

A phylogenetic analysis of the *Cucurbitaceae*: evidences from *matK* nucleotide sequences

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Abstract- MaturaseK gene (*matK*) of chloroplast is highly conserved in plant systematics which is involved in Group-II intron splicing. The size of the gene is approximately 1500 base pairs in length, located with in the intron of *trnK*. In the present study, *matK* gene from *Cucurbitaceae* was taken for the analysis of variants, parsimony site, patterns, transition/ transversion rate and phylogeny. The family *Cucurbitaceae* comprises over 120 genera and 825 species with medicinal values. The *matK* gene sequences have been withdrawn from GenBank and used for the analysis. The multiple sequence alignments were performed by Genebee-ClustalW. The phylogenetic analyses were carried out by MEGA (Molecular Evolutionary Genetics Analysis) software package based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Maximum Parsimony (MP) methods. The results indicated that the *Trichosanthes*, *Austrobryonia*, *Momordica*, *Neoalsomitra*, *Gomphogyne*, *Sicyos* genera showed polyphyletic condition. The overall invariants between the 88 taxa are 28.5%, average composition of nucleotides among taxa are found to be approximately, A-0.30, T-0.37, G-0.15, C-0.17 and the ratio of transition and transversion is 0.824. Phylogenetic tree was designed to identify the ideal regions that could be used for defining the inter and intra-generic relationships. From this study, it could be concluded that, the chloroplast *matK* gene sequences can unambiguously resolve the relationships, as well as provided a good indication of major supra-generic groupings among the selected members of the family *Cucurbitaceae*.

Key Words: *matK* gene, polyphyletic, *Cucurbitaceae*, Phylogenetic relationships, MEGA

INTRODUCTION

Advancement in molecular biology and DNA sequencing techniques has enabled to characterize the genomes of various organisms rapidly. Analyses of the DNA sequences of various species are providing valuable information about their taxonomy, gene makeup and utilizations. The *matK* gene is one of the most rapidly evolving plastid-coding regions [1]. It is approximately 1550 base pairs (bp) long and encodes a maturase involved in splicing type –II introns from RNA transcripts [2]. The *matK* gene sequence has been widely employed as a powerful tool in examining inter- and intragenous phylogeny due to its high substitution rates [3-4]. Recent studies have shown the usefulness of this gene for resolving intergeneric or interspecific relationships among flowering plants. For example, Malphigiaceae [5], Poaceae [6], Cornaceae [7], Orchidaceae [8–9].

Cucurbitaceae is one of the largest families of flowering plants, comprising over 120 genera and 825 species [10]. The family is economically very important being the major source of food and forage and its great diversity (ranked as fifth largest family in flowering plants) has also attracted much interest in ecological as well as systematic studies [11]. The main objective to elucidate the systematic positions of members of the family *Cucurbitaceae* also aimed to generate parallel data sets for representatives of all genera to strengthen the morphology based classification.

METHODOLOGY

Data collection: The entire coding region of *matK* sequences of 88 different species belonging to 54 genera of *Cucurbitaceae* are presented in the table-1. Generic and species information were obtained from taxonomy database of National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) .

Sequence analysis: The data analysis was done for the 88 different species belonging to 54 genera of *Cucurbitaceae* for which the sequences are available in GenBank to find the interspecific and intergeneric relationship among the selected genera. Multiple sequence alignment was performed by using Genebee-ClustalW service (http://www.genebee.msu.su/services/phytree_reduced.htm) to find out the basic sequence statistics including nucleotide frequencies, transition/ transversion (Ts/Tv) ratio and variability in different regions of sequences.

Phylogenetic analysis: The phylogenetic tree was constructed on the basis of *matK* gene sequences ranging between (1051 to 1393) of 88 taxa of 54 genera belong to the family *Cucurbitaceae*, based on UPGMA and MP methods using MEGA software [12]. Tree statistics included the consistency index (CI) [13], retention index (RC) [14] and rescaled consistency index (RCI) for all sites [14]. Branch lengths (ACCTRAN optimization with equal weights) and the level of support for branches of the phylogenetic trees was evaluated with the bootstrap analysis [15] to verify the strength of the branches based on 100 replicates, using

branch and bound search. Bootstrap percentages are described as high (85-100%), moderate (75 – 84%) or low (50 – 74%) [16]. The number of nucleotide substitutions per site was estimated by Kimura's two parameter method [17] with the DNADIST program.

RESULTS AND DISCUSSION

The study presented here is a preliminary attempt to employ plastid DNA sequence of a few selective genera in *Cucurbitaceae* to address interfamilial relationships; it is evidenced from the Fig-1. The results of the multiple sequence alignment studies demonstrated that, 695 variable sites and 257 parsimony sites. The overall invariants between the 88 taxa are 28.5%, average nucleotide composition in 88 taxa found to be approximately, A-0.30, T-0.37, G-0.15, C-0.17 and transition and transversion ratio is 0.824. Phylogenetic tree was designed to identify the ideal regions that could be used for defining the inter and intra-generic relationships. Phylogenetic tree of family *Cucurbitaceae* of 88 taxa were inferred using UPGMA (MP) method. The length of the tree was found to be 18473. The consistency index is 0.161641, the retention index is 0.657458 and the rescaled consistency index for all sites is 0.106272. All positions containing gaps and missing data were eliminated from the dataset. A total of 1000 residues were used from the sequences of 88 selected taxa to construct a phylogenetic tree, out of which 257 are invariants and potentially informative. The family tree obtained from the UPGMA method was split into fourteen well supported clades and also noticed that, the few selected genera namely, *Trichosanthes*, *Austrobryonia*, *Momordica*, *Neoalsomitra*, *Gomphogyne*, *Sicyos* showed polyphyletic condition, indicating that the members of these genera are evolved several times during evolutionary processes.

The UPGMA method of phylogenetic analysis strongly suggests that, the Clade – I consists of two subclades, which are sisterly origin and majority of the taxa present in this clade consists of 1146 nucleotide residues. Subclade-I consists of six species, of which, five belongs to tribe Trichosantheae, namely, *Trichosanthes ovigera*, *T. cucumerina*, *T. bracteata*, *T. subvelutina*, *Hodgsonia heteroclite* and are commonly sharing certain features like pollen striate, smooth or verrucose, flower longer, stamens united with triplicate thecae. The *Acanthosicyos naudianianus* and *Cucumeropsis manii* belongs to tribe Melothrieae and Benincaeseae respectively, and sharing certain common features like tendrils proximally 2-7 fid or simple, seeds unwinged and reticulated pollen. Where as, the members of subclade-II of clade –I are belongs strictly to the tribe Zanonieae of sub family Zanonioideae, sharing commonly the small

and striate pollen grains, tendrils distally bifid, seeds mostly winged.

In case of clade-II, only *Gomphogyne cissiformis* belongs to the tribe Zanonieae showing the typical character is tendrils distally bifid, seeds mostly winged. Where as, remaining two taxa, *Sicyos hillebrandii* and *S. baderoa* belongs to tribe Sicyeae sharing common features like trichomatous nectary, 4 to 10 – colporate pollen grains, ovules solitary, pendulous, pollen spinulose, polyporate. They are closely related and this relationship is strongly supported by high bootstrap values (98%).

The clade-IV is also divided into 2 subclades, the subclade-II consists of total four taxa, of which *Luffa graveolens* and *L. echinata* belongs to tribe Benincaeseae, sharing the common features like ovules many, horizontal, pollen reticulate, triporate and their association is strongly supported by very high bootstrap, values, 97%. Where as, *Trichosanthes schlechteri* and *Gymnopetalum chinense* belongs to subtribe Trichosantheinae of tribe Trichosantheae, are sisterly originated with the *Luffa* species, and the relationship among these two tribal members is supported by very low bootstrap values i.e., 44%. These Trichosantheae tribal members sharing commonly pollen striate, smooth or verrucose, flower longer stamens united with triplicate thecae characters.

In case of clade-V, the *Tricyclandra leandrill* and *Trochomeria macrocarpa* belongs to tribe Trichosantheae and Melothrieae respectively, sharing some of the common features like, styles united into a single column, receptacle tube relatively longer, alike in male and female flowers. Other four members of the same clade namely, *Austrobryonia centralis*, *Polyclathra cucumerina*, *Scopellaria marginata*, *Schizocarpum palmeri* belongs to the subfamily Cucurbitoideae, exhibiting common features are styles united into a single column, ovules many, horizontal or 1 to few ascending pollen, spinose pentaporate.

In clade-VI, out of three species, *Sicyosperma gracile* and *Rytidostylis ciliate* are belongs to subtribe Cyclantherinae of tribe Sicyeae and exhibiting strictly some common characters are trichomatous nectary, 4 to 10 colporate pollen grains, ovules solitary, pendulous, pollen spinulose, polyporate and is strongly supported by high bootstrap values 96%. Where as, *Nothoalsomitra suberosa* belongs to subtribe Benincasiniae of tribe Benincaeseae and is distantly related with above two species. This is also confirmed by poor bootstrap values only 13%.

Similarly, clade –VII consists of two subclades, subclade-I comprises *Melothrianthus smilacifolius*, *Trochomeriopsis diversifolia*, *Psiguria umbrosa* belongs strictly to the tribe Melothrieae and sharing features commonly are

pollens usually reticulate, flowers small, stamens usually free with simple thecae. However, subclade-2 consists of *Cogniauxia trilobata* and *Peponopsis adhaerens* belongs to tribe Benincaseae and Cucurbitaceae respectively, and sharing certain features commonly are petals without a fringe or ventral scales, receptacle tube usually relatively short, if long in male flowers then short in female flowers. Clade-VIII consists of total six taxa and are belongs to the tribes Benincaseae, Joliffieae and Melothtrieae of subfamily Cucurbitaceae, sharing common features are styles united into a single column, tendrils proximally 2-7 fid or simple seeds unwinged.

In case of clade-IX, all the members are strictly belongs to the tribe Zanonieae of subfamily Zanonioideae and sharing common characters are small striate pollen grains, tendrils distally bifid, seeds mostly winged. The members of the clade-XII belong to the subfamily Cucurbitaceae and exhibiting commonly styles united into a single column. In clade-XIII, *Sechiopsis tetraptera*, *Sechium edule*, *Sicyos angulatus*, are closely related and strictly belongs to tribe Sicyeae and sharing common features like, trichomatous nectary, 4 to 10 colporate pollen grains, ovules solitary, pendulous, pollen spinulose, polyporate. The another member *Selysia prunifera* belongs to tribe Cucurbitaceae is also originated sisterly with above three Sicyeae tribal members, and their relationship is strongly supported by high bootstrap values (905%) and sharing some of its characters are receptacle tube usually relatively short, if long of male flowers then short in female flowers. Tendrils proximally 2 -7 fid or simple, seeds unwinged.

In case of clade-XIV, consists of total ten species strictly belongs to subfamily Cucurbitaceae, of which *Trichosanthes ovigera* and *T. kirilowii* belongs to subtribe Trichosantheae of tribe Trichosantheae, sharing pollen striate, smooth, flowers larger, stamens united with triplicate thecae. The members of the same clade like *Siraitia grosvenorii*, *Thladiantha davidii*, *Momordica foetida*, *M. calantha* belongs to tribe Joliffieae sharing some common characters are petals fringed or with ventral scales, in addition to receptacle tube usually relatively short, if long in male flowers then short in female flowers. The members of the *Austrobryonia pilbarensis*, *A. micrantha* and *A. argillicola* exhibiting highest level of similarity (both) morphologically, location and sequence similarity.

In assessing the results presented here, are compared with the most recent system of classification of the family *Cucurbitaceae* as given by Charles Jeffrey as of 1990 [18 - 19]. This new technology affords the opportunity to apply methods of molecular analysis, especially DNA sequencing to Plant systematics.

The *matK* gene is an ORF (open reading frame) embedded in the intron of the *trnK* gene coding the transfer RNA for lysine (tRNA^{Lys}^{UUU}). The exon of the *trnK* gene consists of only 70 base nucleotides and is divided into two parts by a long intron of about 2500 bp in length [20]. Based on the phylogenetic analysis, the *matK* gene showed higher support for individual clades, subclades and better resolution within the family *Cucurbitaceae* than that obtained from *rbcL* [11]. The plastid gene *matK* has proven useful in addressing Phylogenetic relationships at a variety of taxonomic levels within a number of taxonomic groups.

CONCLUSION

Overall results of the present studies clearly indicated that, the amount of sequence divergence exhibited by the *matK* is sufficient and appropriate for addressing the evolutionary relationships within the family *Cucurbitaceae*. Here *matK* gene sequences provided valuable information for phylogenetic relationships among the different taxa included here. The members clustered into different clades and subclades based on sequence similarity are also strongly correlating the evolutionary relationships exhibited at generic, suprageneric and interfamilial levels as the classification made based on the morphological similarities as well as other traditional methods in the family *Cucurbitaceae*. Thereby, it is confirmed that, these molecular analysis further strengthening the traditional method of systematic classification. Because of the few sequences analyzed, the subfamilial relationships within the *Cucurbitaceae* could not be inferred conclusively that, *matK* gene is a good candidate for DNA barcoding of Cucurbitaceae family members. It is hoped that, the results of this study and its interpretation will stimulate future research.

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Table: List of the matK nucleotide sequences for analysis of phylogenetic relationships in the family Cucurbitaceae.

Sl. No	GenBank Accession Number	No of base pairs	TAXA USED	REFERENCES
01	DQ491025	1219	<i>Helmontia leptantha</i>	Kocyan <i>et al.</i> , (2007)
02	DQ491024	1234	<i>Zombitsia lucorum</i>	Kocyan <i>et al.</i> , (2007)
03	DQ491023	1206	<i>Tricyclandra landrii</i>	Kocyan <i>et al.</i> , (2007)
04	DQ491022	1231	<i>Thladiantha hookeri</i>	Kocyan <i>et al.</i> , (2007)
05	DQ491021	1264	<i>Telfairia pedata</i>	Kocyan <i>et al.</i> , (2007)
06	DQ491020	1263	<i>Telfairia occidentalis</i>	Kocyan <i>et al.</i> , (2007)
07	DQ491019	1232	<i>Momordica charantia</i>	Kocyan <i>et al.</i> , (2007)
08	EU155611	1146	<i>Trichosanthes subvelutina</i>	Schaefer <i>et al.</i> , (2008)
09	EU155610	1149	<i>Trichosanthes schlechteri</i>	Schaefer <i>et al.</i> , (2008)
10	EU155609	1146	<i>Trichosanthes cucumerina</i>	Schaefer <i>et al.</i> , (2008)
11	EU155608	1146	<i>Trichosanthes bracteata</i>	Schaefer <i>et al.</i> , (2008)
12	EU155607	1146	<i>Hodgsoniaheteroclita</i>	Schaefer <i>et al.</i> , (2008)
13	EU155606	1149	<i>Gymnopetalum chinense</i>	Schaefer <i>et al.</i> , (2008)
14	EF066331	1128	<i>Ampeloscycios scandens</i>	Kocyan <i>et al.</i> , (2007)
15	DQ521608	1251	<i>Ampeloscycios humblotii</i>	Kocyan <i>et al.</i> , (2007)
16	EU590121	1227	<i>Papuascycosm papuanus</i>	Schaefer <i>et al.</i> , (2009)
17	EU541412	1256	<i>Zehneria anomala</i>	Schaefer <i>et al.</i> , (2009)
18	EU436422	1143	<i>Zanonia indica</i>	Schaefer <i>et al.</i> , (2009)
19	EU436420	1149	<i>Trochomerina polymorpha</i>	Schaefer <i>et al.</i> , (2009)
20	EU436419	1122	<i>Sicyos hillebrandii</i>	Schaefer <i>et al.</i> , (2009)
21	EU436418	1149	<i>Puthalicia egladulosa</i>	Schaefer <i>et al.</i> , (2009)
22	EU436417	1137	<i>Pseudosicydium acariianthum</i>	Schaefer <i>et al.</i> , (2009)
23	EU436416	1194	<i>Neosalsomitra trifoliolata</i>	Schaefer <i>et al.</i> , (2009)
24	EU436415	1194	<i>Neosalsomitra stephensiana</i>	Schaefer <i>et al.</i> , (2009)
25	EU436414	1047	<i>Neosalsomitra stephensiana</i>	Schaefer <i>et al.</i> , (2009)
26	EU436413	1146	<i>Neosalsomitra integrifoliola</i>	Schaefer <i>et al.</i> , (2009)
27	EU436412	1146	<i>Neosalsomitra angustipetala</i>	Schaefer <i>et al.</i> , (2009)
28	EU436411	1125	<i>Muelleragia jeffreyana</i>	Schaefer <i>et al.</i> , (2009)
29	EU436407	1134	<i>Hemsleya aff ambilis</i>	Schaefer <i>et al.</i> , (2009)
30	EU436406	1122	<i>Gomphogyne cissiformis</i>	Schaefer <i>et al.</i> , (2009)
31	EU436409	1149	<i>Luffa graveolens</i>	Schaefer <i>et al.</i> , (2009)
32	EU436408	1149	<i>Luffa echinata</i>	Schaefer <i>et al.</i> , (2009)
33	EU436405	1146	<i>Gomphogyne cirromitrata</i>	Schaefer <i>et al.</i> , (2009)
34	EU436402	1146	<i>Cucumeropsis manii</i>	Schaefer <i>et al.</i> , (2009)
35	EU436401	1152	<i>Corallocarpus triangularis</i>	Schaefer <i>et al.</i> , (2009)
36	EU436400	1064	<i>Cogniauxia trilobata</i>	Schaefer <i>et al.</i> , (2009)
37	EU436399	1159	<i>Alanthosicyos naudinianus</i>	Schaefer <i>et al.</i> , (2009)
38	EU436398	1149	<i>Apodanthera sagittifolia</i>	Schaefer <i>et al.</i> , (2009)
39	EU436397	1149	<i>Anangia microsepala</i>	Schaefer <i>et al.</i> , (2009)
40	EU037012	1158	<i>Trichosanthes ovigera</i>	Schaefer <i>et al.</i> , (2008)
41	EU037005	1393	<i>Trichosanthes villosa</i>	Schaefer <i>et al.</i> , (2008)
42	EU037001	1175	<i>Trichosanthes amara</i>	Schaefer <i>et al.</i> , (2008)
43	EF487562	1175	<i>Neoachmandra cunninghamii</i>	Schaefer <i>et al.</i> , (2008)
44	EF487561	1144	<i>Momordica colchinensis</i>	Schaefer <i>et al.</i> , (2008)
45	EF487560	1154	<i>Austrobryonia pilbaren</i>	Schaefer <i>et al.</i> , (2008)
46	EF487559	1154	<i>Austrobryonia micrantha</i>	Schaefer <i>et al.</i> , (2008)
47	EF487557	1148	<i>Austrobryonia centralis</i>	Schaefer <i>et al.</i> , (2008)
48	EF487556	1154	<i>Austrobryonia argillicola</i>	Schaefer <i>et al.</i> , (2008)
49	DQ536755	1103	<i>Zygosicyos tripartitus</i>	Kocyan <i>et al.</i> , (2007)
50	DQ536754	1156	<i>Zehneria bodinieri</i>	Kocyan <i>et al.</i> , (2007)
51	DQ536753	1157	<i>Neoachmandra japonica</i>	Kocyan <i>et al.</i> , (2007)
52	DQ536752	1157	<i>Neoachmandra indica</i>	Kocyan <i>et al.</i> , (2007)
53	DQ536751	1145	<i>Scopellaria marginata</i>	Kocyan <i>et al.</i> , (2007)
54	DQ536750	1151	<i>Xerosicyos pubescens</i>	Kocyan <i>et al.</i> , (2007)
55	DQ536746	1160	<i>Trochomeriopsis diversifolia</i>	Kocyan <i>et al.</i> , (2007)

56	DQ536745	1148	<i>Trochomerina macrocarpa</i>	Kocyan <i>et al.</i> , (2007)
57	DQ536740	1051	<i>Thladiantha dubia</i>	Kocyan <i>et al.</i> , (2007)
58	DQ536688	1154	<i>Trichosanthes pubera</i>	Kocyan <i>et al.</i> , (2007)
59	DQ536742	1154	<i>Trichosanthes kirilowii</i>	Kocyan <i>et al.</i> , (2007)
60	DQ536739	1154	<i>Thladiantha davdii</i>	Kocyan <i>et al.</i> , (2007)
61	DQ536737	1119	<i>Solena heterophylla</i>	Kocyan <i>et al.</i> , (2007)
62	DQ536736	1154	<i>Siraitia grosvenorii</i>	Kocyan <i>et al.</i> , (2007)
63	DQ536735	1151	<i>Siolmatra brasiliensis</i>	Kocyan <i>et al.</i> , (2007)
64	DQ536734	1090	<i>Sicyosperma gracile</i>	Kocyan <i>et al.</i> , (2007)
65	DQ536733	1122	<i>Sicyos baderoa</i>	Kocyan <i>et al.</i> , (2007)
66	DQ536732	1130	<i>Sicyos angulatus</i>	Kocyan <i>et al.</i> , (2007)
67	DQ536731	1142	<i>Sicydium tamnifolium</i>	Kocyan <i>et al.</i> , (2007)
68	DQ536730	1142	<i>Syidium diffusum</i>	Kocyan <i>et al.</i> , (2007)
69	DQ536729	1154	<i>Sciana odorifera</i>	Kocyan <i>et al.</i> , (2007)
70	DQ536728	1130	<i>Selysia prunifera</i>	Kocyan <i>et al.</i> , (2007)
71	DQ536727	1130	<i>Sechium edule</i>	Kocyan <i>et al.</i> , (2007)
72	DQ536726	1130	<i>Sechiopsis tetraptera</i>	Kocyan <i>et al.</i> , (2007)
73	DQ536725	1145	<i>Schizocarpum palmeri</i>	Kocyan <i>et al.</i> , (2007)
74	DQ536724	1090	<i>Rytidostylis cliata</i>	Kocyan <i>et al.</i> , (2007)
75	DQ536717	1147	<i>Polyclathra cucumerina</i>	Kocyan <i>et al.</i> , (2007)
76	DQ536719	1157	<i>Praecitrullus fistulosus</i>	Kocyan <i>et al.</i> , (2007)
77	DQ536721	1150	<i>Psiguria umbrosa</i>	Kocyan <i>et al.</i> , (2007)
78	DQ536722	1121	<i>Pteropepon parodii</i>	Kocyan <i>et al.</i> , (2007)
79	DQ536716	1037	<i>Peponopsis adharens</i>	Kocyan <i>et al.</i> , (2007)
80	DQ536715	1157	<i>Peponium vogelii</i>	Kocyan <i>et al.</i> , (2007)
81	DQ536714	1157	<i>Peponium caledonicum</i>	Kocyan <i>et al.</i> , (2007)
82	DQ536707	1153	<i>Neosalsomitra clavigera</i>	Kocyan <i>et al.</i> , (2007)
83	DQ536708	1122	<i>Neosalsomitra schefferiana</i>	Kocyan <i>et al.</i> , (2007)
84	DQ536709	1129	<i>Neosalsomitra suberosa</i>	Kocyan <i>et al.</i> , (2007)
85	DQ536703	1154	<i>Momordica foetida</i>	Kocyan <i>et al.</i> , (2007)
86	DQ536702	1154	<i>Momordica calantha</i>	Kocyan <i>et al.</i> , (2007)
87	DQ536700	1162	<i>Melothrianthus smilacifolius</i>	Kocyan <i>et al.</i> , (2007)
88	DQ536698	1157	<i>Marah fabaceus</i>	Kocyan <i>et al.</i> , (2007)

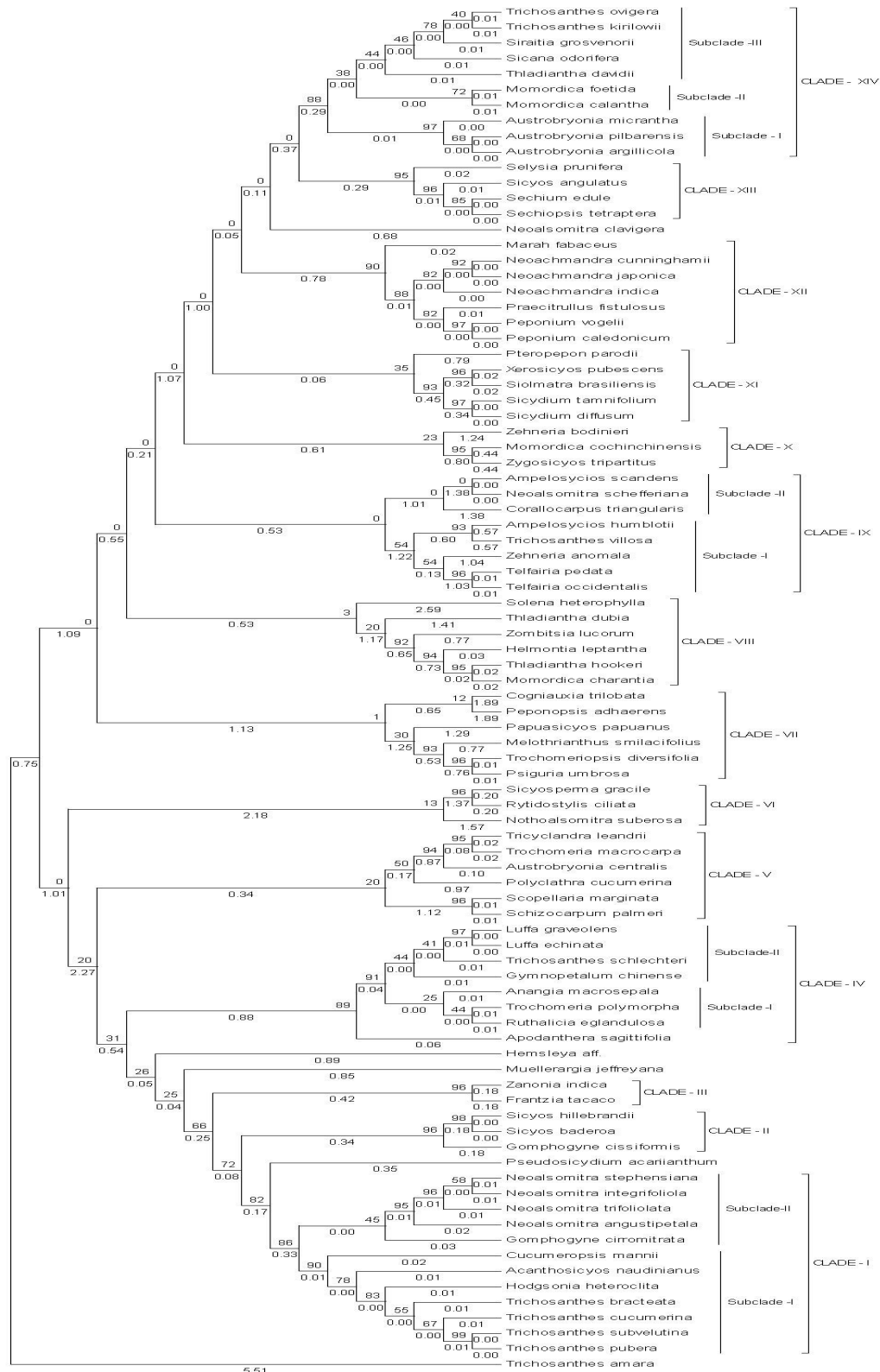


Fig -1 Phylogenetic tree from matK sequences representing 88 taxa of family *Cucurbitaceae*, were inferred using UPGMA method. Bootstrap percentages and branch lengths are indicated above and below the branches, respectively.

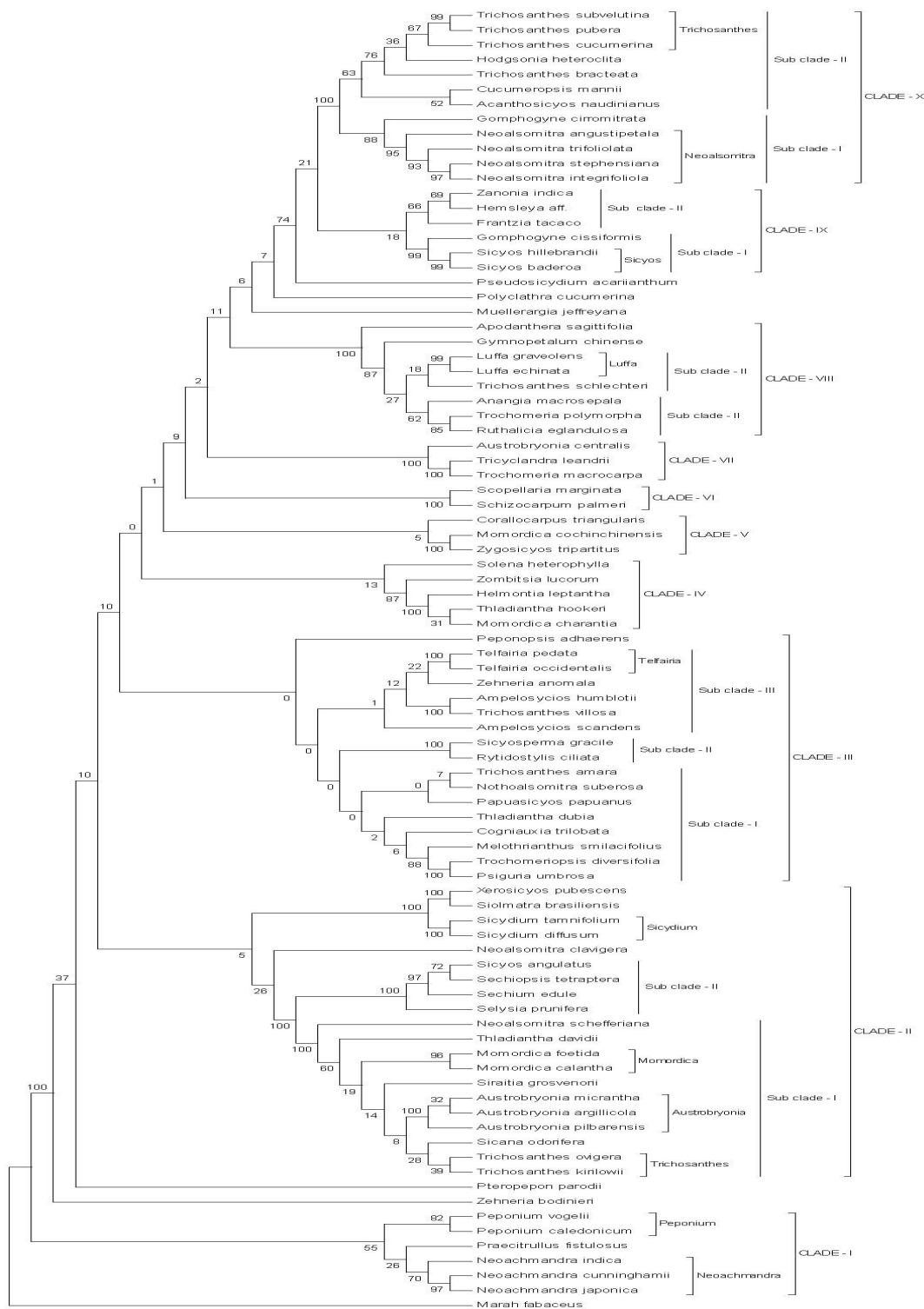


Fig -2 Combined Phylogenetic tree of family *Cucurbitaceae* (Evolutionary relationships of 88 taxa were inferred using Maximum Parsimony method. The length of the tree is 18473 is shown. The consistency index is 0.161641, the retention index is 0.657458 and the rescaled consistency index for all sites is 0.106272, . All positions containing gaps and missing data were eliminated from the dataset. There were a total of 1000 residue were used to construct a phylogenetic tree, out of which 257 are invariants and potentially informative