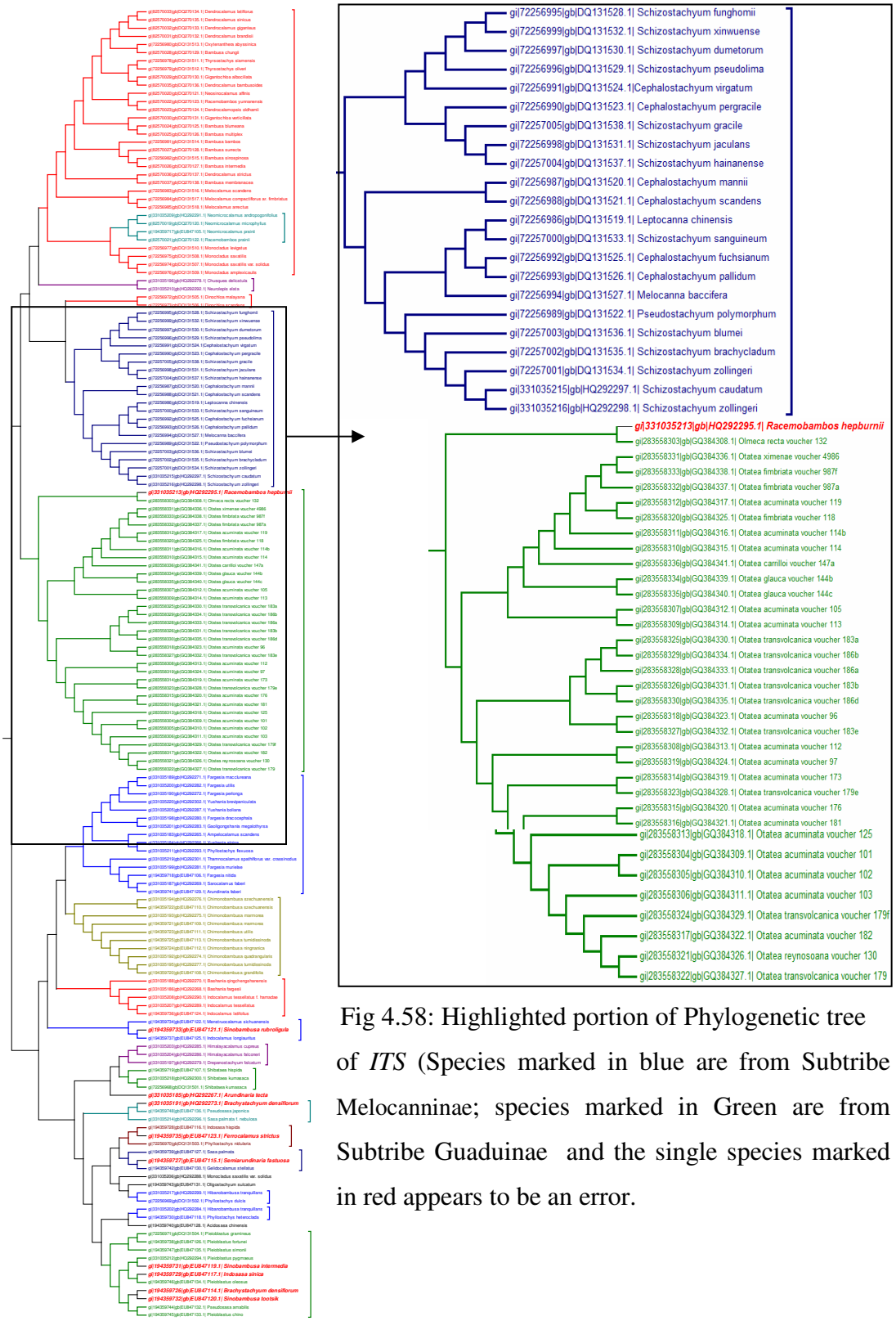


Fig 4.58: Highlighted portion of Phylogenetic tree of *ITS* (Species marked in blue are from Subtribe Melocanninae; species marked in Green are from Subtribe Guaduinae and the single species marked in red appears to be an error.

Fig 4.57: Phylogenetic tree of *ITS*

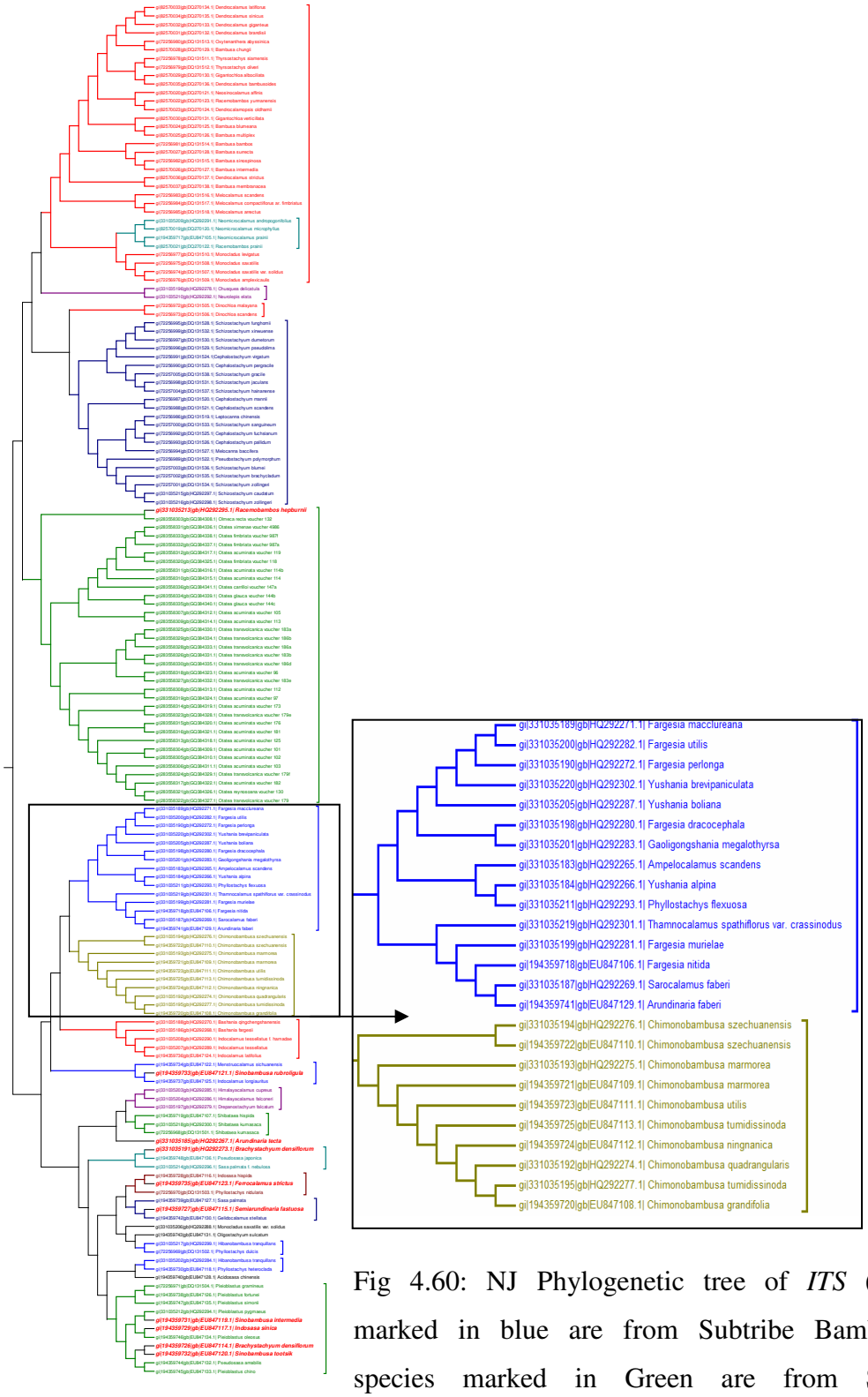


Fig 4.60: NJ Phylogenetic tree of *ITS* (Species marked in blue are from Subtribe Bambusinae; species marked in Green are from Subtribe Shibataeinae.

Fig 4.59: Phylogenetic NJ tree of ITS