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# Systematic studies on some West African species of the Tribe Bauhinieae (Cercidioideae)

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**Abstract.** The tribe Bauhinieae is the largest and most taxonomically complex group within the subfamily Cercidoideae. They possess the most distinguishable morphological features but are the most variable group. Here, we explore the phylogenetic relationship of the tribe Bauhinieae using morphological, anatomical and molecular data (ITS, rbcL, trnL-F, ITS+rbcL+trnL-F). Relationships inferred from morphological, anatomical and molecular data revealed congruent result, a non-monophyletic *Bauhinia* and *Piliostigma* group. The leaf epidermal shape in all *Bauhinia* species examined are polygonal with straight cell walls except *B. tomentosa*, which has an undulating cell wall. Stomatal types observed vary between the two genera studied. *Bauhinia* species has paracytic stomata while *Piliostigma* species exhibited hemiparacytic stomata. Dendrogram generated revealed the interrelationship between the species at a distance value of 80. Bayesian analysis revealed a high resolution of species and posterior probability. The strict consensus tree for all the tested gene regions revealed a polyphyletic *Bauhina* divided into three major clades. The *Piliostigma* group exhibited a paraphyletic and polyphyletic relationship within the *Bauhinia* group at high support values. *B tomentosa* exhibited a closer relationship with *Piliostigma* species. These results support the proposition to divide members of the large *Bauhinia* s.s group into subclades. This study has attempted to elucidate the unresolved species and genus level taxonomy of the tribe Bauhinieae. However, more variable gene regions in addition to broader species sampling should be considered for further phylogenetic patterns of this taxon.

**Keywords:** *Bauhinia*, *Piliostigma*, ITS, *rbcL*, *trnL-F*, molecular phylogeny, plant anatomy.

## INTRODUCTION

The plant family Fabaceae is the third largest angiosperm family only after Orchidaceae and Asteraceae. They vary in habit from herbs to shrubs, vines, lianas, and trees, with an extremely high diversity of 651 living genera and 19,500 species across different habitats of the world (Wang et al. 2014). The family was formerly divided into three subfamilies, Mimosideae, Ceasalpinioideae and Papilionoideae. Upon recent reclassification, the family is now divided into 6 sub-families (LPWG 2017): a recircumscribed Caesalpinioideae DC., Cercidoideae Legume Phylogeny Working Group (stat. nov.), Detari-

oideae Burmeist., Dialioideae Legume Phylogeny Working Group (stat. nov.), Duparquetioideae Legume Phylogeny Working Group (stat. nov.), and Papilionoideae DC. Since then, studies are ongoing to revise and ratify the classification of the new subfamilies (Estrella et al. 2018). Amongst the sub family Cercidoideae, the taxonomic and phylogenetic relationships among members of the tribe Bauhinieae has remain challenging and been the subject of recent studies (Zhang 1995; Sinou et al. 2020).

The tribe Bauhinieae possess the most distinguishable morphological features but are the most variable group among the cercidoideae (Meng et al. 2014), owing to their bilobate, bifoliolate, or unifoliolate pulvinate leaves with basal actinodromous or acrodromous venations. They exhibit seeds with a crescent-shaped hilum and an aril-lobed funiculus (Wunderlin et al. 1987; Sinou et al. 2020); leaves are mostly simple (entire to bilobed) or bifoliolate with zygomorphic flowers. Specifically, these species exhibit wide-ranging distribution and eclectic morphological variability (Hao et al. 2003; Sinou et al. 2009). Members of the tribe Bauhinieae are disjunctly distributed in tropical and sub-tropical regions of Africa such as Sudan, Ivory Coast, South Africa, Algeria and even in Eastern and South-Western Nigeria. They are found in nearly all ecosystems, including forests (Amazonian, Atlantic, gallery forests), savannas (cerrados, campos rupestres) and caatinga (dry deciduous forest of the semi-arid Brazilian Northeast). The formerly recognized tribe Cercioideae (now subfamily Cercidioideae) was divided into the subtribes Cercidiinae and Bauhiniinae (now elevated to tribal rank). Across West African countries, the former comprises the genera *Cercis*, *Adenolobus* and *Griffonia* while the subtribe Bauhiniinae contains the genera *Bauhinia*, *Barklya*, *Brenierea*, *Gigasiphon*, *Lysiphyllum*, *Phanera*, *Piliostigma* and *Tylosema* (Wunderlin et al. 1987), out of which *Bauhinia* and *Piliostigma* are the only West African species. Many members are of huge economic import (Burkhil 2000), usually cultivated as ornamental trees worldwide and known to be medicinally significant. Species can be used for prevention of tumours, are antihaemorrhagic, control levels of glucose in the blood, and used for the treatment of constipation and other gastro-intestinal infections (Larsen and Larsen 1991; Duarte-Almeida et al. 2015).

According to LPWG (2017), the taxonomic history and classification of the *Bauhinia* group in its broadest circumscription comprising about 300 to 350 species is likewise complex and particularly difficult to delimit. It is the largest and most taxonomically complex group within the subfamily Cercidoideae (Wunderlin et al. 1987). Within the Bauhinieae, an unresolved species- and genus-level taxonomy has hindered the understanding of the taxonomic significance of the varied morphological and anatomical features and been the subject of a number of regional studies (Wunderlin et al. 1987; Lewis and Forest 2005; Queiroz 2006; Vaz 2010; Wunderlin 2011). Until date, no comprehensive species-level overview has been published. Furthermore, previous analyses using morphological and molecular data suggest contrasting relationships with complicated and poorly resolved evolutionary relationships in this lineage (Bruneau et al. 2001; Sinou et al. 2009). The tribe is currently the subject of much phylogenetic research and combining both molecular and anatomical examination (Banks et al. 2014) will provide useful information on the diagnostic characters at generic and infrageneric taxonomic level. All of the previous phylogenetic studies have concluded that the *Bauhinia* group is non-monophyletic and represents an artificial grouping that could be divided into several genera (Bandyopadhyay and Ghoshal 2015; Mackinder and Clark 2014; Trethowan et al. 2015; Clark et al. 2017). However, Sinou et al. (2020) reported that the subtribe Bauhinieae is weakly supported as monophyletic based on plastid and duplicated nuclear gene sequences. The study portrayed a superfluous taxonomic relationship in the Cercidoideae. Hence, using a single and multi-tiered datasets of three different gene regions in addition to both micro and macro-morphological data, this study present a systematic studies of the tribe Bauhinieae so as to further elucidate on the body of knowledge surrounding this taxon group.

## MATERIALS AND METHODS

### *Plant material*

Twenty-two samples of *Bauhinia* and *Piliostigma* species representing 7 species were collected from selected sites in Nigeria in addition to the outgroup species *Detarium macrocarpum* Harms. The outgroup taxon was selected based on results of previous studies, which indicate members of the Detaroidieae is sister to the subfamily Cercidioideae (LPWG 2017). Additional sequences used were downloaded for GenBank. Collected samples were identified and authenticated at the University of Lagos Herbarium (LUH). The voucher number and other information about samples are given in Table 1.

### *Morphology*

A morphologically description of the species was done using their qualitative and quantitative characteristics. Observed qualitative characters include leaf apex,

**Table 1.** Details about the source of the plant samples used for the study.

| S/N | Plant species | Locality | GPS location | Collector's name | Collector's number | GenBank number (ITS) | GenBank number (rbcL) | GenBank number (trnL-F) |
|---|---|---|---|---|---|---|---|---|
| 1 | Bauhinia monandra Kurz. | Kamuku National Park, Kaduna State | 10°47'49"N 6°18'20"E | Dr. Aramide Igbari | LUH 9663 | KX057835 | KX119264 | KX268152 |
| 2 | *Bauhinia tomentosa* L. | Kainji National Park, Niger State | 9°59'56"N 4°17'10"E | Dr. Aramide Igbari | LUH 9664 | KX057838 | KX119268 | KX268155 |
| 3 | *Bauhinia rufescens* Lam. | Yankari Game reserve National Park, Kastina State | 9°45'24"N 10°30'34"E | Mr. Daramola | LUH 5124 | KX057837 | KX119266 | KX268154 |
| 4 | *Bauhinia purpurea* L. | Ahmadu Bello University, Zaria, Kaduna State | 11°15'12"N 7°64'46"E | Dr. Aramide Igbari | LUH 9675 | KX057836 | KX119265 | KX268153 |
| 5 | *Bauhinia vahlii* Wight & Arn. | Ahmadu Bello University, Zaria, Kaduna State | 11°15'12"N 7°64'46"E | Dr. Aramide Igbari | LUH 9664 | - | KX119267 | KX268137 |
| 6 | *Piliostigma thonningii* (Schum.) Milne-Redh. | Kainji National Park, Niger State | 9°59'56"N 4°17'10"E | Dr. Aramide Igbari | LUH 8518 | - | KX119320 | KX268205 |
| 7 | *Piliostigma reticulatum* (DC.) Hochst | Yankari Game reserve National Park, Kastina State | 9°45'24"N 10°30'34"E | Dr. Aramide Igbari | LUH 9684 | KX057894 | KX119319 | KX268204 |

leaf base, leaf venation, leaf shape, leaf margin while stem length, petiole length, leaf length, leaf are some of the quantitative characteristics recorded.

*Anatomy*

Dried specimens from median portion of the leaves near the midrib were carefully cut, and soaked in concentrated nitric acid inside McCartney bottles for about 2-6 hours to macerate the mesophyll and bleach the leaf portions. Tissues disintegration was noticed by bubbles and the epidermal layers were separated and transferred into petri dishes containing water for cleansing and then separated with forceps. Separated strips of adaxial and abaxial surfaces of the leaves were stained with safranin following standard protocols and viewed under the microscope following Ogundipe et al. (2009); Onuminya et al. (2020). The diagnostic features of the adaxial and abaxial surface of the leaves were photographed using Motic image plus version 2.0 mm with MC camera mounted on an Olympus compound light microscope at a magnification of 9600. In addition, the number, length and width of the stomata, and epidermal cells were recorded using a calibrated micrometer eyepiece.

*Statistical analysis*

The descriptive statistics of the mean, standard deviation, standard error, minimum and maximum value were calculated for all variables. The Stomata Index (S.I) was calculated using the formula of Metcalfe and Chalke (1979):

$$({}^{S}/_{S+E}) \times 100$$

Where, S denotes the number of stomata per unit area and E is the number of epidermal cells of the same area.

In addition, the sequential, hierarchical and nested (SAHN) clustering analysis was done using PAST V4.0 software package on both anatomical and morphological characters. Dendrograms were generated based on Nei genetic distances following Sneath and Sokal (1973).

*DNA extraction and amplification*

Total genomic DNA was isolated from approximately 0.0300 g of silica-gel dried and 0.0180 g of herbarium plant material following a modified 2X CTAB protocol of Doyle and Doyle (1987). Herbarium samples were precipitated for one week while silica dried for 1hr. Extracted DNA was stored at -20°C prior subsequent use. Polymerase chain reaction (PCR) was performed in 50 µl reaction mixtures containing 25 µl biomix, 1 µl BSA, 2 µl DMSO, 1.75 µl of 10 µM of each primer, 17.5 µl of millipore H2O and 1 µl of 30-50 ng template DNA. Primers according to Sun et al. (1994), Olmstead et al. (1992) and Taberlet et al. (1991) were used for ITS, rbcL and trnL-F regions respective-

**Table 2.** Amplification profiles.

| Region | Initial denaturing Temp./time | Denaturation Temp./time | Annealing Temp./time | Extension Temp./time | Final extension Temp./time | No. of cycles |
|---|---|---|---|---|---|---|
| ITS | 97°C/2:00 | 97°C/1:00 | 55°C/0:45 | 72°C/0:45 | 72°C/7:00 | 30 |
| *matK* | 94°C/5:00 | 94°C/0:40 | 48°C/0:40 | 72°C/0:40 | 72°C/7:00 | 30 |
| trnL-F | 94°C/2:00 | 94°C/1:00 | 55°C/1:00 | 72°C/2:00 | 72°C/10:00 | 30 |

ly. PCR profiles run for each region are given on Table 2. Amplifications were run on a Veriti® 96 well thermal cycler. Each PCR product was run on 1% agarose gel stained in ethidium bromide and successful amplified products were sent to Source Bioscience (UK) for bidirectional sequencing using the same primer used in PCR.

*Phylogenetic analysis*

Chromatographic traces and contiguous alignments were edited using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan). Any uncertain base positions, generally located close to the priming sites, and regions of uncertain alignment were excluded from the phylogenetic analysis. Sequences were aligned and edited in Bioedit (Hall 1999). Informative insertion/deletion events (indels) were identified and coded as binary characters, and gaps were treated as missing data. All three regions were analyzed separately. Less than 1% of the data were scored as missing. A Bayesian analysis (Ronquist et al. 2012) was carried out by first determining the optimal substitution model using MrModeltest v2.3 (Nylander 2004) and the Akaike information criterion. The general reversible model with a gamma shape (GTR+G) was selected for the nuclear ITS region, Hasegawa–Kishono–Yano with a proportion of invariable sites and gamma shape (HKY+I+G) for rbcL region and Hasegawa–Kishono–Yano with a gamma shape (HKY+G) for trnL-F region. Four discrete states were used for the gamma substitution. The data were therefore partitioned into two for the Bayesian analysis and the correct substitution model as specified by MrModeltest was specified for each partition. The partitions were unlinked so that each parameter could be specified separately. Analysis was run for 75,000,000 generations with sampling every 75,000 generations. The first 18,000,000 samples trees were discarded as 'burn in' while the remaining trees were used to build a 50% majority rule consensus tree with posterior probability for nodes.

## RESULTS

*Morphological and anatomical studies*

The qualitative and quantitative foliar morphological characters of the species (Fig. 1) are presented in Tables 3 and 4. All of the species examined have a bifoliate leaf shape except *Bauhinia purpurea* that has orbiculate leaves. Although, there were variations in lobe division of each leaf, their leaf base ranges between cordate and subcordate with entire leaf margin. A palmate reticulate leaf venation, leathery and glabrous leaf surface distinguishes members of *Philiostigma* from *Bauhinia* morphologically. Anatomically, there are variations in the epidermal cells of the species examined; all epidermal cells are polygonal with straight, curved, wavy or undulating anticlinal wall patterns. The two *Piliostigma* species has distinct anticlinal wall patterns (Tables 5 and 6). The stomata shape for *Bauhinia* species are paracytic while *Piliostigma* species possess hemiparacytic stomata. Observed foliar trichomes were mostly non-glandular (*Piliostigma* species and *B. vahlii* and *B. refuscens*), conical and unicellular (*Bauhinia purpurea*) and glandular, unicellular (*Bauhinia tomentosa*). Trichomes were present on both adaxial and abaxial leaf surfaces of the species (Fig. 2). Cluster analysis based on distance matrix revealed similarities and differences among the species. Dendrogram generated from both morphological and anatomical data obtained showed the interrelationship between the species studied at a distance value of 80 (Fig. 3). It revealed the closeness similarity of species to each other based on the examined features. *B. rufescens* showed to be closest to *B. vahlii*, *P. reticulatum* closest to *B. monandra* while *B. purpurea* is closest to *P. thonnongii*.

*Molecular studies*

The strict consensus tree for all the tested gene regions revealed a polyphyletic *Bauhina* group divided into three major clades. In the ITS gene tree, *B. tomentosa* is clustered with *Piliostigma reticulata* as well as


**Figure 1.** A = *Bauhinia monandra*, B- *Bauhinia tomentosa*; C = *Bauhinia rufescens*; D = *Bauhinia purpurea*; E = *Bauhinia vahlii*; F=*Piliostigma thonningii*; G = *Piliostigma reticulatum*.

**Table 3.** Qualitative foliar morphological characteristics of the selected species of the tribe Bauhinieae.

| Species | Leaf shape | Leaf apex | Leaf margin | Leaf base | Venation | Leaf surface |
|---|---|---|---|---|---|---|
| *Bauhinia monandra* | Bifoliate and folded in the centre | Rounded and split up to 1/3 leaf length | Entire | Sub cordate | Palmate | Glabrous |
| *Bauhinia tomentosa* | Bifoliate and elliptic | Acuminate and split up to ½ leaf length | Entire | Sub cordate | Palmate | Glabrous |
| *Bauhinia rufescens* | Bifoliate | Cordate rounded, and split up to ¾ leaf length | Entire | Sub cordate | Palmate | Glabrous |
| *Bauhinia purpurea* | Orbiculate | Emarginate | Cleft, lobed | Cordate | Palmate | Glabrous |
| *Bauhinia vahlii* | Bifoliate | Apiculate | Entire | Cordate | Palmate | Hairy |
| *Piliostigma thonningii* | Bifoliate | Acuminate and split up to 1/8 leaf length | Entire | Cordate | Palmate Reticulate | Leathery and finely pubescent beneath |
| *Piliostigma reticulatum* | Bifoliate | Rounded to cuneate | Entire | Cordate | Palmate reticulate | Leathery and glabrous beneath |

**Table 4.** Quantitative foliar morphological characteristics of the selected species of the tribe Bauhinieae Min (Mean ± S.E) Max.

| Species | Stem length (cm) | Leaf length (cm) | Leaf width (cm) | Leaf blade (cm) | Petiole length (cm) |
|---|---|---|---|---|---|
| *Bauhinia monandra* | 21.6 (23.1 ± 0.8) 25.4 | 8.2 (8.6 ± 0.2) 9.1 | 8.9 (9.6 ± 0.3) 10.2 | 29.8 (32.9 ± 0.8) 34.4 | 3.6 ( 3.8± 0.1 ) 4.0 |
| *Bauhinia tomentosa* | 39.9 ( 41.8 ± 0.8 ) 44.2 | 4.8 (5.3 ± 0.3) 6.5 | 4.9 (5.4 ± 0.2) 5.9 | 16.9 (18.8 ± 0.9) 21.8 | 1.7 (3.8 ± 0.1) 2.1 |
| *Bauhinia rufescens* | 39.9 (43.4 ± 0.9) 45.4 | 1.5 (1.6 ± 0.1) 1.8 | 1.9 (2.1 ± 0.1) 2.3 | 3.9 (4.5 ± 0.3) 5.7 | 0.6 ( 0.8 ± 0.1 ) 1.1 |
| *Bauhinia purpurea* | 47.2 (47.6± 0.7) 50.1 | 11.9 ( 13.1 ± 0.3) 13.8 | 10.8 (12.6 ± 0.7) 15.1 | 39.6 (42.2 ± 0.8) 44.7 | 3.5 (3.8 ± 0.1) 4.2 |
| *Bauhinia vahlii* | 38.4 (40.8 ± 0.9 ) 43.1 | 5.9 (6.5 ± 0.2 ) 7.1 | 10.9 ( 11.6 ± 0.3)12.3 | 17.9 (18.8 ± 0.3) 19.6 | 2.8 (3.1 ± 0.1) 3.5 |
| *Piliostigma thonningii* | 36.8( 38.5 ± 0.7)40.5 | 10.5 (10.8 ± 0.1 ) 11.2 | 11.9 (12.6 ± 0.2) 13.2 | 33.7 (36.6 ± 0.9) 38.7 | 3.6 (3.9 ± 0.1) 4.2 |
| *Piliostigma reticulatum* | 39.4 (40.3 ± 0.3) 41.1 | 5.9 (6.9 ± 0.4) 8.1 | 8.9 (11.5 ± 0.8) 13.2 | 19.1 (20.6 ± 0.5 ) 22.1 | 4.9 (5.9 ± 0.5) 7.3 |

**Table 5.** Qualitative Foliar Anatomical characteristics of the selected species of the tribe Bauhinieae.

| Species | Cell wall Shape | | Anticlinal Wall shape | | Stomata type | | Trichome type | |
|---|---|---|---|---|---|---|---|---|
| | Abaxial | Adaxial | Abaxial | Adaxial | Abaxial | Adaxial | Abaxial | Adaxial |
| *Bauhinia monandra* | Polygonal | Polygonal | Straight | Straight | Paracytic | None | Glandular | Glandular |
| *Bauhinia tomentosa* | Polygonal | Polygonal | Curved | Curved | Paracytic | None | Glandular, Unicellular | Glandular, Unicellular |
| *Bauhinia rufescens* | Polygonal | Polygonal | Straight | Straight | Paracytic | None | Non glandular | Non glandular |
| *Bauhinia purpurea* | Polygonal | Polygonal | Straight | Straight | Paracytic | None | Conical and unicellular | Conical and unicellular |
| *Bauhinia vahlii* | Polygonal | Polygonal | Straight | Straight | Paracytic | Paracytic | Non glandular | Non glandular |
| *Piliostigma thonningii* | Polygonal | Polygonal | Wavy | Wavy | Hemiparacytic | None | Non glandular | Non glandular |
| *Piliostigma reticulatum* | Polygonal | Polygonal | Undulating | Undulating | Hemiparacytic | None | Non glandular | Non glandular |

with other *Piliostigma* species. (Fig. 4). The strict consensus *rbcL* gene tree (Fig. 5) also revealed a polyphyletic *Bauhinia* group; however, *Piliostigma* species were distributed in a different clade but with *B. rufescens* and *B. tomentosa*. In the *trnL-F* consensus gene tree, *Piliostigma* species were clustered among the three clades of *Bauhina* species, exhibiting closest relationships with *B. rufescens, B. tomentosa* and *B. blakeana* (Fig. 6). The two *P. thonningii* samples were nested in two different *Bauhinia* clades. The phylogram for the concatenated matrix exhibited a similar tree topology to the *trnL-F* gene tree. *Bauhinia* group is polyphyletic while *Piliostigma* species were clustered within the three Bauhinia clades (Fig. 7) also suggesting *B tomentosa* exhibited a closer relation-

**Table 6.** Quantitative Foliar Anatomical characteristics of the selected species of the tribe Bauhinieae.

| Species | Epidermal cell number Min.(mean±S.E)max | Epidermal cell length (µm) Min.(mean±S.E)max | Epidermal cell width (µm) Min.(mean±S.E)max | Epidermal cell wall thickness (µm) Min.(mean±S.E)max |
|---|---|---|---|---|
| *B. monandra* | | | | |
| Adaxial | 100.00(103.80±0.91)110.00 | 10.00(12.50±0.60)15.00 | 10.00(11.40±0.34)13.00 | 1.00(1.20±0.13)2.00 |
| Abaxial | 60.00(72.70±3.03)88.00 | 12.00(17.30±0.97)20.00 | 14.00(16.60±0.52)18.00 | 1.00(1.50±0.17)2.00 |
| *B. tomentosa* | | | | |
| Adaxial | 100.00(105.70±1.26)112.00 | 12.00(18.90±1.06)24.00 | 11.00(12.80±0.53)16.00 | 1.00(1.00±0.00)1.00 |
| Abaxial | 102.00(116.80±2.98)130.00 | 11.00(14.10±0.90)18.00 | 10.00(11.80±0.61)14.00 | 1.00(1.00±0.00)1.00 |
| *B. rufescens* | | | | |
| Adaxial | 40.00(53.60±2.38)62.00 | 56.00(87.20±7.42)140.00 | 56.00(79.80±3.98)96.00 | 11.00(17.80±1.31)22.00 |
| Abaxial | 40.00(60.30±3.61)76.00 | 29.00(62.20±4.58)76.00 | 32.00(47.00±3.29)68.00 | 7.00(14.00±2.12)25.00 |
| *B. purpurea* | | | | |
| Adaxial | 98.00(108.70±2.71)120.00 | 7.00(9.40±0.82)16.00 | 7.00(8.50±0.56)13.00 | 1.00(1.60±0.16)2.00 |
| Abaxial | 98.00(103.40±1.10)110.00 | 16.00(20.10±0.75)24.00 | 11.00(13.40±0.69)16.00 | 1.00(1.40±0.16)2.00 |
| *B. vahlii* | | | | |
| Adaxial | 7.00(9.80±0.59)12.00 | 50.00(60.80±3.00)77.00 | 38.00(47.50±2.58)57.00 | 6.00(8.00±0.47)10.00 |
| Abaxial | 6.00(9.50±0.79)12.00 | 52.00(64.20±3.17)80.00 | 31.00(50.10±6.53)89.00 | 7.00(9.50±0.5)11.00 |
| *P. thonningii* | | | | |
| Adaxial | 91.00(102.70±1.66)110.00 | 16.00(18.90±0.69)22.00 | 11.00(13.40±0.54)15.00 | 1.00(1.50±0.17)2.00 |
| Abaxial | 72.00(88.6±3.58)107.00 | 12.00(13.70±0.42)16.00 | 11.00(13.00±0.56)16.00 | 1.00(1.00±0.00)1.00 |
| *P. reticulatum* | | | | |
| Adaxial | 93.00(104.70±1.96)120.00 | 18.00(19.80±0.79)32.00 | 11.10(14.70±0.65)17.50 | 1.30(1.90±0.27)4.80 |
| Abaxial | 78.00(66.8±5.58)107.00 | 13.00(15.70±0.84)17.90 | 14.00(12.50±0.56)16.00 | 1.51(1.40±0.31)1.70 |

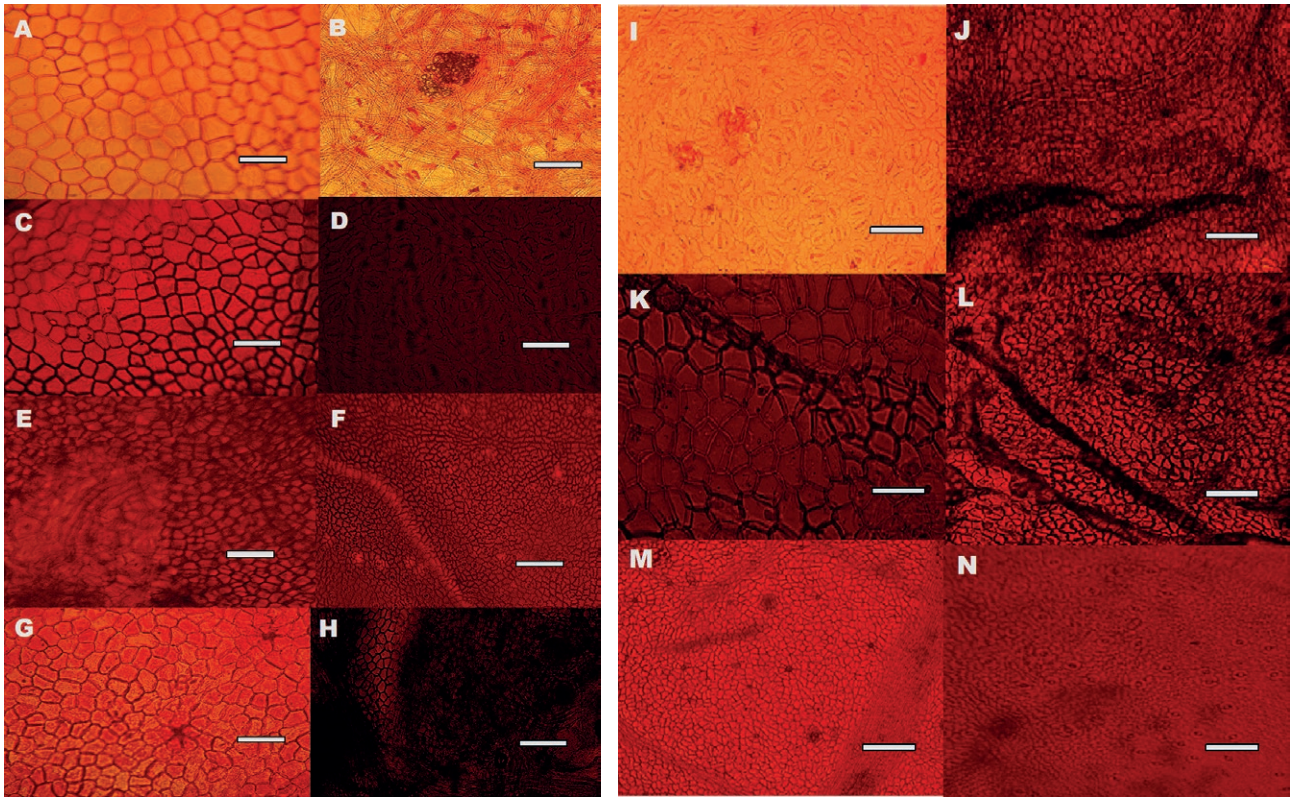


**Figure 2.** Leaf epidermal of members of the Bauhininieae: adaxial on the left, abaxial on the right, A,B- *Bauhinia monandra*, C,D- *B. tomentosa*, E, F- *B. rufescens*, G, H- *B. purpurea*, I,J- *B. vahlii*, K,L- *Piliostigma thonningii*, M, N- *P. reticulatum* Scale bars: 50µm.

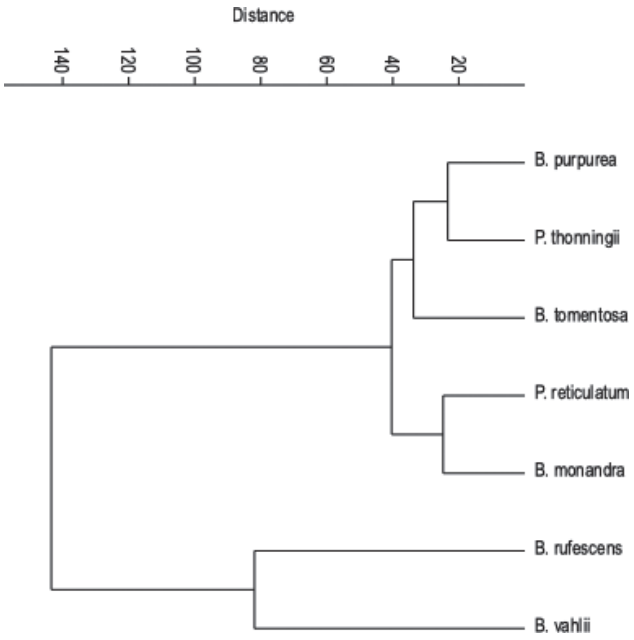


**Figure 3.** UPGMA similarity tree showing relationships amongst members of the tribe Bauhinieae studied based on combined morphological and anatomical data.

ship with *Piliostigma* species. The robustness of most clades were at high bayesian inference of >9 indicating a higher resolution of species cluster at distinct node with a high posterior probability.

## DISCUSSION AND CONCLUSIONS

The systematic studies of the tribe Bauhinieae was elucidated based on morphological and molecular data in order to unravel the relationship among members of this group. Both anatomical and morphological data were analyzed in addition to molecular data using two chloroplast regions (*rbcL*, *trnL-F*) and the nuclear ITS region. Results from both data revealed a polyphyletic *Bauhinia* and *Piliostigma* group, some *Bauhinia* species were clustered among *Piliostigma* species.

Morphologically, members of the genus *Bauhinia* generally possess bilobate leaves with glabrous surface; amongst all species examined, only *B. vahlii* possess hairy leaves supporting descriptions provided by Elbanna et al. (2016). The present study showed that the epidermal shape in all *Bauhinia* species are polygonal and the cell walls are straight except *B. tomentosa*, which has an undulating cell wall supporting Duarte-Almeida et al. (2015). Vaz and Tozzi (2005) confirmed the stomatal types observed among the two genera studied. According to Carpenter and Smith (1975), variations in stomatal frequencies have taxonomic importance at a generic level. After the quantitative investigations of stomatal frequency and index of the species examined, there was a remarkable variation between the two genera showing that these characters were significant at the genus level supporting of Patil and Patil (1987), Ogundipe at al. (2009), Onuminya et al. (2020).

Carlquist (1961) emphasizes the contribution of stomatal size variation in delimiting species within a genus. Major variations in stomatal frequencies of *B. monandra* and *B. tomentosa* are also notable; the distribution of stomata is likewise specific in *B. purpurea*, with amphistomatic stomata, while other studied species exhibited hypostomatic stomata supporting Metcalfe and Chalk (1979) and Albert and Sharma (2013). *B. vahlii* is characteristically distinct in its leaf margins and veins. Some dissimilarities were observed in the trichome index of the species studied. *Bauhinia* species possess both long and short hairs, but with variations in size and morphology of the hair, this corroborates a proposed hypothesis of Pereira et al. (2018). Trichomes observed are mainly unicellular, long, and tapers to a pointed tip except *B. tomentosa* whose hairs are non-glandular, while both *P. thonningii* and *P. reticulatum* lack trichomes as illustrated by Bannerje et al. (2002). These results confirms the importance of trichomes in taxonomic studies. Hence, based on the observed foliar morphological and anatomical features, a diagnostic key is proposed as below:

1a. Leaf bifoliate, palmate veination with paracytic stomata..... ..... ***Bauhinia*** L.

2a. Polygonal cell wall with straight anticlinal cell wall ..... 3

2b. Polygonal cell wall with curved anticlinal cell wall ..... ..... ***B. tomentosa***

3a. Leaf surface glabrous, each leaf lobe rounded and split up to 1/3 of leaf length..... ***B. monandra***

3b. Leaf surface glabrous, leaf lobe cordate or rounded and split up to 3/4 of leaf length..... ***B. rufescens***

4a. Leaf apex emarginate with conical and unicellular trichomes..... ***B. purpurea***

4b. Leaf apex apiculate with nonglandular trichomes. ***B. vahlii***

1b. Leaf bifoliate, palmate reticulate venation with hemiparacytic stomata..... ***Piliostigma*** Hochst.

6a. Leaf finely pubescent beneath, and leaf apex acuminate ..... ..... ***P. thonningii***

6b. Leaf glabrous beneath and leaf apex rounded to cuneate..... ..... ***P. reticulatum***

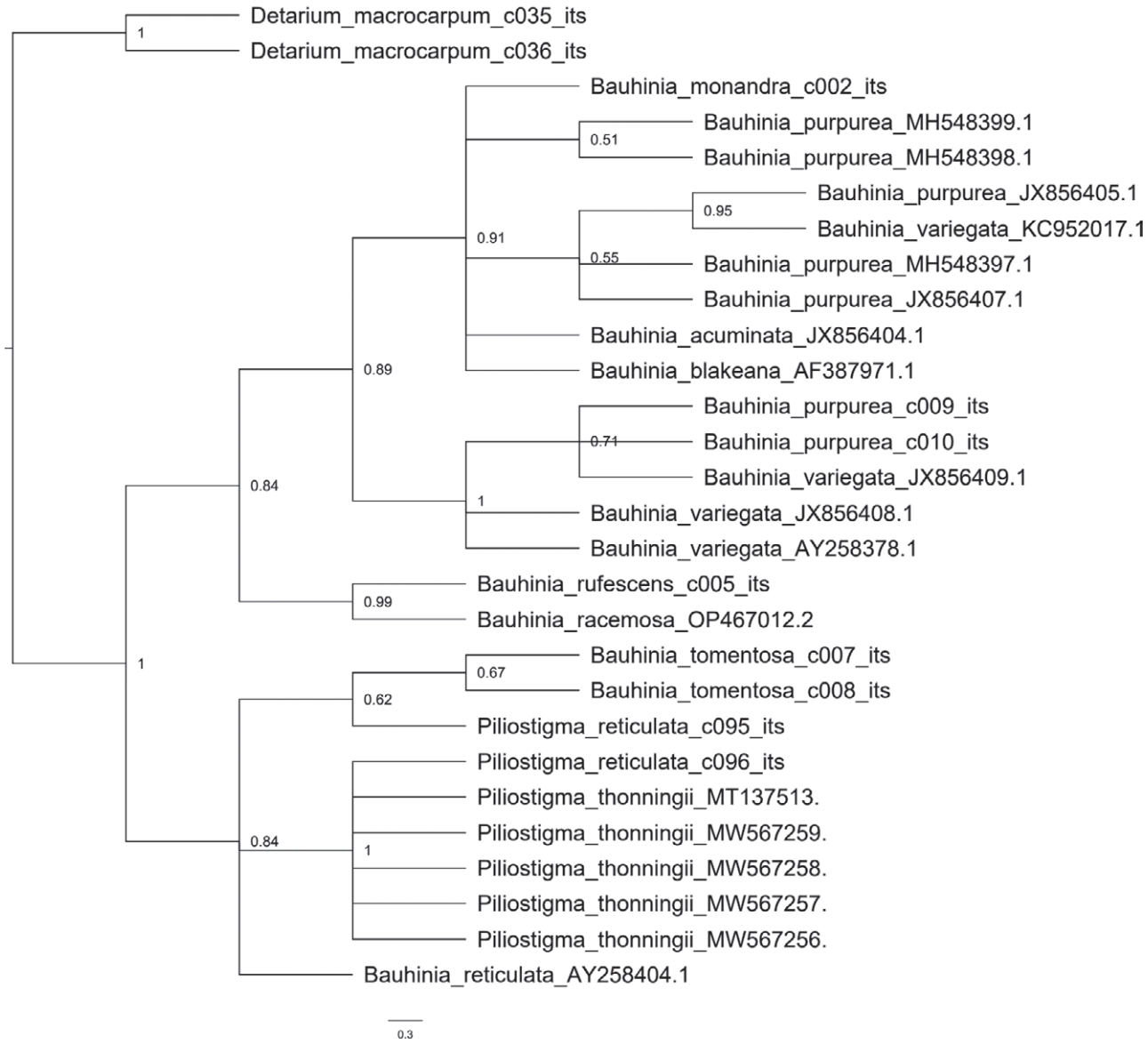


**Figure 4.** Phylogram inferred by Bayesian analysis (ITS), numbers at node indicate posterior probability value.

The phylogenetic pattern of the Bauhinieae has always been controversial. In this study, based on three gene regions, we explored the generic patterns of this taxon. In previous studies, *Piliostigma* group has been debated to be monophyletic (Hao et al. 2003; Sinuou, 2020), this submission contradicts our findings. The ITS and concatenated matrix phylo-tree exhibited a polyphyletic relationship with the *Bauhinia* ss group. Although, *Piliostigma* species has distinct morphological features, but a polyphyletic group was observed from both morphological and anatomical data. Hence, employing morphological, anatomical and molecular data *Piliostigma* species exhibits a close relationship with *B. rufescens*, *B. tomentosa* and *B. purpurea*. This supports the reports of some authors who propose *Piliostigma* as a section of *Bauhinia*, rather than as a separate genus (Bentham 1865; Wunderlin et al. 1987; Zhang 1995; Hao et al. 2003).

In addition, results from this study observed a complex phylogenetic pattern among the *Bauhinia* s.s. group and these results support previous works and proposition to divide members of this large group into subclades. In this study, the *Bauhina* species were divided into 3 subclades at weak to strong bayesian inference. The dendrogram using morphological and anatomical

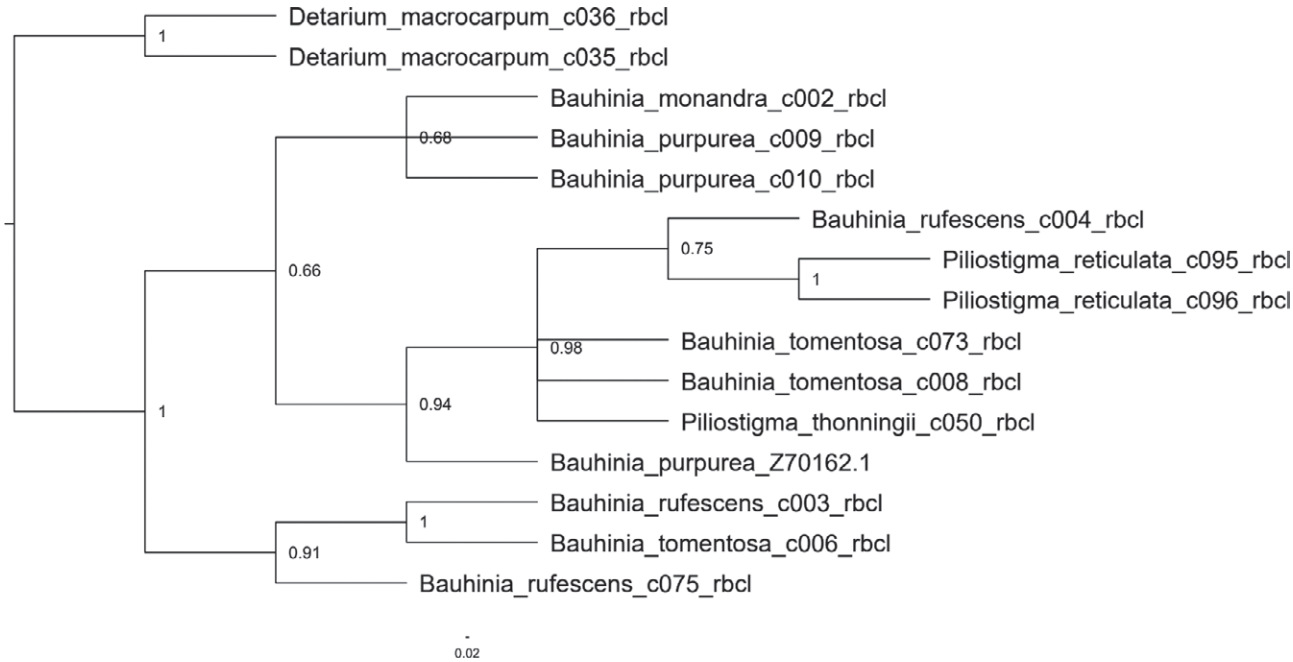


**Figure 5.** Phylogram inferred by Bayesian analysis (*rbcL*), numbers at node indicates posterior probability value.

data also presented 3 clades; *B. rufescens* and *B. vahlii*; *B. tomentosa* and *B. purpurea* and *B. monandra* groups. The ITS phylo-tree presented two clades comprising clade 1: *B. purpurea*, *B. monandra, B. acuminata, B. variegata* and *B. blakeana;* clade 2: *B. rufescens* while the rbcL phylo-tree exhibited clades comprising *B. purpurea*, *B. monandra* and *B. rufescens*, *B. tomentosa* groups. A similar complex topology was observed for trnL-F phylo-tree and the concatenated matrix phylo-tree. This corroborates Sinou et al. (2020) that posited that the subtribe Bauhinieae is weakly supported as monophyletic. Although Sinou et al. (2020) made a proposition for a geographical distribution of species into groups, suggesting species from each region to be grouped into a clade. Within the West African members of the tribe Bauhinieae, results from this study revealed a polyphyletic relationship. This could probably be as a result of the limited sampling of this taxon as well as the poor performance of some species during amplication of the selected gene regions. Similarly, it was observed that some species were phylogenetically divergent in relationship with members of other species e.g. *P. thonningii*, *B. pupurea* and *B. variegata*, this could be as a result of different localities of sampling or misrepresentation of samples. A powerful solution would likely be found in a denser sampling and highly variable character selection for better species resolution.

In summary, the phylogeny based on both chloroplast and nuclear DNA as well as morphological and anatomical data confirms the polyphyly of Bauhinieae. Our results show that similarities in the morphological and anatomical structures of members of this taxon were due to some evolutionary processes and this has posed a complexity in their classification. Furthermore, the monophyly of the *Piliostigma* group exhibited a paraphyletic and polyphyletic relationship with the *Bauhinia* group at high support values. The relationship among the West African *Bauhinia* species is polyphyletic and remain unresolved. This study has attempted to elucidate the unresolved species- and genus-level taxonomy of the tribe Bauhinieae. However, more variable gene regions in addition to broader species sampling should be considered for further phylogenetic patterns of this taxon.

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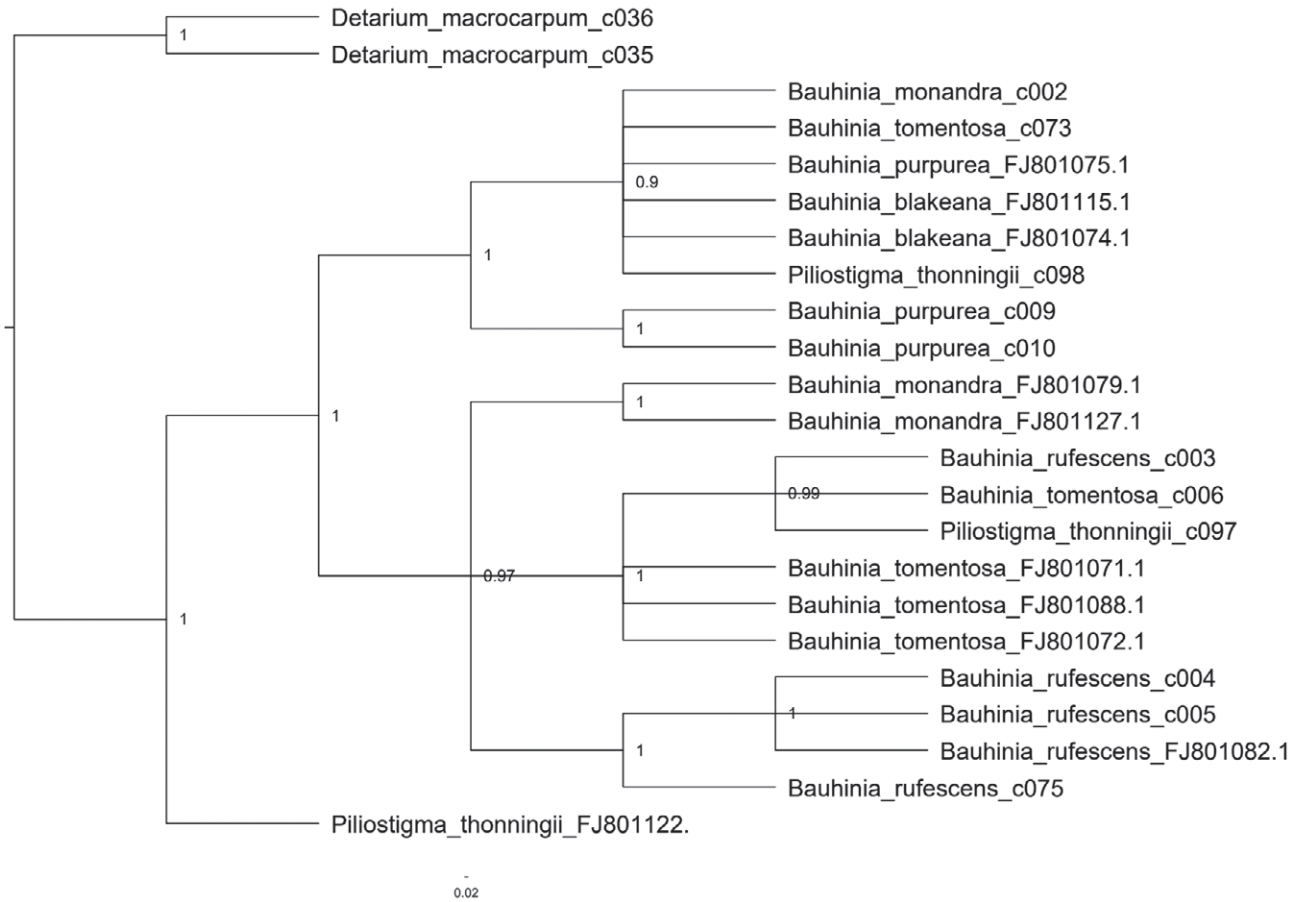


**Figure 6.** Phylogram inferred by Bayesian analysis (*trnL-F*), numbers at node indicates posterior probability value.

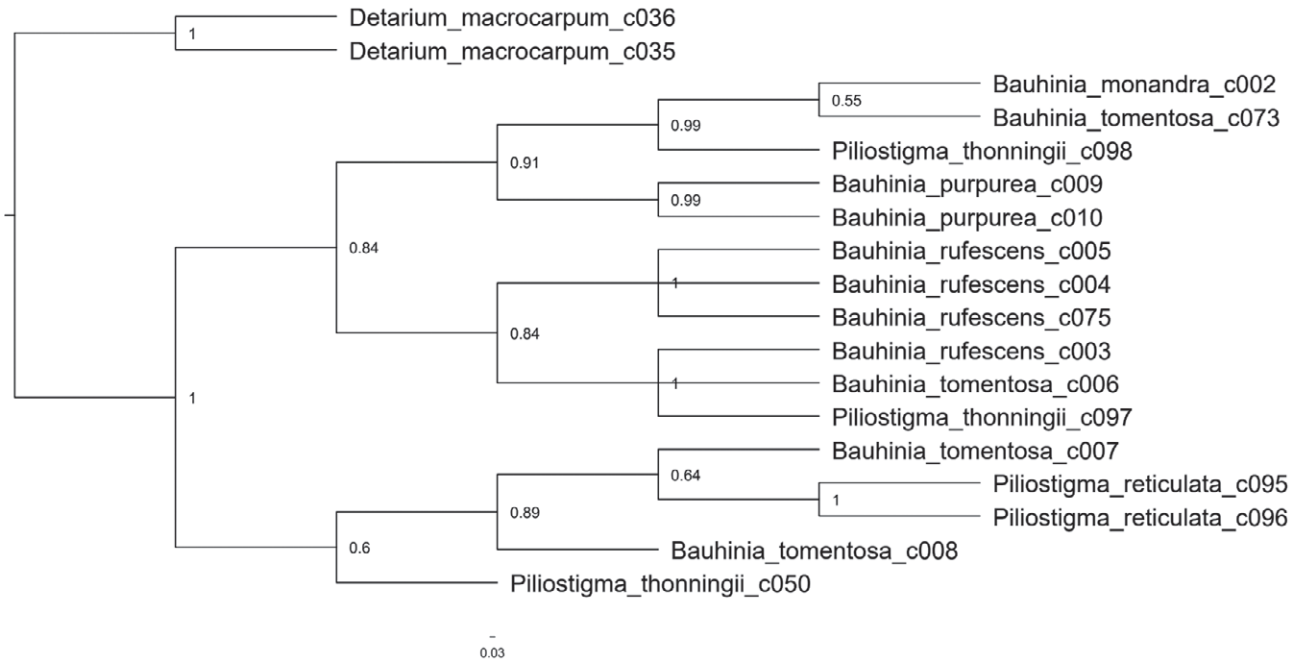


**Figure 7.** Phylogram inferred by Bayesian analysis (ITS+*rbcL*+*trnL-F*), numbers at node indicates posterior probability value.

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