



Lectotypification of *Pseudoglochidion anamalayanum* Gamble and its taxonomic position under the genus *Phyllanthus* (Phyllanthaceae)

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Abstract

Pseudoglochidion anamalayanum Gamble (Phyllanthaceae), the only species of the genus is presently treated under *Phyllanthus* L. It is a narrow endemic of Western Ghats, India and is known from very few collections from Anamalais, Coimbatore district, Tamil Nadu. It has been assigned the threat status ‘Critically Endangered’ (CR) by World Conservation Monitoring Centre (1998). In the present study, its taxonomic position has been evaluated using molecular sequences of internal transcribed spacer (ITS) and chloroplast *matK* genes with a matrix of ITS and *matK* sequences of *Phyllanthus*. The results obtained from MEGA and Bayesian analyses have shown that *Pseudoglochidion* is nesting among the *Phyllanthus* species of subgenus *Isocladus*, which itself is polyphyletic. The morphological characters also support the taxonomic position of the species in *Phyllanthus*. A detailed description is provided and lectotype for the name *Pseudoglochidion anamalayanum* (= *Phyllanthus anamalayanus*) is designated here.

Keywords: ITS, lectotype, *matK*, phylogeny, *Phyllanthus*, *Pseudoglochidion*

Introduction

Pseudoglochidion Gamble (1925a, 1925b) has been considered a monotypic genus till Webster (1994) merged the only species *Pseudoglochidion anamalayanum* Gamble under the genus *Phyllanthus* L. However, he has not provided any reasons for its merger. This treatment was followed by Balakrishnan & Chakrabarty (2007) and Chakrabarty *et al.* (2012). This species is endemic to Western Ghats of India and its distribution is restricted to above 1400 m in Anamalais (Tamil Nadu). IUCN Red List assigns ‘Critically Endangered’ (CR) status [B1+2c ver 2.3] to this species (World Conservation Monitoring Centre 1998).

Since it is a ‘CR’ species, its taxonomic treatment as a species of one of the largest genera of angiosperms has different conservation implications. Recently, the family Phyllanthaceae has been a subject of molecular phylogenetic studies. Earlier molecular phylogeny works (Wurdack *et al.* 2004; Kathriarachchi *et al.* 2005, 2006; Samuel *et al.* 2005; Varontsova *et al.* 2007) did not deal with the genus *Pseudoglochidion* probably due to lack of material for study. Hence, phylogenetic studies using ITS of nuclear rDNA and chloroplast *matK* has been attempted to confirm its taxonomic position.

Taxonomically *Phyllanthus* has always been in some sort of confusion regarding its circumscription. This confusion may be due to the limited number of morphological characters used to define or describe species of one of the largest genera. Detailed studies carried out by Webster (1955, 1956a, 1956b, 1957, 1958, 1967, 1970, 1994), Webster and AiryShaw (1971) have set the base for understanding of the genus. Studies by Balakrishnan & Chakrabarty (2007) and Chakrabarty *et al.* (2012) have put *Phyllanthus anamalayanus* (Gamble) G.L.Webster (= *Pseudoglochidion anamalayanum* Gamble) under the subgenus *Isocladus* G.L.Webster. In the present study molecular evidences have been sought to confirm the taxonomic position of *Phyllanthus anamalayanus* and lectotype has been designated for its basionym *Pseudoglochidion anamalayanum* Gamble.

Material & Methods

Plant material and DNA extraction

Leaf sample was collected from the naturally growing population from Iyerpadi, Valparai, Tamil Nadu, India and stored in silica gel (Chase and Hills 1991) for DNA isolation. Voucher specimen *Janarthanam et al.*, 4951 has been deposited in MH.

The *matK* gene and ITS region were sequenced using universal primers. The ITS region was sequenced with ITS5-F (GGAAGTAAAAGTCGTAACAAGG) and ITS4-R (TCCTCCGCTTATTGATATGC). The *matK* gene was sequenced with forward primer 3F-KIM-F (CGTACAGTACTTTTGTGTTTACGAG) and reverse primer 1R-KIM-R (ACCCAGTCCATCTGGAAATCTTGGTTC). The ITS and *matK* gene sequences are deposited in NCBI and accession numbers are given in Table 1.

TABLE 1. Sequences deposited at NCBI

Gene	<i>Phyllanthus anamalayanus</i> (Gamble) G.L. Webster (submitted as <i>Pseudoglochidion anamalayanum</i> Gamble)	<i>Phyllanthus talbotii</i> Sedgw.
ITS	KC414629	KC414630
<i>matK</i>	KC514100	KC514101

Sequence analysis, alignment and phylogenetic tree

The ITS and *matK* sequences of *Pseudoglochidion anamalayanum* were analysed with the data set from NCBI (Table 2) with *Glochidion acuminatum* Müll.Arg. as an outgroup. The sequence alignment was done using clustalX (ver. 2.0.12). The individual data set of the ITS and *matK* sequences are joined together in Sequence matrix (1.7.8) and exported in MEGA and NEXUS formats. The most suitable substitution models for the respective datasets were selected by using JModeltest (2.1.3) (Darriba *et al.* 2012) and model which shows lowest likelihood scores (Table 3) was used for Bayesian analysis using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and MEGA 5.2 (Kumar *et al.* 2008) analysis.

TABLE 2. Accession numbers of ITS and *matK* sequences data set of NCBI and infrageneric classification of *Phyllanthus* species used.

Subgenus	Section	Taxa	Location	ITS	<i>matK</i>	Clade
		<i>Phyllanthus vakinankaratrae</i> Leandri	Madagascar	AY936737	AY936638	I
		<i>Phyllanthus andalangiensis</i> Leandri	Madagascar	AY936670	AY936576	I
<i>Conami</i>	<i>Conami</i>	<i>Phyllanthus acuminatus</i> Vahl	Guatemala	AY936667	AY936573	D
	<i>Nothoclema</i>	<i>Phyllanthus graveolens</i> Kunth	Ecuador	AY936696	AY936600	D
<i>Emblica</i>	<i>Emblica</i>	<i>Phyllanthus emblica</i> L.	India	AY936689	AY936594	K
		<i>Phyllanthus oxyphyllus</i> Miq.	Thailand	AY936719	AY936621	K
<i>Eriococcus</i>	<i>Eriococcus</i>	<i>Phyllanthus cinereus</i> Müll.Arg.	Sri Lanka	AY936682	AY936587	B
		<i>Phyllanthus pulcher</i> (Baill.) Wall. ex Müll.Arg.	Sri Lanka	AY936726	AY936627	B
		<i>Phyllanthus sikkimensis</i> Müll.Arg.	India	AB550102	--	B
	<i>Eriococcodes</i>	<i>Phyllanthus ruber</i> Spreng.	China	AY765298	--	B
		<i>Phyllanthus pulchroides</i> Beille	Mahaxai	--	FJ235273	B
		<i>Phyllanthus talbotii</i> Sedweg.	India	KC414630	KC514101	B
		<i>Phyllanthus gracilipes</i> Müll.Arg.	Thailand	AB550095	--	K
		<i>Phyllanthus ovalifolius</i> Forssk.	South Africa	--	JX518152	A

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TABLE 2. (Continued)

Subgenus	Section	Taxa	Location	ITS	<i>matK</i>	Clade
<i>Gomphidium</i>	<i>Adenoglochidion</i>	<i>Phyllanthus liukuensis</i> Hayata	Japan	--	FJ235272	B
		<i>Phyllanthus aeneus</i> Baill.	Japan	--	FJ235260	G
		<i>Phyllanthus caudatus</i> Müll.Arg.	Japan	--	FJ235259	H
		<i>Phyllanthus aff. moorei</i> M.Schmid	New Caledonia	AY936710	AY936614	H
		<i>Phyllanthus bourgeoisie</i> Baill.	Japan	--	FJ235256	H
		<i>Phyllanthus chamaecerasus</i> Baill.	New Caledonia	AY936678	AY936583	H
		<i>Phyllanthus gneissicus</i> S.Moore	Japan	--	FJ235263	G
		<i>Phyllanthus guillauminii</i> Däniker	Japan	--	FJ235261	G
		<i>Phyllanthus mangenotii</i> M.Schmid	Japan	--	FJ235257	H
		<i>Phyllanthus pancherianus</i> Baill.	New Caledonia	AY936721	AY936623	H
		<i>Phyllanthus vulcani</i> Guillaumin		--	FJ235262	G
		<i>Phyllanthus loranthoides</i> Baill.	New Caledonia	AY936705	AY936607	G
		<i>Phyllanthus favieri</i> M.Schmid	New Caledonia	AY936691	AY936596	G
		<i>Isocladus</i>	<i>Antipodanthus</i>	<i>Phyllanthus calycinus</i> Labill.	Australia	AY936674
<i>Caramanthus</i>	<i>Phyllanthus cochinchinensis</i> Spreng.		China	AY936684	AY936589	C
	<i>Phyllanthus welwitschianus</i> Müll.Arg.		Tanzania	AY936739	AY936640	C
<i>Diandri</i>	<i>Phyllanthus diandrus</i> Pax		Gabon	AY936687	AY936592	C
<i>Loxopodium</i>	<i>Phyllanthus caroliniensis</i> Walter		Guyana	AY936675	AY936580	D
<i>Macraea</i>	<i>Phyllanthus chrysanthus</i> Baill.		New Caledonia	AY936680	AY936585	C
	<i>Phyllanthus gardnerianus</i> Baill. ex Müll.Arg.		Sri Lanka	AY936694	AY936598	C
	<i>Phyllanthus myrtifolius</i> Moon		Sri Lanka	AY936712	AY936616	C
	<i>Phyllanthus virgatus</i> G.Forst.		Australia	AY936738	AY936639	C
	<i>Phyllanthus wheeleri</i> G.L.Webster		Sri Lanka	AY936740	AY936641	C
<i>Paraphyllanthus</i>	<i>Phyllanthus maderaspatensis</i> L.		Australia	AY936707	AY936609	C
	<i>Phyllanthus columnaris</i> Müll.Arg.		Thailand	AB550094	--	K
	<i>Phyllanthus kozhikodianus</i> Sivar. & Manilal		India	KF312395	--	E
	<i>Phyllanthus polyphyllus</i> Willd.		Sri Lanka	AY936725	AY830278	K
	<i>Phyllanthus rheedii</i> Wight		Sri Lanka	AY936729	AY936630	E
	<i>Phyllanthus scabrifolius</i> HooK.f.		India	KF003023	--	E

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TABLE 2. (Continued)

Subgenus	Section	Taxa	Location	ITS	<i>matK</i>	Clade
		<i>Phyllanthus ussuriensis</i> Rupr. & Maxim.	China & Japan	AY765295	FJ235274	K
		<i>Phyllanthus anamalyanus</i> (Gamble) G.L. Webster (submitted as <i>Pseudoglochidion anamalyanum</i> Gamble)	India	KC414629	KC514100	K
<i>Kirganelia</i>	<i>Anisonema</i>	<i>Phyllanthus casticum</i> P. Willemet	Aldabra, Seychelles	AY936676	AY936581	A
		<i>Phyllanthus matitanensis</i> Leandri	Madagascar	--	AY936610	A
		<i>Phyllanthus reticulatus</i> Poir.	Sri Lanka	AY936728	AY936629	A
	<i>Aporosella</i>	<i>Phyllanthus chacoensis</i> Morong	Paraguay	AY936677	AY936582	J
	<i>Chorisandra</i>	<i>Phyllanthus pinnatus</i> (Wight) G.L. Webster	Zimbabwe	AY936724	AY936626	J
	<i>Cicca</i>	<i>Phyllanthus acidus</i> (L.) Skeels	Thailand	AY936666	AY936572	J
	<i>Floribundi</i>	<i>Phyllanthus muellerianus</i> (Kuntze) Exell	Cameroon and Ghana	AY936711	AY936615	A
	<i>Pentandra</i>	<i>Phyllanthus nummulariifolius</i> Poir.	Madagascar	AY936716	AY552444	F
		<i>Phyllanthus pentandrus</i> Schumach. & Thonn.	Zambia	AY936722	AY936624	F
		<i>Phyllanthus tenellus</i> Roxb.	Mayotte, Comoro Is	AY936733	AY936634	F
		<i>Phyllanthus ciccoides</i> Müll.Arg.	Papua New Guinea	DQ499082	--	A
		<i>Phyllanthus flexuosus</i> (Siebold & Zucc.) Müll.Arg.	China & Japan	EU279432	FJ235269	A
		<i>Phyllanthus glaucus</i> Wall ex. Müll.Arg.	China	AY765291	--	A
		<i>Phyllanthus oligospermus</i> Hayata	Japan	--	FJ235268	A
		<i>Phyllanthus pervilleanus</i> (Baill.) Müll.Arg.	Mayotte, Comoro Is	AY936723	AY936625	A
<i>Macraea</i>		<i>Phyllanthus betsileanus</i> Leandri	Madagascar	AY936673	AY936578	I
<i>Pentaglochidion</i>		<i>Phyllanthus kanalensis</i> Baill.	New Caledonia	AY936701	AY936604	G
<i>Phyllanthodendron</i>		<i>Phyllanthus mirabilis</i> Müll.Arg.	Thailand	HM132100	AY936613	L
		<i>Phyllanthus roseus</i> (Craib & Hutch.) Beille	Thailand and Japan	AB550101	FJ235240	F
<i>Phyllanthus</i>	<i>Phyllanthus</i>	<i>Phyllanthus clausenii</i> Müll.Arg.	Brazil	AY936683	AY936588	D
		<i>Phyllanthus debilis</i> J.G.Klein ex Willd	Sri Lanka	AY936686	AY936591	E
		<i>Phyllanthus amarus</i> Schumach. & Thonn.	Trinidad	AY936668	AY936574	F

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TABLE 2. (Continued)

Subgenus	Section	Taxa	Location	ITS	matK	Clade
		<i>Phyllanthus fraternus</i> G.L.Webster	India	EU876846	--	F
	<i>Phyllanthus</i> Subsec. <i>Swartziani</i>	<i>Phyllanthus gossweileri</i> Hutch.	Gabon	AY936695	AY936599	E
		<i>Phyllanthus lokohensis</i> Leandri	Madagascar	AY936704	AY936606	E
		<i>Phyllanthus madagascariensis</i> Müll.Arg.	Madagascar	AY936706	AY936608	E
	<i>Phyllanthus</i> Subsec. <i>Odontadenii</i>	<i>Phyllanthus sepialis</i> Müll.Arg.	Kenya	AY936732	AY936633	E
		<i>Phyllanthus mannianus</i> Müll.Arg.	Cameroon	AY936708	AY936611	E
	<i>Phyllanthus</i> Subsec. <i>Niruri</i>	<i>Phyllanthus niruri</i> L.	Costa Rica	AY936713	AY936617	D
	<i>Salviniopsis</i>	<i>Phyllanthus fluitans</i> Benth. ex Müll.Arg.	Netherlands	AY936693	AY936597	D
	<i>Urinaria</i>	<i>Phyllanthus hookeri</i> Müll.Arg.	Taiwan	AY831634	--	K
		<i>Phyllanthus urinaria</i> L.	Mayotte, Comoro Is	AY936735	AY936636	K
		<i>Phyllanthus embergeri</i> Haicour & Rossignol	Taiwan	AY725463	--	K
		<i>Phyllanthus parvifolius</i> Buch.- Ham. ex D.Don	China	AY765294	--	K
		<i>Phyllanthus hutchinsonianus</i> S.Moore	Zimbabwe	AY936697	AY936601	E
		<i>Phyllanthus arenarius</i> Beille	China	AY765300	--	F
		<i>Phyllanthus clarkei</i> Hook.f.	China	HM106989	--	C
		<i>Phyllanthus guangdongensis</i> P.T.Li	China	AY765297	--	B
		<i>Phyllanthus klotzschianus</i> Müll. Arg.	Brazil	AY936702	AY936605	D
		<i>Phyllanthus lepidocarpus</i> Siebold & Zucc.	Japan	--	FJ235252	K
		<i>Phyllanthus taxodiifolius</i> Beille	Thailand	AB550083	--	B
<i>Tenellanthus</i>		<i>Phyllanthus kaessneri</i> Hutch.	Tanzania	AY936700	AY936603	E
<i>Xylophylla</i>	<i>Astrandra</i>	<i>Phyllanthus juglandifolius</i> Willd.	Ecuador	AY936699	AY936602	D
	<i>Elutanthos</i>	<i>Phyllanthus pachystylus</i> Urb.	Cuba	AY936720	AY936622	D
	<i>Glyptothamnus</i>	<i>Phyllanthus chryseus</i> R.A.Howard	Cuba	AY936681	AY936586	D
	<i>Orbicularia</i>	<i>Phyllanthus salviifolius</i> Kunth	Ecuador	AY936730	AY936631	D
		<i>Phyllanthus</i> cf. <i>chamaecristoides</i> Urb.	Cuba	AY936679	AY936584	D
		<i>Phyllanthus comosus</i> Urb.	Cuba	AY936685	AY936590	D

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TABLE 2. (Continued)

Subgenus	Section	Taxa	Location	ITS	<i>matK</i>	Clade
		<i>Phyllanthus orbicularis</i> Kunth	Cuba	AY936718	AY936620	D
	<i>Xylophylla</i>	<i>Phyllanthus angustifolius</i> (Sw.) Sw.	RBG Kew	AY936671	AY936577	D
		<i>Phyllanthus epiphyllanthus</i> L.	US & Cuba	AY663604	AY936595	D
	<i>Williamia</i>	<i>Phyllanthus discolor</i> Spreng.	Cuba	AY936688	AY936593	D
		<i>Phyllanthus microdictyus</i> Urb.	Cuba	AY936709	AY936612	D
		<i>Phyllanthus sellowianus</i> Müll.Arg.	RBG Kew	AY936731	AY936632	D
		<i>Phyllanthus purpusii</i> Brandegees	Mexico	AY936727	AY936628	*
<i>Unplaced taxa</i>		<i>Phyllanthus sauropodoides</i> Airy Shaw	Australia	EU623558	EU643745	G
		<i>Phyllanthus unifoliolatus</i> M.Schmid	New Caledonia	AY936734	AY936635	G
		<i>Phyllanthus meghalayensis</i> Chakrab. & N.P.Balkr.	Japan	KC913130	--	M

In Bayesian analysis, number of generations for individual and combined analysis was given in Table 3 to obtain frequency < 0.01. The first 25% trees were discarded and remaining trees were used to generate consensus tree. The final tree was viewed and read in Mesquite (2.75).

In MEGA 5.2.2, the confidence limits (bootstrap percentage) for clades were assessed by performing 1000 replicates of bootstrapping (Felsenstein 1985). The individual as well as combined analyses were performed for Maximum Likelihood analyses. The bootstrap values are presented in the Bayesian analysis below the branch.

Results

Phylogeny

The ITS and *matK* gene sequences of *Pseudoglochidion anamalayanum* were analysed with the ITS and *matK* sequence of the taxa given in Table 2. The characteristics and statistics for the Bayesian and MEGA analysis of individual as well as combined data set are given in Table 3. The result of combined analysis (Fig. 3) is used for the discussion of phylogenetic relationship.

Analysis of ITS

The aligned ITS matrix consisted of 834 base pairs of which 103 are conserved characters. The parsimony informative sites were 427 and 91 taxa were analysed for phylogeny. The strict consensus tree with posterior probability and bootstrap percentage is presented in Fig. 1. In ITS consensus tree *Pseudoglochidion* is nested in polyphyletic *Isocladus* subgenus clade which also includes subgenera *Emblica*, *Phyllanthus* and *Eriococcus*. *Pseudoglochidion* is sister to *P. gracilipes*, *P. columnaris*, *P. oxyphyllus* and *P. polyphyllus* with good posterior probability (0.94) but moderate bootstrap value (76).

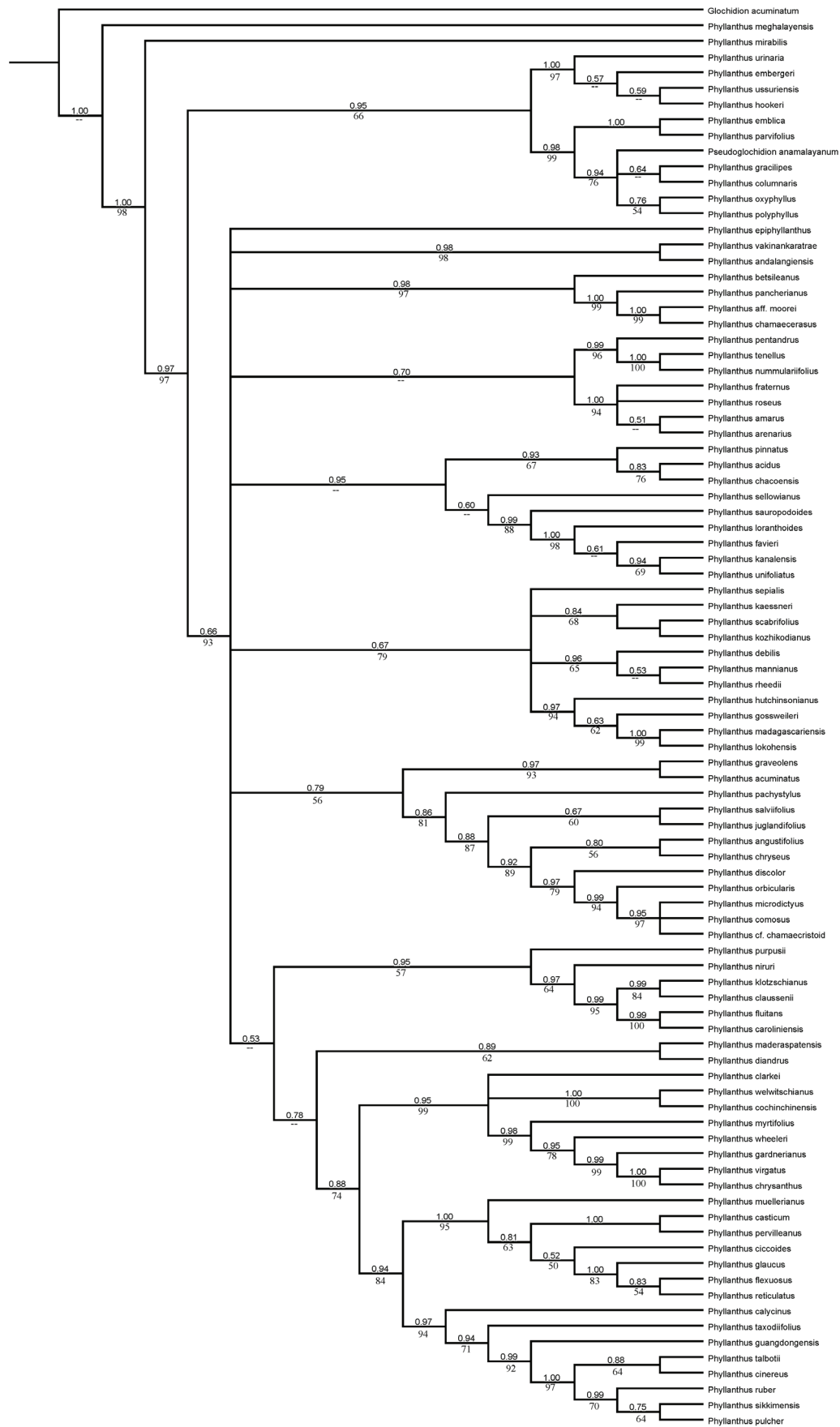


FIGURE 1. Bayesian Phylogram based on ITS sequence data. Bayesian posterior probabilities, bootstrap values for ML shown above and below of the branch respectively. Posterior probability <50 were shown as dash.

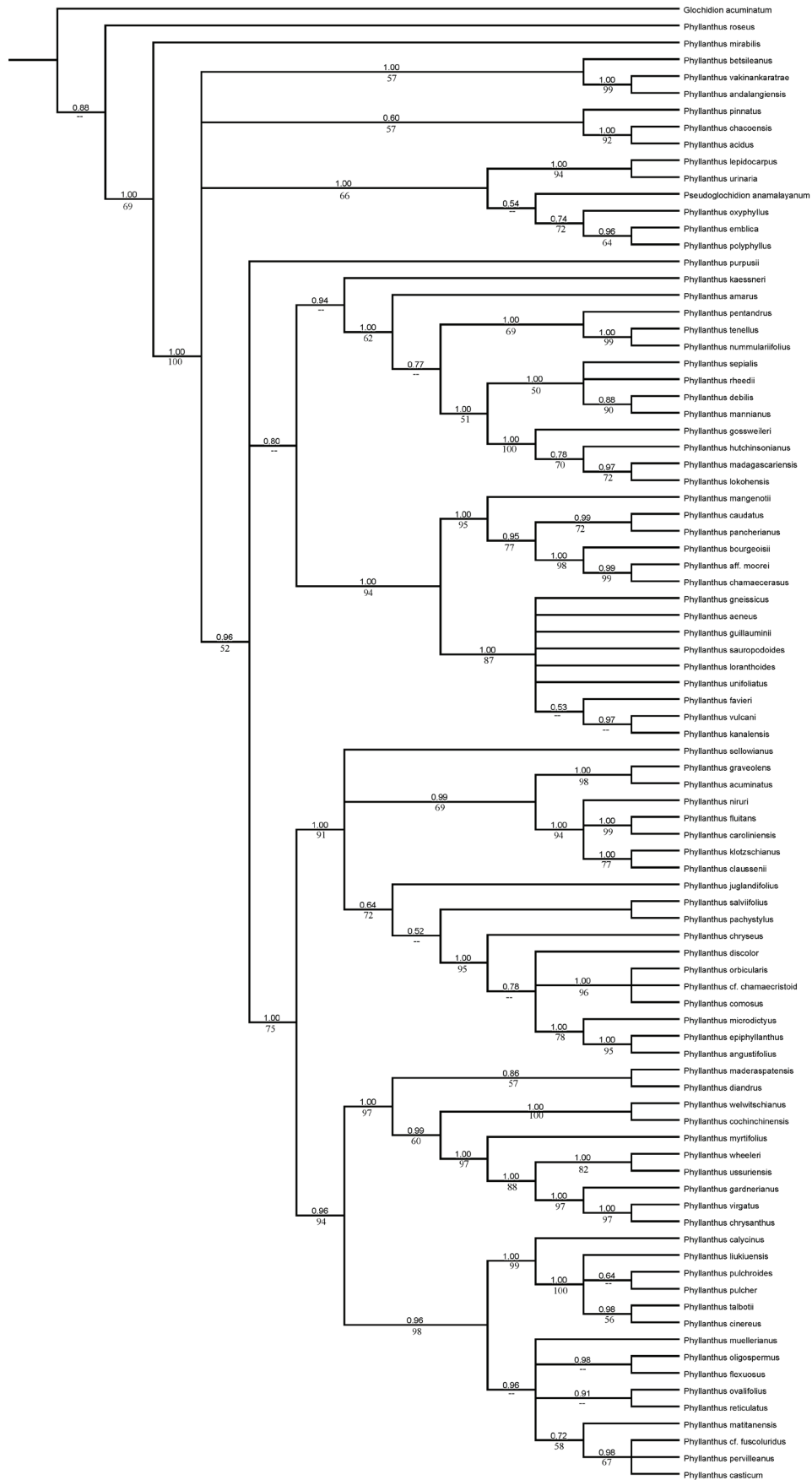


FIGURE 2. Bayesian Phylogram based on *matK* gene sequence data. Bayesian posterior probabilities, bootstrap values for ML shown above and below of the branch respectively. Posterior probability <50 were shown as dash.

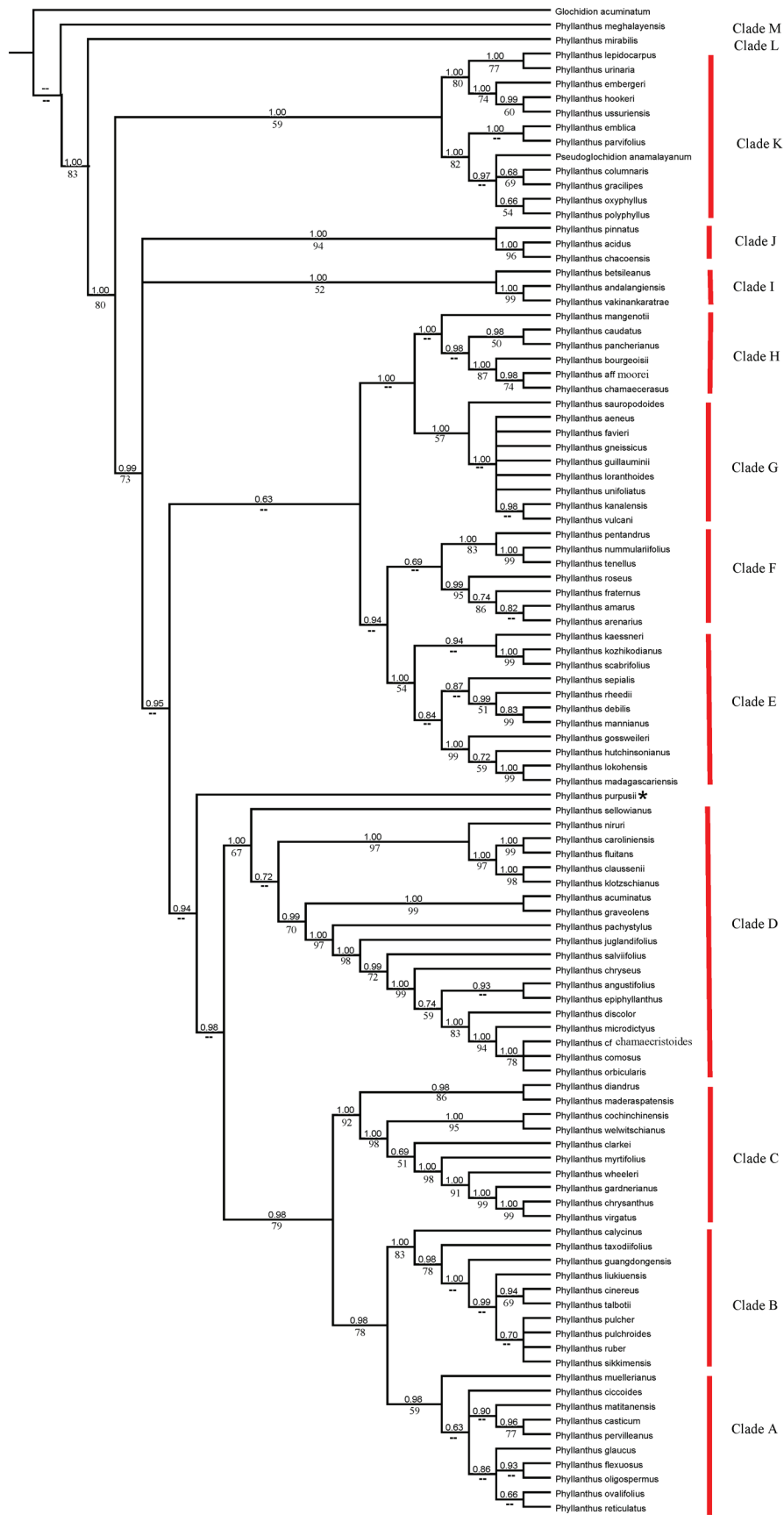


FIGURE 3. Bayesian Phylogram based on combine data set of ITS + *matK* sequence data. Bayesian posterior probabilities, bootstrap values for ML shown above and below of the branch respectively. Posterior probability <50 were shown as dash.

TABLE 3. Matrix and tree statistics for combined and independent analysis

Data Set	ITS	<i>matK</i>	Combined (ITS+ <i>matK</i>)
No. of taxa without-group	91	88	104
Matrix length (aligned) (bp)	834	1983	2817
Conserved sites (C)	103	1057	1162
Variable sites (V)	715	816	1529
Parsim-informative sites (Pi) (%)	427 (51.19)	442 (22.28)	1529 (54.27)
Single tone sites	272	371	869
Substitution model with rates	GTR with Invgamma	GTR with Invgamma	GTR with Invgamma
No. of generations	1500000	1250000	2100000
No. of initial trees discarded	25%	25%	25%

Analysis of *matK*

The aligned *matK* matrix consisted of 1983 base pairs of which 1057 are conserved characters. The parsimony informative sites were 442. In all 88 taxa were analysed using *matK* gene sequences. The strict consensus tree with posterior probability and bootstrap percentage is presented in Fig. 2. In *matK* consensus tree analysis *Pseudoglochidion* is nested in polyphyletic *Isocladus* subgenus along with subgenera *Emblica*, *Phyllanthus* and *Urinaria*. *Pseudoglochidion* is sister to *P. lepidocarpus*, *P. urinaria*, *P. oxyphyllus*, *P. emblica* and *P. polyphyllus* with good posterior probability (1.00) but moderate bootstrap value (66).

Analysis of ITS + *matK*

The combined aligned ITS and *matK* matrix consisted of 2817 base pairs of which 1162 are conserved characters. The parsim-informative sites were 1529. In all 104 taxa were used in combined (ITS + *matK*) phylogenetic analysis. The strict consensus tree with posterior probability and bootstrap percentage is presented in Fig. 3. *Pseudoglochidion* is nested in polyphyletic clade which includes subgenera *Emblica*, *Eriococcus*, *Phyllanthus* and *Isocladus* with good posterior probability (0.97) but poor bootstrap value (below 50).

Discussion

This study based on molecular phylogenetic analysis presents first time report of confirming the taxonomic position of monotypic genus *Pseudoglochidion* Gamble under genus *Phyllanthus* as treated by Webster (1994), Balakrishnan & Chakrabarty (2007) and Chakrabarty *et al.* (2012). The nuclear gene internal transcribed spacer (ITS) as well as chloroplast gene *matK* sequences of *Pseudoglochidion anamalayanum* aligned and analysed with highly similar sequences, confirm its taxonomic position. *Pseudoglochidion* is embedded among *Phyllanthus oxyphyllus*, *P. gracilipes*, *P. columnaris*, *P. polyphyllus* and *P. emblica* belonging to subgenera *Emblica*, *Eriococcus*, *Phyllanthus* and *Isocladus*. All the *Phyllanthus* subgenera are polyphyletic in nature (Fig. 3).

Present study substantiate the earlier works (Wurdack *et al.* 2004; Kathriachchi *et al.* 2005, 2006; Samuel *et al.* 2005; Varontsova *et al.* 2007). The present phylogenetic analysis of tribe Phyllantheae is in agreement with Kathriachchi *et al.* (2006) confirming the polyphyletic nature of subgenera such as *Isocladus*, *Kirganelia*, *Phyllanthus*, *Eriococcus* and *Gomphidium*.

In large genera such as *Phyllanthus* and its other allied genera morphological variations are often misunderstood and in those cases molecular studies come handy. Varnostsova *et al.*, (2007) carried out detailed morphological studies of *Andrachne cuneifolia* Britton in comparison with *Phyllanthus pachystylus* and transferred the former to *Phyllanthus* with additional evidences from ITS and *matK* sequence analysis. Rajkumar (2001) supported the transfer of *Pseudoglochidion* to *Phyllanthus* by Webster (1994) through morphological studies. The present phylogenetic analysis using ITS and *matK* fully supports its position in the genus *Phyllanthus*.

Taxonomic treatment

Phyllanthus anamalayanus (Gamble) G.L. Webster in J. Ann. Missouri Bot. Gard. 81: 45. 1994; Balakr. & Chakrab., Euphorbiaceae in India 377. 2007; Chakrab. *et al.*, in Balakr. *et al.*, Tribe Phyllanthae: Euphorbeaceae . 462. 2012; *Pseudoglochidion anamalayanum* Gamble in Kew Bull. Misc. Inf. Kew 1925: 329–330. 1925 & Fl. Madras 2(7): 1285. 1925; Chandrabose in Henry *et al.*, Fl. Tamil Nadu Ser. 1, 2: 239. 1987; Nayar & Sastry in, Red Data Book of Indian Plants 3: 126. 1990.

Lectotype (first step by Gamble, 1925b): Aiyarpadi, March 1912, C.E.C. Fischer 3301 ([K0000246606, K0000246607] [image!]).

Lectotype (second step, **designated here**):—INDIA. ‘aiyerpadi’ (Iyerpadi): ‘anamalais’ (Anamalai hills), 4000 ft., 30.03.1912, Fischer 3301 [K0000246607 image!] Fig. 4.

A monoecious shrub/tree, up to 10 m high; branches slender, irregular; branchlets phyllanthoid. Leaves 10–14 per branchlet, distichous, ovate to lanceolate, 4–9 x 1.5–3 cm, rounded to acute at base, entire along margins, acute to acuminate at apex, glabrous, lateral nerves regular on both the sides; stipules subulate, up to 4 mm long; petioles up to 2.5 mm long. Flowers either axillary or on leafless branchlets; flowering branchlets up to 14 cm long, might continue to grow and produce leaves after flowering. Staminate flowers: in clusters; bracts and bracteoles subulate, at the base of pedicel; pedicel filiform, 5–6 mm long; tepals 6, biseriate, imbricate, ovate-lanceolate, c. 3 x 1 mm; stamens 3, joined at base by short filaments; anthers 3, c. 2 x 0.5 mm with prominent apiculate connective, yellow, extrorse; glands 6, conspicuous. Pistillate flowers: solitary or in clusters of 2–3, pedicel c. 1 mm long, thick; tepals 6, biseriate, as in staminate flowers, often unequal; ovary 3-locular, ovules 2 in each; styles fused to form a thick column of up to 3 mm when young; stigma 6-toothed; disc minute, annular. Capsules 6-lobed when mature, 8–10 mm in diameter, subglobose, glabrous, depressed at apex. Seeds three sided, dorsally curved, 4–5 mm long, dotted, hilum black spotted.

Fl. and Fr.: Throughout the year.

Distribution: Above 1400 m; restricted to Anamalai Tiger Reserve of Anamalais, Coimbatore district, Tamil Nadu (India).

Specimen examined: INDIA. **S. India:** Anamalai Hills, Monica, 1300 m. alt., 18.10.1901, C.A. Barber 3807 (MH00002524!); Anamalai Hills, Aiyarpadi, 30.03.1912, C.E.C. Fischer 3301 (K000246606 image!; K000246607 image!); **Tamil Nadu:** Coimbatore, Valparai, Iyerpadi, 23.1.2010, Janarthanam *et al.* 4951 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Janarthanam *et al.* 4952 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Naik *et al.* 4953 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Naik *et al.* 4954 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Janarthanam *et al.* 4955 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Janarthanam *et al.* 4956 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Janarthanam *et al.* 4957 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Naik *et al.* 4958 (MH); Coimbatore, Valparai, Iyerpadi, 23.1.2010, Janarthanam *et al.* 4959 (MH).

Notes

Gamble (1925 b) described the species based on collections made from South India by Barber 3807 from Anamalai hills, Monica, 1300 m. alt., and C.E.C. Fischer 3301 from Aiyerpadi. In protologue, author cited Fischer’s collection as ‘type’ and hence Barber’s collection, the other original material, is considered as paratype in accordance with Article 9.6 of the Melbourne Code (McNeill *et al.* 2012). There are two specimens of C.E.C. Fischer 3301 [K0000246606, K0000246607] housed at Kew. These two specimen are treated here as syntypes and it warrants lectotypification according to the Article 9.17 of the Melbourne Code (McNeill *et al.* 2012). On one herbarium specimen (K0000246607) author’s annotation “Phyllanthae genus nov.?” is seen. On this sheet, illustrations are also elaborate as compared to the other and specimen is better and more representative than the other. Hence, C.E.C. Fischer 3301 (K0000246607) at K (<http://specimen.kew.org/herbarium/K0000246607>) has been selected here as lectotype at second step.

Conclusion

The confirmation of the status of *Pseudoglochidion* under *Phyllanthus* is significant as World Conservation Monitoring Centre (1998) treated it as ‘CR’ under the former, since the ‘CR’ category of any higher rank such as genus/ family takes priority for conservation over species. The lectotypification and additional information provided herein will help in planning conservation programme.

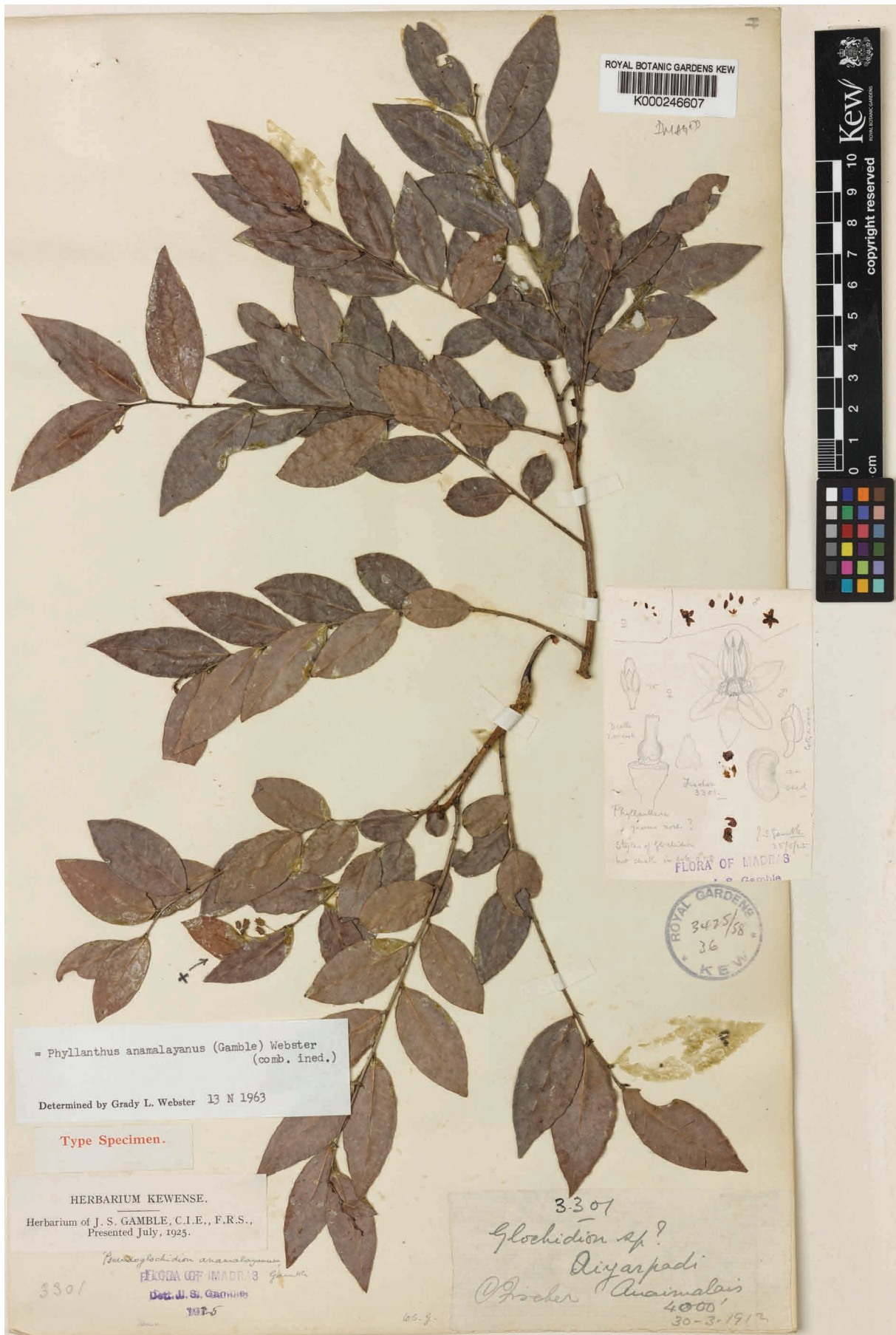


FIGURE 4. *Pseudoglochidion anomalayanum* Gamble (Fischer 3301 (K0000246607), K; Lectotype) (<http://specimens.kew.org/herbarium/K000246607>) [© RBG, Kew].

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References

- Balakrishnan, N.P. & Chakrabarty, T. (2007) *The family Euphorbiaceae in India: A synopsis of its profile, taxonomy and bibliography*, Bishen Singh Mahendra Pal Singh, India.
- Chakrabarty, T., Gangopadhyay, M. & Balakrishnan, N.P. (2012) Tribe Phyllanthae: *In*: Balakrishnan, N.P., Chakrabarty, T., Sanjappa, M., Lakshminarsimhan, P. & Singh, P. (Eds.) *Flora of India*, Vol. 23. Botanical Survey of India, Kolkata, 462 pp.
- Chase, M.W. & Hills, H.G. (1991) Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.
<http://dx.doi.org/10.2307/1222975>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9 (8): 772.
<http://dx.doi.org/10.1038/nmeth.2109>
- Felsenstein, J. (1985) confidence limits on phylogenetics: an approach using bootstrap. *Evolution* 39: 783–791.
<http://dx.doi.org/10.2307/2408678>
- Gamble, J.S. (1925a) *Flora of the Presidency of Madras*. Vol VII., Botanical Survey of India, Calcutta, 899 pp.
- Gamble, J.S. (1925b) Decades Kewenses. Plantarum Novarum in Herbario Horti Regii Conservatorum. Decas CXII Reviewed work(s): *Bulletin of Miscellaneous Information. Royal Gardens, Kew* 1925: 329–330.
- Kathriarachchi, H., Samuel, R., Hoffmann, P., Mlinarec, J., Wurdack, K.J., Ralimanana, H., Stuessy, T.F. & Chase, M.W. (2006) Phylogenetics of tribe Phyllanthae (Phyllanthaceae; Euphorbiaceae *sensu lato*) based on nrITS and plastid *matK* sequence data. *American Journal of Botany* 93 (4): 637–655.
<http://dx.doi.org/10.3732/ajb.93.4.637>
- Kathriarachchi, H., Hoffmann, P., Samuel, R., Wurdack, K.J. & Chase, M.W. (2005) Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid *atpB*, *matK*, 3′*ndhF*, *rbcL* and nuclear *PHYC*). *Molecular Phylogenetics and Evolution* 36: 112–134.
<http://dx.doi.org/10.1016/j.ympev.2004.12.002>
- Kumar, S., Nei, M., Dudley, J. & Tamura, K. (2008) MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9: 299–306.
<http://dx.doi.org/10.1093/bib/bbn017>
- McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Prud'homme van Reine, W.F., Smith, G.F., Wiersma, J.H. & Turland, N.J. (2012) *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code)*. Regnum Vegetabile 154. Koeltz Scientific Books, Königstein.
- Rajkumar, S. (2001) Studies on systematics and biology of endemic tree genera of Western Ghats India. Ph.D. Thesis submitted to Goa University, Goa, India.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Samuel, R., Kathriarachchi, H., Hoffmann, P., Barfuss, M.H.J., Wurdack, K.J., Davis, C.C. & Chase, M.W. (2005) Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* 92:132–141.
<http://dx.doi.org/10.3732/ajb.92.1.132>
- Varnostsova, M.S., Hoffman, P., Kathriarachchi, H., Kolterman, D.A. & Chase, M.W. (2007) *Andrachne cuneifolia* (Phyllanthaceae; Euphorbiaceae *s.l.*) is a *Phyllanthus*. *Botanical Journal of Linnean Society* 155: 519–525.
<http://dx.doi.org/10.1111/j.1095-8339.2007.00726.x>
- Webster, G.L. (1955) Studies of the Euphorbiaceae, Phyllanthoideae I. Taxonomic notes on the West Indian species of *Phyllanthus*. *Contributions from the Gray Herbarium of Harvard University. Cambridge* 176: 45–63.

- Webster, G.L. (1956a) Studies on the Euphorbiaceae, Phyllanthoideae II. The American species of *Phyllanthus* described by Linnaeus. *Journal of the Arnold Arboretum* 37 (1):1–14.
- Webster, G.L. (1956b) A monographic study of the West Indian species of *Phyllanthus*. *J Journal of the Arnold Arboretum* 37 (2): 91–122, 217–263, 340–359.
- Webster, G.L. (1957) A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* 38: 51–64, 170–198, 295–373.
<http://dx.doi.org/10.5962/bhl.part.9105>
- Webster, G.L. (1958) A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* 39: 49–200, 111–212.
<http://dx.doi.org/10.5962/bhl.part.19111>
- Webster, G.L. (1967) Genera of Euphorbiaceae in Southern United States. *Journal of the Arnold Arboretum* 48: 303–361; 363–430.
- Webster, G.L. (1970) A revision of *Phyllanthus* (Euphorbiaceae) in the continental United States. *Brittonia* 22: 44–76.
<http://dx.doi.org/10.2307/2805721>
- Webster, G.L. (1994) Synopsis of the genus and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* 81: 33–144.
<http://dx.doi.org/10.2307/2399909>
- Webster, G.L. & Airy-Shaw, K.H. (1971) A provisional synopsis of the New Guinea taxa of *Phyllanthus* (Euphorbiaceae). *Kew Bulletin* 26 (1): 85–109.
<http://dx.doi.org/10.2307/4117335>
- Wurdack, K, Hoffmann, P., Samuel, R., Bruijn, A., Bank, M. & Chase M.W. (2004) Molecular phylogenetic analysis of Phyllanthaceae (Phanthoideae Pro Parte, Euphorbiaceae sensu lato) using plastid rbcL Dna sequences. *American Journal of Botany* 91 (11): 1882–1900.
<http://dx.doi.org/10.3732/ajb.91.11.1882>
- World Conservation Monitoring Centre (1998) *Pseudoglochidion anamalayanum*. *The IUCN Red List of Threatened Species* 1998: e.T33643A9800121.