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RESEARCH ARTICLE

Phylogenetic relationships of *Gordonia* Ellis (Theaceae) species endemic to Sri Lanka

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Abstract: A study of the genus *Gordonia* Ellis was carried out using morphological data to determine the phylogenetic relationships of the four endemic species, *Gordonia ceylanica* Wight, *G. elliptica* Gardner, *G. speciosa* (Gardn.) Choisy, and *G. dassanayakei* Wadhwa *et* Weerasooriya. Vegetative and reproductive features were studied in detail from live and herbarium specimens in order to select characters and character states. The phylogenetic analysis was performed using PAUP* computer soft ware. The present analysis using 77 morphological characters recovered a strongly supported monophyletic genus with the endemic *Gordonia* species. Even though most of the past taxonomic treatments recognized *G. ceylanica* Wight and *G. elliptica* Gardner as two distinct species, both species did not receive any support as being monophyletic. *G. speciosa* (Gardn.) Choisy and *G. dassanayakei* Wadhwa *et* Weerasooriya were recovered as monophyletic groups. The study revealed several confusing character combinations that overlap among these species, especially between *G. elliptica* and *G. ceylanica* questioning their species limits. Therefore, this study emphasizes the necessity of carrying out further studies on species limits of *Gordonia* with more informative characters such as molecular data.

Keywords: Cladistics, *Gordonia* Ellis, monophyly, morphological data, Sri Lanka

INTRODUCTION

Family Theaceae harbours approximately 22 genera and about 610 species in the tropical and warm temperate areas of the world[1]. In Sri Lanka, the family is represented by five genera and 12 species, where the well-known tea plant *Camellia sinensis* (L.) Kuntze is also a member. The genus *Gordonia* is not endemic to Sri Lanka and is distributed in South East Asia and America. About 40 species are found from India, Sri Lanka, Burma, Thailand, Indo-China, South China, Taiwan, Malaysia, Philippine Islands and New Guinea. Thirty species are also present in North and South America, and the West Indies. The four species that occur in Sri Lanka[2] are endemic to the island and includes *G. ceylanica* Wight, *G. elliptica* Gardner, *G. speciosa* (Gardn.) Choisy, and *G. dassanayakei* Wadhwa *et* Weerasooriya. Of the four species *G. ceylanica* and *G. elliptica* show a wide distribution when compared with the other two species. Both *G. speciosa* and *G. dassanayakei* are not common and are confined to a few localities. Apart from these four species, several young plants of *G. axillaries* (Ker-Gawl.) D. Diter. have been reported from the forest adjoining the Hakgala Botanical Garden testifying the naturalization of the species within the country[3]. Further, all four species of *Gordonia* are confined to the upper montane areas of the country.

Based on recent developments, circumscription of the traditional Theaceae has been changed. Theaceae is classified under the monophyletic order Ericales under the asterid clade[4]. The family as traditionally circumscribed, probably are not monophyletic[4,5]. Based on molecular based cladistic analyses the family is restricted to the genera traditionally placed in Theoideae[5-7]. Further three monophyletic tribes *Theeae*, *Gordonieae* (including the genus *Gordonia*) and *Stewartieae* have been identified.

G. ceylanica was first described by Wight in 1840. Seven years later, in 1847, George Gardner, the then Director of the Royal Botanic Gardens, Peradeniya, described two new species, *Carria speciosa* from the Ramboda area and *G. elliptica* from Elephant plains -Nuwara-Eliya district. *Carria speciosa* was later recognized as a member of *Gordonia* in 1855 and was named accordingly as *G. speciosa*[2]. Thwaites in 1858[8] reduced *G. elliptica* as a variety under *G. ceylanica*; *G. ceylanica* var. *elliptica* (Gardner) Thw. He also recognized two

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species, i.e. *G. ceylanica* and *G. speciosa* and included *G. elliptica* under *G. ceylanica* as a variety. During the recent revision of the flora Wadhwa (1996), identifies four species of *Gordonia*; *G. ceylanica*, *G. speciosa*, *G. elliptica* and a newly recognized fourth species *G. dassanayakei* [2]. This species with pink flowers has been named in honour of Prof. M.D. Dassanayake, for his valuable contribution to the plant taxonomy of Sri Lanka.

The presence of large fragrant flowers is a characteristic feature of all *Gordonia* species. *G. ceylanica* and *G. elliptica* both bear white coloured flowers that closely resemble the flowers of *C. sinensis* (tea plant). The shape of their leaves is the character used to distinguish the two species from each other[2]. *G. elliptica* bear elliptic leaves as the name implies while *G. ceylanica* bear lanceolate leaves. *G. speciosa* bears the most remarkable bright crimson colored large flowers, which easily distinguishes them from the other 3 species. *G. dassanayakei* is also distinguished from others through its flowers, which are pink in colour (Figures 1-4).

METHODS AND MATERIALS

Materials: All recorded locations for the occurrence of *Gordonia* species together with other possible locations were visited periodically in order to collect plants with vegetative and reproductive parts. In addition, three herbarium specimens for each taxa were also included in the study. *Symplocos cochinchinensis* (Lour.) S. Moore (Symplocaceae) was selected as the out-group based on a study on the phylogenetic relationships of Theaceae[5]. The details of herbarium materials and voucher specimens are given in Table 1.

Methods: Vegetative and reproductive features were studied in detail in order to select characters and character states. Flowers were immersed in 70% alcohol at the time of collection, to preserve characters. All the four floral appendage series were studied separately in each flower, and characters were recorded. Observations were done under the light and stereo microscope as well as under the dissecting microscope. A total of 93 characters were coded into discrete states. Some characters were coded as binary variables and most as multi-state due to extended variation (Table 2). Of the 93 coded characters, only 77 characters were used in the analysis, as the others were not informative.

Data coding and cladistic analysis: The characters were coded into a data matrix using the MacClade 3.04 program[9]. Phylogenetic trees were constructed using the PAUP* 4d55 for Macintosh[10]. For all analyses heuristic searches were performed initially under the unordered and equal weighting criteria of Fitch parsimony[11] with 500 replicates, random sequence

Figure 1: Flower of *G. ceylanica* Wight

Figure 2: Flower of *G. speciosa* (Gardn.) Choisy

Figure 3: Flower of *G. dassanayakei* Wadhwa *et* Weerasooriya

Figure 4: Flower of *G. elliptica* Gardner

additions, tree bisection-reconnection (TBR) branch swapping and MULPARS in effect, steepest descent on. Ten trees were held for each step. Strict consensus and 50% majority rule consensus trees were obtained and branch lengths and tree scores were calculated using ACCTRAN (accelerated transformation optimisation). The initial trees found with equal (Fitch) weights were used as the basis for successive weighting. Successive weighting was carried based on the Retention Index. Re-weighting was continued until the same length was obtained in two successive rounds. Bootstrap analysis was carried out to evaluate the support for the groupings[12].

Table 1: Location and the date of collection of the field specimens and the voucher information of the herbarium specimens, National Herbarium, Royal Botanic Gardens, Peradeniya used for the morphological analyses. The herbarium specimens are indicated by the abbreviation PDN in brackets.

Species	Voucher/specimen number	Location	Date of collection
G. ceylanica Wight	GC1	Knuckels Range (Corbet's Gap)	18.02.2004
	GC2	Morning Side-Sinharaja	06.03.2004
	GC3	Piduruthalagala	31.10.2004
	GC4	Adisham-Haputhle	01.02.2004
	GC5	Adisham-Haputhale	03.05.2005
	GC6	Thangamali Plains - Haputhale	03.05.2005
	GC7	Hakgala	03.05.2005
	GC8	Mahakoodagala Estate - Kandapola	03.05.2005
	GC9	Mahakoodagala Estate	03.05.2005
	GC10	Nuwara-Eliya (Keena Rd.)	22.05.2005
	GC11	Nuwara- Eliya (Keena Rd.)	22.05.2005
	Jayasuriya & Bandaranayake, 1763 (PDN)	Midlands, Matale	23.07.1974
	Nowicke & Jayasuriya 7945 (PDN)	Hakgala, Nuwara Eliya	30.06.1973
	Jayasuriya & Karunaratne 8252 (PDN)	Kandapola-Sita Eliya Forest Reserve	07.09.1994
G. elliptica Gardner	GE1	Knuckles Range (Corbet's Gap)	09.05.2005
	GE2	Rattota-Illukkumbura Rd.	07.05.2005
	GE3	Rattota-Illukkumbura Rd.	07.05.2005
	GE4	Thangamali Plains - Haputhale	03.05.2005
	Huber,797 (PDN)	Hoolankande Estate, Madulkele	20.08.1978
	Kostermans 25066 (PDN)	Knuckles, Madulkele	12.06.1973
	Wadhwa, Weerasooriya & Samarasinghe 501 (PDN)	Rattota-Illukkumbura Road	23.11.1994
G. speciosa(Gardn.) Choisy	GS1	Fishing Huts - Maskeliya	15.05.2004
	GS2	Adams Peak trail *via*. Rathnapura	13.02.2005
	GS3	Adams Peak trail *via*. Kuruwita	13.02.2005
	Jayasuriya, Balasubramaniam, Greller, S. & N. Gunatilleke 2835 (PDN)	Peak Wilderness, Meriyakota	16.08.1984
	Balakrishnan 592 (PDN)	Moray Estate, Maskeliya	03.02.1971
	Jayasuriya & Sumithraarachchi 1563 (PDN)	Adams Peak Wilderness, Moray Estate Estate	27.03.1974
G. dassanayakei Wadhwa *et* Weerasooriya	GD1	Thangamali Plains - Haputhale	03.02.2005
	GD2	Thangamali Plains	03.02.2005
	GD3	Thangamali Plains	03.02.2005
	Huber 685 (PDN)	West slopes of Knuckles - Bambarella	22.11.1977
	Wadhwa &Weerasooriya 380 (PDN)	Namunukula Hill forest	05.12.1992
	Wadhwa &Weerasooriya 126 (PDN)	Namunukula Hill forest	16.10.1992

Table: 2: Qualitative and quantitative characters assessed for the phylogenetic analyses

Character number	Description
Vegetative characters	
1.	colour of the dry bark
2.	colour of the live bark
3.	fissured bark
4.	vertical ridges
5.	branchlets pilose
6.	leaves crowed at extremities
7.	leaf arrangement
8.	leaf shape
9.	leaf tapering
10.	texture of the leaf
11.	leaf margin
12.	leaf apex
13.	glandular point
14.	mid-rib channeled on the upper surface
15.	hairs along the mid-rib
16.	veins obsolete
17.	hairs on the upper surface of the lamina
18.	hairs on the lower surface of the lamina
19.	coloured flush
20.	hairs present on the young leaves
21.	hairs present on the mid-rib of the flush
22.	hairs on the leaves unicellular unbranched hairs
23.	nature of the petiole
24.	length of the petiole
Floral characters	
25.	axillary flowers
26.	solitary flowers
27.	flower diametre
28.	flower pedicle
29.	average length of the pedicle
30.	flower colour
31.	sepal number
32.	orbicular sepals
33.	texture
34.	persistent sepals
35.	concave sepals
36.	two large inner sepals
37.	sepal arrangement
38.	petal shape
39.	petal number
40.	petal arrangement
41.	pubescent present on the upper surface
43.	pubescent present on the lower surface
44.	connate at the base
45.	Stamens numerous

Character number	Description
46.	Stamens clustered
47.	Stamens in 3 whorls
48.	stamens free
49.	stamens adnate to the base of the petals
50.	stout filaments
51.	length of the filaments
52.	pubescent on the filaments
53.	shape of the anthers
54.	versatile anthers
55.	connective broad
56.	ovary position
57.	no. of locules
58.	hairs on the ovary
59.	unicellular, unbranched hairs
60.	ovule arrangement
61.	arrangement of ovules in two rows
62.	style length
63.	stout style
64.	fused style
65.	hairs on the style
66.	no. of lobes of the stigma
67.	capsule texture
68.	capsule shape
69.	no. of angles in the capsule
70.	persistent sepals
71.	hairs on the fruit
72.	unicellular, unbranched hairs
73.	size of the fruit
74.	fruit apiculate
75.	dehiscence
76.	winged seeds
77.	flattened seeds

RESULTS

Heuristic search under the Fitch criterion yielded 42 most parsimonious trees (MPTs) with 220 steps, Consistency Index (CI) = 0.447 and Retention Index (RI) = 0.686 (figures not shown). The successive weighting resulted in a single most parsimonious tree with a length of 143.498, CI of 0.321 and RI of 0.910 (Figure 9). The genus is recovered as a monophyletic group with strong support (Bootstrap value = 100%). The four species of *Gordonia* has not been recovered as monophyletic. Only *G. speciosa* and *G. dassanayakei* were recovered as monophyletic groups with 96% and 73% bootstrap support respectively. Majority of *G. ceylanica* individuals were recovered as monophyletic but with no support. However, a small monophyletic group of four individuals receives a 60% bootstrap support. This large group also included an individual of *G. elliptica*. The other two individuals were scattered, where one occurred as the sister to the large monophyletic clade *G. dassanayakei* and the large clade that includes the majority of *G. ceylanica*, while the other occurs as the basal most taxa for the genus. Majority of *G. elliptica* individuals occur as monophyletic clade together with *G. speciosa* which is nested as a strongly supported group. The rest of the individuals occur as basal taxa for the genus while one occurs within the *G. ceylanica* clade.

DISCUSSION

The present analyses using 77 morphological characters recovered a strongly supported monophyletic genus with the endemic *Gordonia* species. Although most of the past taxonomic treatments recognized *G. ceylanica* and *G. elliptica* as two distinct species, both species did not receive any support as being monophyletic. *G. speciosa* and *G. dassanayakei* were recovered as monophyletic groups. Even though a majority of *G. ceylanica* is recovered an a monophyletic group, this clade does not receive support. Further an authenticated herbarium specimen of *G. elliptica* (Huber, 797) is within the clade. It is interesting to note that the scattered individuals of both *G. ceylanica* (Jayasuriya and Bandaranayake, 1763) and *G. elliptica* (Huber, 797 and Kostermans, 25066, Wadhwa, Weerasooriya & Samarasinghe 501) are herbarium specimens that were cited under each of these species as 'examined specimens' during the revision of the family for the Revised Handbook to the Flora of Ceylon[2]. Considering the two taxa, *G. elliptica* individuals are the most displaced. Several reasons may attribute to this situation. In many circumscriptions, the distinction between the two species are based on few characters; the latest revision employs the leaf shape and the presence of hairs on the young leaves and branches to distinguish *G. ceylanica* from *G. elliptica* and *G. dassanayakei*. However, during the present study, populations of *G. ceylanica* were encountered with glabrous young leaves and branches (specimens collected from Pidurutalagala). During these instances, the leaf shape will be the only character that will distinguish the two species. During the detailed study of morphological characters too it was noted that many characters overlap. Distinguishing between *G. elliptica* and *G. dassanayakei* is straight forward with the white flowers; elliptic leaves and the slightly revoluted margin in the lower part of the leaves of the former. Considering the leaf shapes, although shape seems to be consistent for a given species, during coding of data it was apparent that the shapes showed a continuum. Further, the leaf shapes of *G. elliptica* and *G. dassanayakei* were overlapping in many instances, with the only difference being the difference in size. In such a case as this, the flower colour becomes the only parameter that differentiates *G. elliptica* and *G. dassanayakei*. Studying herbarium specimens in detail at the National Herbarium, Royal Botanic Gardens, Peradeniya also had proved this argument. Several specimens that had been identified/ authenticated for species level during the revision of the flora had key characters that were overlapping. The specimen that is identified as *G. ceylanica*, (Weerasooriya, Samarasinghe and Karunaratne, 116) is similar to *G.dassanayakei* in all aspects except the leaf shape, which is lanceolate. Even the flower colour is indicated as pink. One may argue that this should remain under *G.ceylanica* since the leaf shape is lanceolate, but then the question arises as to whether the flower colour should not be taken into consideration when assigning a species? Further, *G. ceylanica* (Kostermans

Figure 5: Fruits of *G. dassanayakei* Wadhwa *et* Weerasooriya

Figure 6: Fruits of *G. elliptica* Gardner

Figure 7: Fruit of *G. ceylanica* Wight

Figure 8: Fruit of *G. speciosa* (Gardn.) Choisy

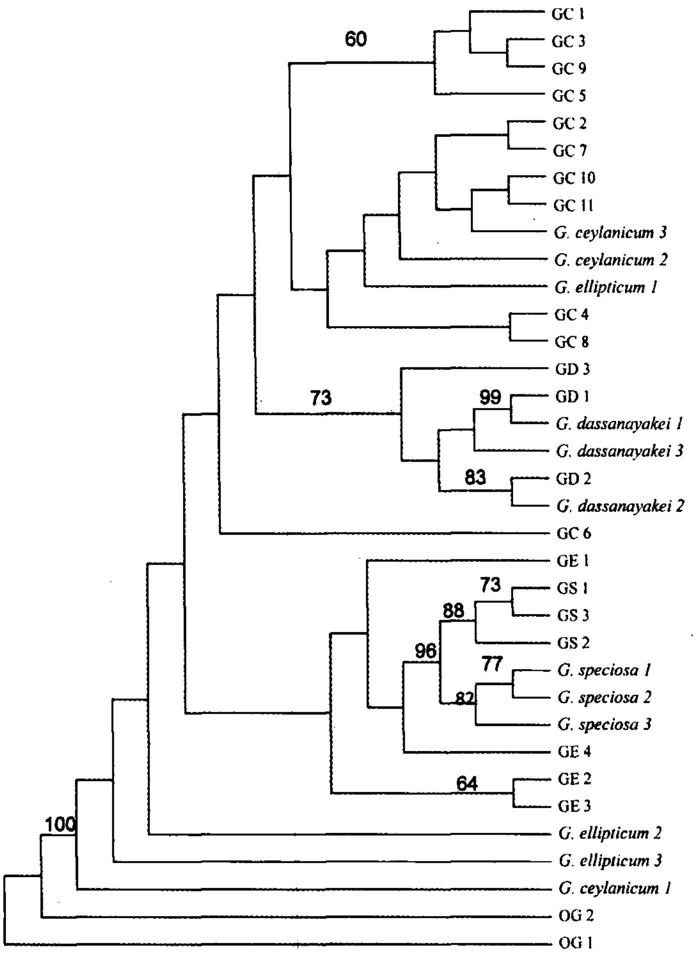

Figure 9: The single most parsimonious tree recovered during the successive weighting of the morphological data. Length = 143.498, steps, CI = 0.321 and RI = 0.910. The bootstrap support values are shown above the branches. GC 1-3, GE 1-3, GS 1-3 and GD 1-3 - field collected specimens of *G. ceylanica*, *G. elliptica*, *G. speciosa* and *G. dassanayakei* respectively while the others are herbarium specimens, where the details are given in Table 1. OG – out group taxa.

25099) is another specimen with the flower colour indicated as purple. Nooteboom 3413 is identified as *G. ceylanica* but this specimen bearing elliptic leaves shows close resemblance to *G. elliptica*. Similarly, Waas 1614 is determined as *G. elliptica*. But it is similar to *G. ceylanica* in all aspects. Even the leaf shape is lanceolate. Therefore, it is evident that the identification of *G. elliptica, G. ceylanica* and *G. dassanayakei* does have problems with certain morphological characters. These problems of overlapping characters would have been one reason for not recovering monophyletic groups. Further, these characters which were considered may not have evolutionary significance. *G. dassanayakei* is recovered with moderate support. *G. dassanayakei* together with a majority of *G. ceylanica* contributes to a larger monophyletic group with no support. *G. speciosa* has received strong support as monophyletic. This group is placed once again within a large monophyletic group together with *G. elliptica,* where the large clade has not received support.

According to the present study and detailed character analysis, only *G. speciosa* could be unequivocally placed into a species. *G. speciosa* had clear-cut, well defined characteristics of its own, while the other three species had characteristics that seemed to overlap with each other. Considering the leaf shapes, *G. speciosa* leaves had a consistent, unique, characteristic appearance with highly revolute margins and a thick, shiny leaf lamina, which set it apart from the rest. Comparing the fruit shapes (Figures 5-8) it could be seen that the fruits of *G. speciosa* had a unique pyramidal shape, while the fruits of *G. elliptica*, *G. dassanayakei* and *G. ceylanica* all had a similar globose appearance. Comparison of flowers of the four species further emphasizes on the above-mentioned argument. Flowers of *G. speciosa* are very large when compared to the flowers of the other three species and are very attractive and conspicuous with a bright crimson colour. But the flowers of the other three species did not vary much from each other. As it can be seen from Figures 1-4, the basic shape of the three flowers other than *G. speciosa* are the same with *G. elliptica* and *G. ceylanica* being almost identical. *G. dassanayakei* varies only by its colour.

In 1847, Gardner recognized *G. elliptica* first as a *Carria* species and next as a *Gordonia*, but later Thwaites reduced this species to a variety[2]. This was also adopted by Trimen[13]. After hundred and three years, it was only during the revision of the Trimen's Flora that the species was resurrected[2]. The positions of both *G. elliptica* and *G. ceylanica* are not supported during the analysis. Therefore, based on the present study, re-evaluation of the species limits of the *Gordonia* is suggested as it is the basis of biodiversity conservation and management plans.

CONCLUSION

The phylogenetic analyses of the endemic *Gordonia* species using morphological data recovered a strongly supported monophyletic genus. *G. speciosa* and *G. dassanayakei* are monophyletic whereas *G. elliptica* and *G. ceylanica* are non-monophyletic.

The study revealed several confusing character combinations that overlap among these species, especially between *G. elliptica* and *G. ceylanica* questioning their species limits. This emphasizes the necessity of carrying out further studies on species limits of *Gordonia* that are endemic to Sri Lanka.

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