

## Cladistic Analysis of Morpho-anatomical Data of Subtribe Coelogyninae of Nepal Himalaya

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### Abstract

A cladistic analysis of subtribe Coelogyninae based on 10 morphological and 19 anatomical characters of 20 species of *Coleogyne*, *Pholidota*, *Panisea* and *Pleione* from Nepal was done. Most parsimonious tree were traced using computer assisted software NONA, Winclada (123 trees with CI = 35 and RI = 57). The analysis confirms the monophyletic origin of the subtribe Coelogyninae and species assorted into two different clades. Clade I is separated by round to oval mesophyll cell shape, such as in the species of *Otochilus*, *Panisea* and *Pleione*. Whereas the clade II is separated by stomatal index value 10-15. The results of cladistic analysis suggest that the subtribe Coelogyninae is monophyletic.

**Keywords:** Anatomical character, Cladistic, Coelogyninae, Morphological

### Introduction

Subtribe Coelogyninae (Orchidaceae) is a significant group belonging under the sub-family Epidendroideae, tribe Arethuseae and subtribe Coelogyninae (Chase et al., 2015). Subtribe Coelogyninae was placed under the tribe Epidendreae by Bentham and Hooker (1883). King and Pantling (1898) also included the member of this subtribe under the tribe Epidendreae. Banerjee and Pradhan (1984) followed the classification of Schlechter (1927) in which subtribe Coelogyninae has been placed under subfamily Monandreae, division Acrotonae, tribe Kerosphaeroideae and series Acranthae. Deva and Naithani (1986) included this subtribe in subfamily Epidendroideae and tribe Epidendreae. Rasmussen (1995) gave the classification of orchids and placed the subtribe under the subfamily Epidendroideae and tribe Coelogyneae. Dressler (1981, 1993) has also classified family Orchidaceae and kept the subtribe Coelogyninae in tribe Coelogyneae and subfamily Epidendroideae. Pearce and Cribb (2002) included this subtribe under subfamily Epidendroideae and tribe Coelogyneae. Taxonomically, this subtribe has been divided and subdivided by numerous taxonomists with different approaches. Bentham (1881) divided the subtribe Coelogyninae into 14 genera including *Coelogyne*, *Otochilus* and *Pholidota*. Kraenzlin (1911) divided it into 15 genera

including *Coelogyne*, *Otochilus*, *Pholidota*, *Panisea*, *Pleione* and *Neogyne*. Butzin (1992) divided it into 15 genera and Gravendeel (2000) divided it into 12 genera including *Coelogyne*, *Otochilus*, *Panisea* and *Pleione*, in which *Neogyne* and *Pholidota* are placed under *Coelogyne*. Out of the total 20 genera as described by Dressler, Nepal harbors only six genera, *Coelogyne*, *Neogyne*, *Otochilus*, *Panisea*, *Pholidota* and *Pleione* (Bajracharya & Shakya, 2002; Hara et al., 1978; Press et al., 2000; Shrestha et al., 2022). The subtribe Coelogyninae includes genus *Aglossorrhyncha*, *Bletilla*, *Bracisepalum*, *Bulleyia*, *Chelonistele*, *Coelogyne*, *Dendrochilium*, *Dichasonia*, *Dilochia*, *Entomophobia*, *Geesinkorchis*, *Glomeria*, *Gynoglottis*, *Ischogyne*, *Nabalua*, *Neogyne*, *Otochilus*, *Panisea*, *Pholidota*, *Pleione*, and *Thunia* (Chase et al., 2015).

Cladistic is accepted as the best method available for phylogenetic analysis as it provides specific and assessable hypothesis of organism relationships. Cladistic is a method of hypothesizing relationships among organisms and in other words a method of reconstructing evolutionary trees. Hennig (1950) published a short book in which he proposed the basic ideas that revolutionized systematics and launched the new science of cladistics. Cladistics takes its name from Hennig's concept of a "clade", which he defined as a group of organisms related

by common descent. The original methods used in cladistic analysis are derived from the work of Hennig (1966) who referred cladistics as phylogenetic systematics. Hennig (1966) argued that classification should reflect the branching pattern of evolution rather than degree of advancement and divergence. He also stated that only such groups are strictly monophyletic, composing ancestral taxon and all of its descendants should be recognized. Sokal and Sneath (1963) mentioned “cladistics relationship refers to the paths of the ancestral lineages and there described the sequence of branching of the ancestral lines”. According to Stuessy (1990), cladistics can be defined as the concepts and methods for determination of branching of evolution.

In the phylogenetic systematics, various characteristics of organisms are referred as “characters” and newly evolved characters are called “derived characters” (Apomorphy). A group composed of an ancestral and all of its descendants are known as monophyletic group, which is recognized on the basis of shared derived characters (synapomorphy). Synapomorphy arose in the ancestral group and are present in all of its members. The evolutionary relationship in general is observed by constructing a phylogenetic tree called as cladogram. The tree should be rooted in order to polarize all the character changes i.e., more recent characters and ancestral characters. Cladograms are commonly rooted using an out-group (Judd et al., 1999).

The basic concept of cladistic is that members of a group share a common evolutionary history which is closely related more to members of same group than other organisms. These shared derived characters are called synapomorphies. Synapomorphies arise in ancestral group and are present in its all members but absent in distant ancestors. The change in characteristic of organisms over time is the most important assumption in cladistics. When there is change in such characteristic, we are able to recognize different lineages or groups. The original state of characteristic is called plesiomorphic and the changed state is apomorphic. The terms primitive and derived have also been used for these terms.

Application of cladistic principle to the study of generic and species relationship is relatively a recent phenomenon. The goal of phylogenetic analysis is to produce classifications that correspond to monophyletic groups and thus convey the maximum amount of information.

Cladistics is a hypothetical relationship among taxa and considered as an alternative method of classification. Classifications based on relative positions of the divisions (branching) lines of descent, which is determined by appropriate study of characters ignoring their similarity or dissimilarity. It requires knowledge or assumptions as to which are ancestral and which are derived states of characters. Hence it is also called as phylogenetic system by several proponents (Hennig, 1966; Michener, 1970).

Until now there has been no morphological or anatomical cladistics analysis of subtribe Coelogyntinae performed in the context of Nepal. Bajracharya (2003) and Bajracharya & Shrestha (2004) performed a cladistic analysis of the genus Himalayan *Eria* on the basis of 35 morphological, three anatomical and two cytological characters scored from 40 species of Himalayan *Eria*. Subedi (2003) performed morphological phylogenetic analysis of genus *Smalix* of Nepal Himalaya. Mishra (2007) performed morphological phylogenetic analysis of genus *Hedychium* of Nepal Himalaya. Pathak (2018) performed morphological phylogenetic analysis of genus *Chremanthodium* of Nepal Himalaya. Subedi (2003), Mishra (2007) and Pathak (2018) confirmed the respective genera as monophyletic on the basis of morphological characters Pradhan and Bajracharya (2020) performed cluster analysis of *Dendrobium* of Nepal Himalaya based on morphological anatomical character of the species in which 104 characters were considered. Among 104 characters, 60 characters are based on anatomical characters. It shows that morphological and anatomical characters are most significant for solving taxonomic problems.

Present paper attempts a morpho-anatomical cladistic analysis of subtribe Coelogyntinae. With the variation in the anatomical characters of roots and leaves, this analysis presents the interrelationship among the closely related taxa within the species and the genera consisted in subtribe Coelogyntinae.

## Materials and Methods

The sampling included 20 species of subtribe Coelogyne and its acronym (Table 1 and 2). The endemic species are not included in this analysis. The member of subtribe showed considerable variation in the morphological as well as anatomical characters. The analysis presents the interrelationship among the closely related taxa. The morphological characters for outgroup were sampled from the subtribe Blettiinae and genus *Phaius* (Pearce & Cribb, 2002). The characters that are used is given in Table 3.

The morpho-anatomical characteristics of the genus were taken from the living and preserved specimens and the characters were coded for analysis. The character codes are shown in Table 3 and the data matrix is shown in Table 4.

Data matrix for cladistic analysis was generated in computer using NONA, Winclada version 1.00.08 developed by K. C. Nixon (1999-2004). The cladistic analysis was performed with the help of closely related taxa *Phaius* as outgroup and 20 species within the subtribe (Table 1). The data coding was carried out selecting the gross anatomical and morphological characters on the basis of their

**Table 2:** Species included and their acronyms for cladistic analysis of subtribe Coelogyne

S.N.	Botanical name	Acronym
1.	<i>Phaius</i>	<i>phaiu</i>
2.	<i>Coelogyne corymbosa</i>	<i>C. corym</i>
3.	<i>C. cristata</i>	<i>C. crist</i>
4.	<i>C. flaccida</i>	<i>C. flacc</i>
5.	<i>C. flavida</i>	<i>C. flavi</i>
6.	<i>C. fuscescens</i>	<i>C. fusce</i>
7.	<i>C. nitida</i>	<i>C. nitid</i>
8.	<i>C. ovalis</i>	<i>C. ovali</i>
9.	<i>C. stricta</i>	<i>C. stric</i>
10.	<i>Otochilus albus</i>	<i>O. albus</i>
11.	<i>O. fuscus</i>	<i>O. fuscus</i>
12.	<i>O. porrectus</i>	<i>O. porre</i>
13.	<i>Panisea demissa</i>	<i>P. demis</i>
14.	<i>P. uniflora</i>	<i>P. unifl</i>
15.	<i>Pholidota articulata</i>	<i>P. artic</i>
16.	<i>P. imbricata</i>	<i>P. imbri</i>
17.	<i>P. protracta</i>	<i>P. protr</i>
18.	<i>P. recurva</i>	<i>P. recur</i>
19.	<i>P. hookeriana</i>	<i>P. hooke</i>
20.	<i>P. humilis</i>	<i>P. humil</i>
21.	<i>P. praecox</i>	<i>P. praec</i>

variation. Each character is divided into different character states for character coding and multistate character coding was done. Those characters and corresponding states are mentioned in Table 3.

**Table 1:** Botanical name and specimens examined

S.N.	Botanical name	Specimen examined
1.	<i>Coelogyne corymbosa</i> Lindl.	Godavari, 1600m, 22.4.2000, D.M. Bajracharya, 316 (ASCOL)
2.	<i>Coelogyne cristata</i> Lindl.	Rajarani, Dhankuta, 500m, 4.6.2003, D.M. Bajracharya, 540 (ASCOL)
3.	<i>Coelogyne flaccid</i> Lindl.	Bhedetar, 1600m, 1.6.2003, D.M. Bajracharya, 503 (ASCOL)
4.	<i>Coelogyne flavida</i> Hook. f. ex Lindl.	Rajarani, Morang, 500m, 14.1.2001, D.M. Bajracharya, 398 (ASCOL)
5.	<i>Coelogyne fuscescens</i> Lindl.	Bhedetar, 1600m, 1.6.2003, D.M. Bajracharya, 502 (ASCOL)
6.	<i>Coelogyne nitida</i> Lindl.	Okhare, Dhankuta, 1600m, 3.6.2001, D.M. Bajracharya, 518 (ASCOL)
7.	<i>Coelogyne ovalis</i> Lindl.	Rajarani, Morang, 500, 14.1.2001, D.M. Bajracharya, 380 (ASCOL)
8.	<i>Coelogyne stricta</i> (D. Don) Schlechter	Godavari, 1600m, 2.5.2000, D.N. Bajracharya, s.n. (ASCOL)
9.	<i>Otochilus albus</i> Lindl.	Hanspokhari, 8000ft, 7.6.1978, P. Pradhan and R. Niraula 469, (KATH).
10.	<i>Otochilus fuscus</i> Lindl.	Bhadaure to Deurali Village, 1800m, 15.1.2001, Subedi 863, (TUCH)
11.	<i>Othochilus porrectus</i> Lindl.	LeleBhanjyang, 7000ft, 27.10.1978, P. Pradhan 661 (KATH)
12.	<i>Panisea demissa</i> (D.Don.) Pfitz.	PanchaseDanda, 2300m, 11.2.2002, Subedi, Chaudhari and Shakya, 1012, (TUCH)
13.	<i>Panisea uniflora</i> (Lindl.) Lindl.	Phulchoki, 7500 ft, 19.4.1978, P. Pradhan 405 (KATH)
14.	<i>Pholidota articulata</i> Lindl.	Letang, Morang, 500m, 8.6.2003, D.M. Bajracharya, 598 (ASCOL)
15.	<i>Pholidota imbricata</i> Hook.	Telok, 5500 ft, 28.6.1969, T. B. Shrestha 15958, (KATH)
16.	<i>Pholidota protracta</i> Hook. f.	Panchase forest, Kaski, 2300m, 15.8.1999, Subedi, A. 251 (TUCH)
17.	<i>Pholidota recurva</i> Lindl.	Makawanpur, 1600m, 24.2.1992, K. J. White, 22 (KATH)
18.	<i>Pleione hookeriana</i> (Lindl.) J. Moore	Maily, Okhaldhunga, 1900m, 28.5.1979, N. P. Manandhar and M. K. Adhikari 1842, (KATH)
19.	<i>Pleione humilis</i> (Sm.) D. Don,	Daman, 2400m, 15.5.2003, D. M. Bajracharya, 486 (ASCOL)
20.	<i>Pleione praecox</i> (Sm.) D. Don	Daman, 2400m, 15.5.2003, D. M. Bajracharya, 490 (ASCOL)

**Table 3:** Characters and character states used in cladistic analysis

Character	Character states		
Leaf texture	(0) coriaceous	(1) membranous	
Stomata type	(0) tetracytic	(1) anomocytic	(2) both
No. of stomata/ sq. mm	(0) 40-80	(1) 80-120	(2) >120
Stomata index	(0) 5-10	(1) 10-15	(2) >15
Strand and interstrand	(0) Not differentiated	(1) Slightly differentiated	(2) Differentiated
Leaf epidermal cell shape	(0) rectangular	(1) polygonal	(2) both
Hypodermis in leaf	(0) absent	(1) present	
No. of mesophyll layer in leaf	(0) <5	(1) 5-10	(2) >10
Mesophyll cell shape	(0) round to oval	(1) oval	
Shape of midrib bundle	(0) round	(1) oval	(2) conical
Fibre cap on midrib bundle	(0) U or V shaped	(1) surrounded	
No of phloem patch in midrib	(0) 1	(1) 1-3	(2) >3
Root hairs	(0) absent	(1) present	
No. of velamen layer	(0) <3	(1) > 3	
Velamen cell type	(0) round to oval	(1) polygonal	
Cortical cell layers	(0) < 5	> 5	
U-shaped endodermal thickening	(0) absent	(1) present	
No. of vascular strand	(0) < 10	(1) > 10	
Habitat	(0) epiphytic	(1) epiphytic +lithophytes	(2) Terrestrial
Stem	(0) Jointed	(1) Pseudobulbs clustered	(2) pseudobulb apart
Shape of pseudobulb	(0) ovoid	(1) cylindrical	
No. of leaves in pseudobulb	(0) 1	(1) 2	
Leaf petiole	(0) sessile	(1) subsessile	(2) petiole
Inflorescence origin	(0) apex of pseudobulb	(1) base of pseudobulb	(2) top of immature leaves
Inflorescence type	(0) heteranthous	(1) proteranthous	(2) synanthus
Inflorescence position	(0) erect	(1) semierect	(2) pendulous
No. of flower in inflorescence	(0) < 5	(1) > 5	
Floral bracts	(0) persistent	(1) deciduous	

### Phylogenetic analysis

The phylogenetic analysis bootstrap and Jackknife consensus were performed on the morphological data with NONA 2000 version (Nixon 1999-2004). All characters were analyzed by using Wagner parsimony as implemented in NONA (Goloboff, 1993). The heuristic search strategy was performed by random addition with ten replicates and TBR (tree bisection reconnection) swapping. The heuristic methods are generally performed when the number of samples in an analysis is very large. Heuristic methods generally use ‘hill climbing technique’ to find optimum cladogram. Two such techniques are stepwise addition and branch swapping. Stepwise addition process adds taxa to the developing cladogram in the initial building phase of an analysis. Initially, a cladogram of three taxa is chosen, then the fourth one is added to one of the three branches. A fifth taxon is then selected and added to the network followed by the rest of the taxa under study. If

random addition is one of the criteria of addition sequence of the remaining taxa, which can adopt a non-rigorous mean to evaluate the effectiveness of heuristic procedures. If a heuristics search is performed with 100 replicate random additions and the same set of most parsimonious tree is obtained each time, then one can be certain that these trees topology represent global optima for the given date set.

Branch swapping is another algorithm that can be used to optimize a cladogram, because manipulation of additional sequence alone generally yields only optimum. This can be done by performing a series of predefined arrangements of the cladograms in a way to find shorter tree topology (Kitching et al., 1998).

Branch swapping algorithms used in the analysis were T-tree bisection. As a measure of character fit, ensemble consistency (CI) and ensemble retention (RI) indices were calculated for the maximally

parsimonious tree by NONA. Bootstrapping (Felsenstein, 1985) was performed as a measure of confidence interval in the phylogenetic trees. Bootstrapping value was obtained from 1000 pseudo-replicates in a parsimony analysis using a heuristic search method of NONA. Bootstrapping is a statistical method, which involved resampling point with replacement, to generate a number of bootstraps sample of the same size as that of the original data set. Each of these replicates is analyzed and variation among the result and estimates considered for the indication of the size of the error in making phylogenetic estimates from the original data. The confidence of the particular clade increases with increasing bootstrap value.

## Results and Discussion

In total, 29 characters has been used for cladistic analysis, 19 characters are based on anatomical and remaining 10 characters are morphological. The data matrix included 29 phylogenetic informative characters of the subtribe Coelogyninae, which yield the maximum parsimonious trees (length = 123 trees with CI = 35 and RI = 57) from maximum tree hold of 10,000. A strict consensus tree the bootstrap consensus topology and corresponding support tree are present in Figure 2. Only the best tree was kept deleting all the suboptimal trees. The dots represent synapomorphies. Numbers above the bar corresponded characters states and below the bars correspond the character codes.

The analysis results in with length of 123 steps, consistency index 35 and retention index of 0.57. Most of the groups had high bootstrap percentage is 100% within the groups (Figure 2).

The cladistic analysis of subtribe Coelogyninae on the basis of anatomical and morphological characters gave a distinct picture of genus differentiation. The tree supported that subtribe Coelogyninae the paraphyly. The first major clade differentiated the genus *Otochilus*, *Panisea* and *Pleione* from other genera confirming it a monophyletic group. Within the genus *Pleione*, *P. hookeriana* and *P. humilis* again form another group excluding *P. praecox*. This

is consistent with the classification of *Pleione* into different sections.

Similarly, the second major clade differentiated the remaining two genera comprising *Pholidota* and *Coelogyne* are again divided into two groups confirming each of them as polyphyletic (Figure 1).

The strict consensus, bootstraps and Jackknife analysis also shows that *Pholidota* is evolved separately whereas *Otochilus*, *Panisea* and *Pleione* evolved separately. Lastly *Coelognae* evolved separately. It shows that on the morphological as well as anatomical characters also help in the delimitation of subtribe too.

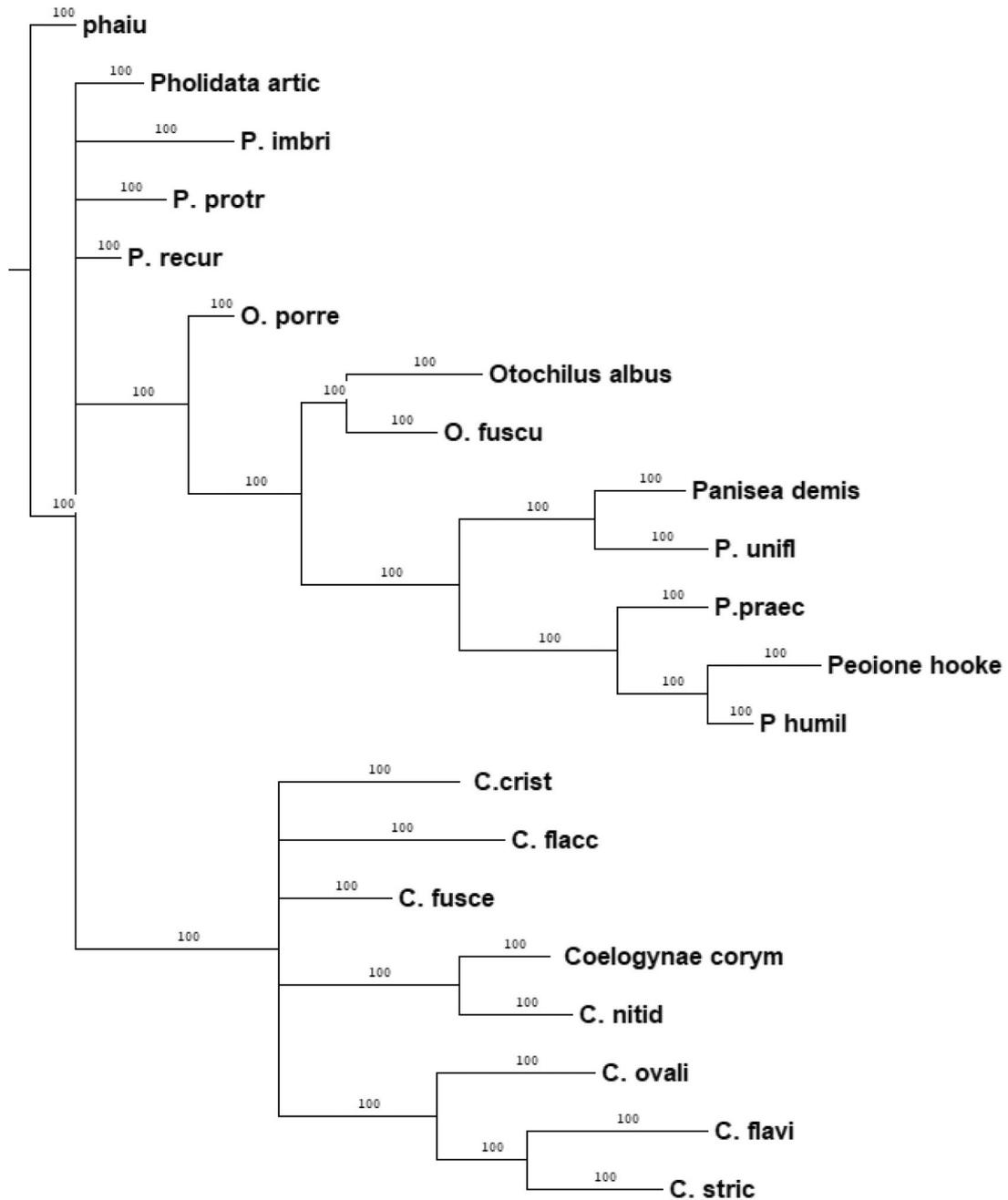
The retention index (0.57) suggested high level of homoplasy in data set resolution and nodal support as defined by the low synapomorphy (Figure 1 and 2), which may be due to number of taxa and characters. Many recent studies indicate that phylogenetic resolution percentage has been improved by directly combining different data sets (Chase & Cox, 1998; DeUeiroz et al., 1995; Wiens, 1998).

The consistency index of 35 represents a high level of homoplasy and may be function of rapid rate of evolution believed to have occurred in Orchidaceae (Dresslar, 1993). The high level of congruence among the anatomical data sets and low number of Maximum Parsimony Tree (MPT) and higher resolution in combined strengthen the confidence in the combined tree as good hypothesis of phylogenetic relationship of sub-tribe Coelogyninae and *Phaius*.

Present study supports the subtribe Coelogyninae as monophyletic origin, because bootstrap and jackknife support for the taxa is high (100%) and synapomorphies included stomata index and no. of phloem patch in midrib (Figure 2).

The cladistic analysis revealed two distinct trees evolved from morphological and anatomical characters of the species. The first clade supported by no. of phloem patch in midrib and second clade supported by stomata index. *Otochilus*, *Panisea* and *Pleione* were separated by the characters of mesophyll cell shape which is oval.





**Figure 2:** Cladistic analysis of subtribe Coelogninae (Bootstraps and Jackknife consensus of 6 trees from morphological and anatomical data are 100%)

**Conclusion**

The result of cladistic analysis suggested that subtribe Coelogninae is monophyletic. Restriction of subtribe Coelogninae has been divided into two clades -clade I and clade II.

Clade I is separated by round to oval mesophyll cell shape in the species of *Otochilus*, *Panisea* and *Pleione*, whereas in clade II of Coelogninae have been separated by the stomatal index value 10-15; with *Pholidota* and *Coelogyne* forming

individual clades respectively. The results of cladistic analysis suggest that the subtribe Coelogyninae is monophyletic.

### Author Contributions

All the authors have equal contribution for the preparation of this manuscript.

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