

---

***Caliciopsis indica* sp. nov. from India**

---

**Pratibha J<sup>1,2</sup>, Amandeep K<sup>3</sup>, Shenoy BD<sup>3\*</sup> and Bhat DJ<sup>1\*</sup>**<sup>1</sup>Department of Botany, Goa University, Taleigao Plateau, Goa - 403 206, India. bhatdj@rediffmail.com<sup>2</sup>MykoTech Pvt. Ltd., Plot No. 12, Mapusa Industrial Estate, Mapusa, Goa – 403 507, India<sup>3</sup>Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39A, Chandigarh – 160036, India. shenoy@imtech.res.in

Pratibha J, Amandeep K, Shenoy BD, Bhat DJ 2010 – *Caliciopsis indica* sp. nov. from India. Mycosphere 1, 65–72.

*Caliciopsis indica* sp. nov. is described from leaf lesions of kokum (*Garcinia indica*, Clusiaceae) from the Western Ghats, India. *Caliciopsis indica* is morphologically similar to *C. myrticola* but differs in having larger ascomata, longer asci and smaller ascospores. Phylogenetic analysis of partial 28S rRNA gene sequence data has confirmed its placement within the Coryneliaceae (Coryneliales, Eurotiomycetes). The ITS/5.8S rRNA gene sequence, however, did not provide any clarity on the species delineation due to lack of reference sequences in GenBank.

**Key words** – biodiversity – bitunicate ascomycete – forest ecology

---

**Article Information**

Received 12 April 2010

Accepted 15 April 2010

Published online 30 April 2010

\*Corresponding author: Bhat DJ – e-mail – bhatdj@rediffmail.com and Shenoy BD –e-mail – shenoy@imtech.res.in

---

**Introduction**

Kokum (*Garcinia indica* Choisy, Clusiaceae) is an economically important plant bearing edible fruits and indigenous to the Western Ghats of India (Korikanthimath & Desai 2005, Bhat et al. 2006). During our studies on foliicolous fungi on forest plants of Goa and neighbouring regions of Western Ghats in southern India (Pratibha et al. 2004, Pratibha & Bhat 2005, Pratibha 2006), we collected an ascomycete on leaf lesions of Kokum. The leaf lesions were apparently formed by insects grazing on mature leaves. The fungus was first collected from Mashem, Canacona in Goa; additional specimens were collected from neighbouring Karnataka (Ankola, Uppinangadi), indicating that the fungus might be distributed in most regions where Kokum is grown. The fungus is characterized by superficial non-setose perithecia with a prominent stalk, and aseptate light brown ascospores enclosed in bitunicate,

saccate, long-pedicellate asci that lack apical structures. Though the fungus is morphologically similar to *Caliciopsis myrticola* Huguenin (Benny et al. 1985), it is described as a novel species of *Caliciopsis* based on differences in dimensions of ascomata, asci and ascospores. A note on its phylogenetic placement based on partial 28S rRNA gene sequence data is also included.

**Methods****Isolates and morphology**

Fresh leaves of kokum with greyish-centered brown patches or spots were collected and taken to the laboratory in zip-lock polythene bags. Fungal material found growing along the brown margin of leaf spots was carefully picked up with a fine-tipped needle and mounted on a slide containing a drop of lactophenol solution or water as mountant and examined under a light microscope. Part of the

material was air-dried and placed in labelled paper bags along with a piece of naphthalene pellet to maintain as herbarium specimens. The specimens were deposited in the Botany Herbarium of Goa University (GUBH). The fungus was isolated in pure culture by ascospore isolation method of Choi et al. (1999). An ascoma was placed in a drop of sterile distilled water and cut open. The ascoma debris was removed from the slide. Mature ascospores were streaked in malt extract agar plates by a fine-tipped needle. Ascospores germinated within 24 h. Colonies developed from germinated ascospores were individually transferred into MEA slants using a fine-tipped needle. A culture was deposited at Fungus Culture Collection of Goa University (GUFCC).

#### DNA extraction, PCR and sequencing

The fungal isolate was grown on potato dextrose agar (PDA) medium for 7 days just before total genomic DNA was extracted using ZR Fungal/Bacterial DNA Kit (Zymo Research, catalogue number D6005). DNA amplification was performed by polymerase chain reaction (PCR). The ITS4-ITS5 and LROR-LR5 (White et al. 1990) primer-pairs were used to amplify the internal transcribed spacers (ITS)/ 5.8S rRNA gene region and partial 28S rRNA gene, respectively. Amplification reactions were performed in a 50 µl reaction volume as outlined by Shenoy et al. (2006). The PCR thermal cycle was as follows: 95°C for 3 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 30s and elongation at 72°C for 1 min, with a final extension step of 72°C for 10 min. The PCR products spanning approximately 900 bp (for 28S rRNA gene) and 600 bp (for ITS/5.8S rRNA gene) were checked on 1% agarose electrophoresis gel stained with ethidium bromide. PCR products were then purified using quick spin column and buffers (washing buffer and elution buffer) according to the manufacturer's protocol (QIA quick gel extraction kit, catalogue number 28706). DNA sequencing was performed using the above mentioned primers in an Applied Biosystem 3130 xlanalyzer at Central DNA sequencing facility of Institute of Microbial Technology, Chandigarh.

#### Sequence alignment and phylogenetic analysis

Sequences obtained from the respective primers were aligned using Sequencher version 4.9 (Gene Codes Corporation) and the consensus sequences were deposited in GenBank with accession numbers GQ259980 (partial 28S rRNA gene) and GQ259981 (ITS/5.8S rRNA gene region). A dataset based on 28S rRNA gene sequence was prepared using MEGA4 (Tamura et al. 2007). Additional reference sequences retrieved from GenBank and their accession numbers are listed in Fig. 3. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007). Phylogenetic relationships of *Caliciopsis indica* were analysed based on Neighbor-Joining method (Saitou & Nei 1987). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option) (Tamura et al. 2007).

#### Taxonomy

*Caliciopsis indica* J. Pratibha & Bhat, **sp. nov.**

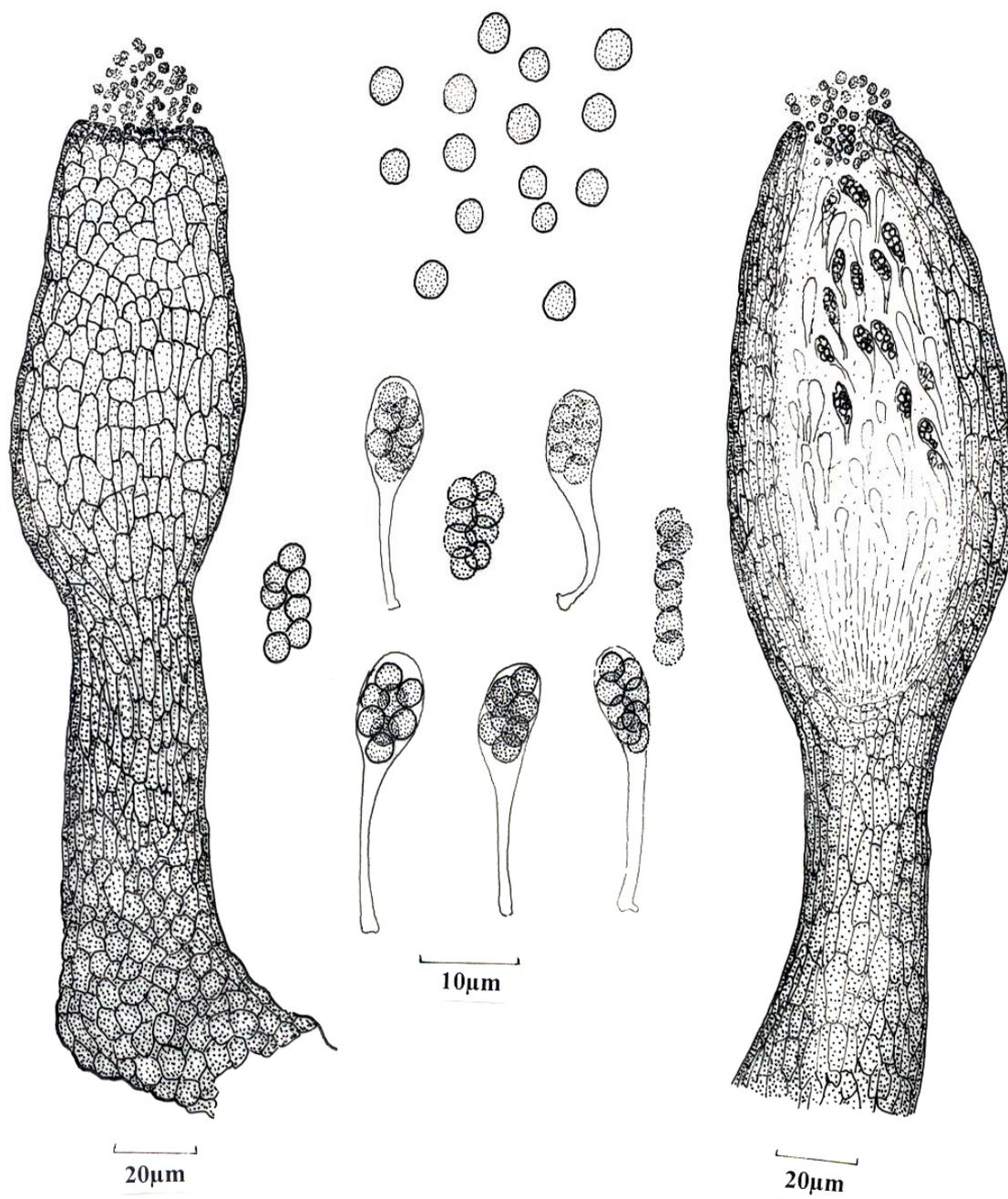
Figs 1–2

Mycobank 501254

Etymology – named for its origin country, India.

Ascomata perithecialis, superficialia, pedunculati, solitaria, clavatum, atro-brunnea, ostiolata, 205–300 × 35–70 µm; papilla absens; setis absens; pedunculus erectus, cylindricus, atro-brunnea; stroma absens. Filamenta hamathecii nulla. Asci bitunicati, octospori, uteriformis, ad apicem rotundus, pedunculati, 18–28 × 4–7 µm, apice non amyloideo, annulo nullo. Ascosporae globosa, laevis, pallide brunnea, unicellulares, 2.5–4.5 µm diam.

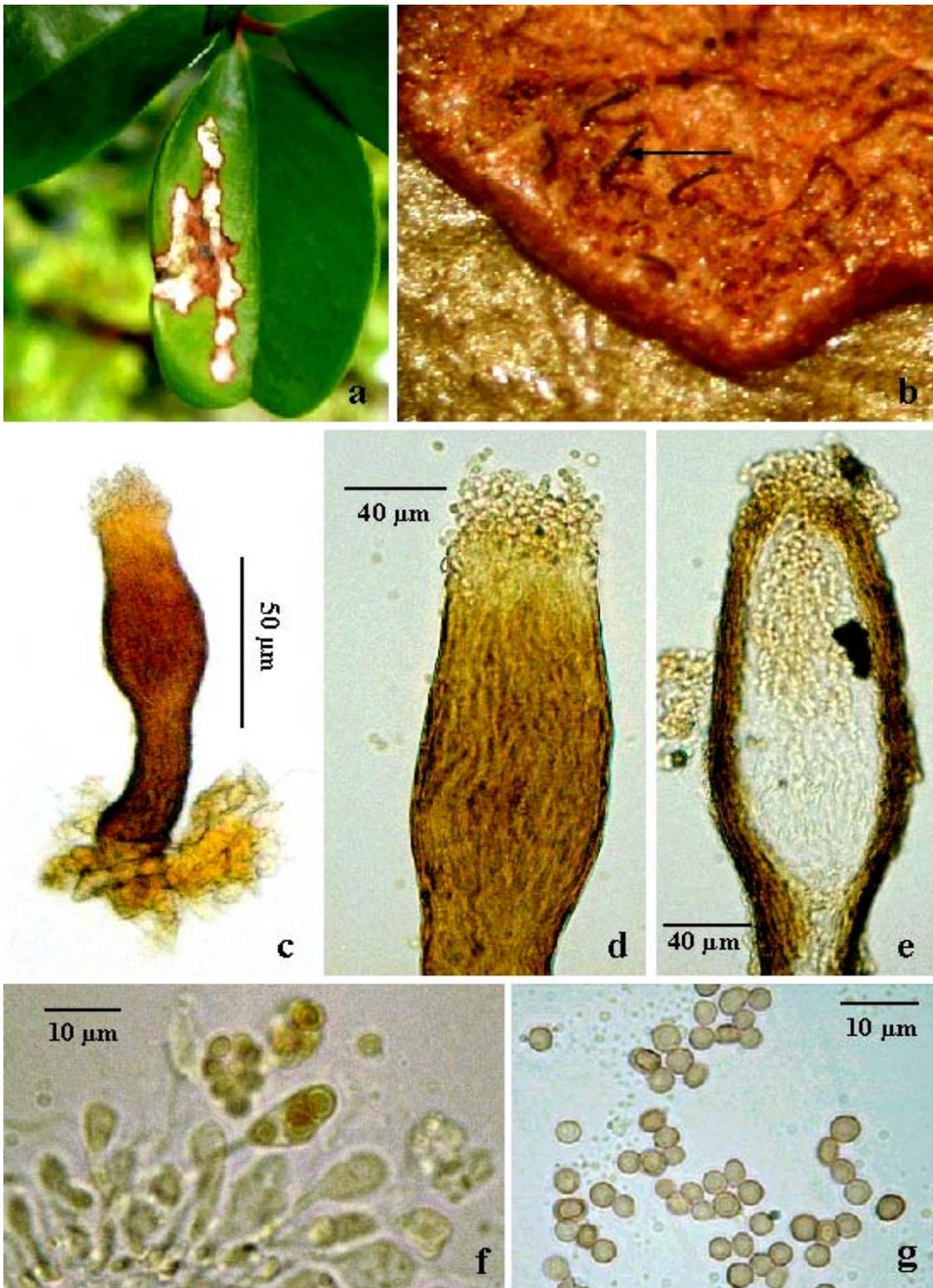
Lesions epiphyllous, irregular, greyish in the center, brown towards the margin, 0.2–1.5 × 0.6–1 cm. Colonies on MEA medium slow-growing, attaining a diameter of 0.4 cm in 7 days, flat, velvety, pale brown, with irregularly serrated margin, reverse medium brown. Mycelium immersed, composed of septate, branched, hyaline to subhyaline, smooth, 2–4 µm wide hyphae. Ascomata perithecial,



**Fig.1.** *Caliciopsis indica*: Stalked ascocarp, L.S. of ascocarp, asci and ascospores.

superficial, with a prominent stalk, solitary, dark brown, velvety, surface rough, with an elongate wide ostiole at the apex,  $205\text{--}300 \times 35\text{--}70 \mu\text{m}$ ; stalk erect, cylindrical, dark brown, slightly wider at the basal region, narrower below the venter (ascoma proper),  $110\text{--}140 \times 35\text{--}40 \mu\text{m}$ ; venter sitting on the end of the stalk, oval, dark brown, wider in the middle and narrow on either ends,  $70\text{--}100 \times 45\text{--}70 \mu\text{m}$ ;

ostiole cylindrical, moderately brown, truncate at the apex,  $25\text{--}55 \times 40\text{--}50 \mu\text{m}$ . Stroma absent. Peridium soft, outwardly composed of 2–3 layers of brown, thick-walled, polygonal cells ( $2\text{--}3.5 \mu\text{m}$  diam.) and inwardly composed of 4–5 layers of thin-walled, elongate cells ( $2\text{--}3 \times 2\text{--}4.5 \mu\text{m}$ ). Interthelial filaments not observed. Asci bitunicate, 8-spored, saccate, apically rounded, pedicellate,



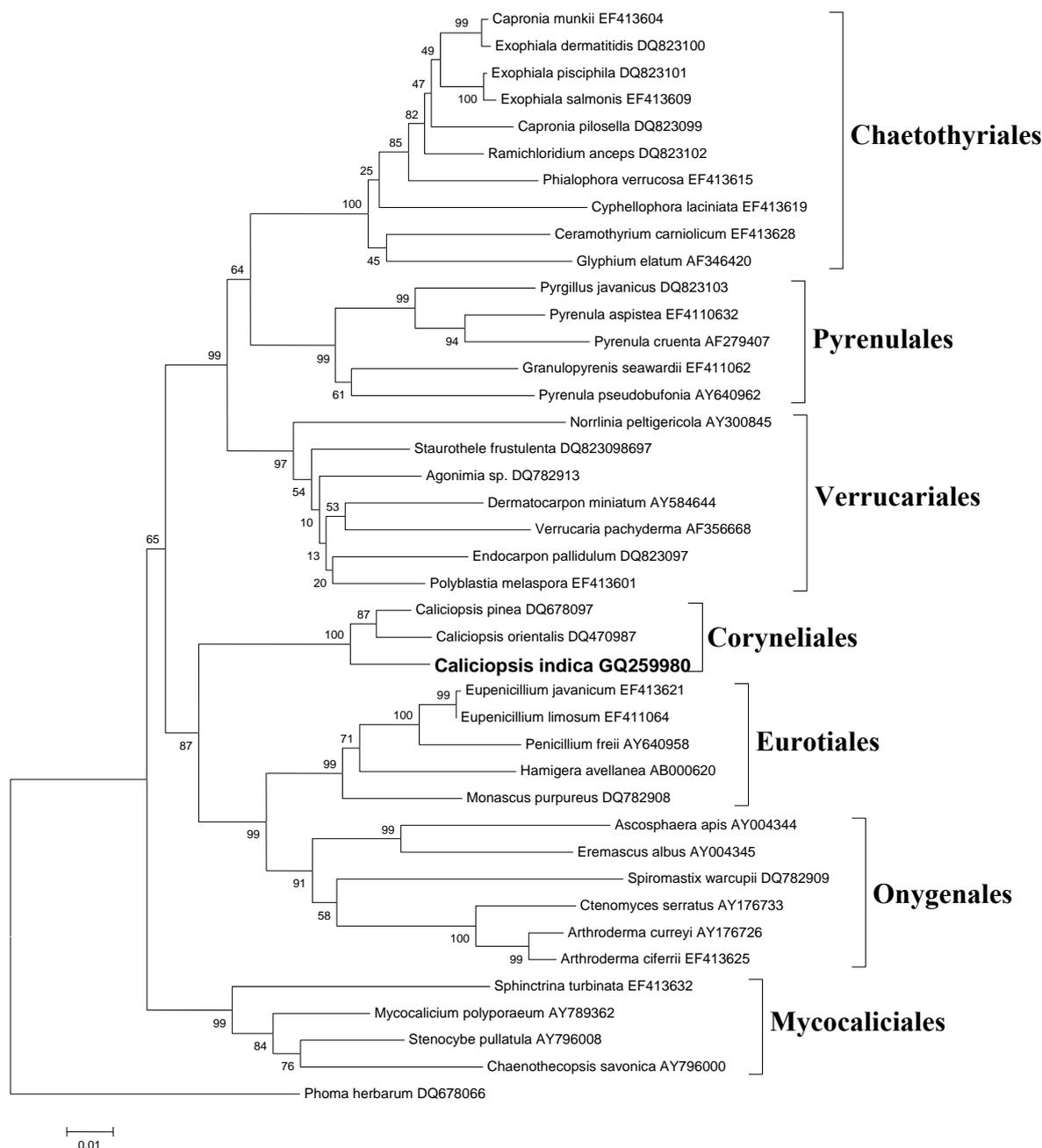
**Fig. 2.** *Caliciopsis indica*, **a.** leaf lesion, **b.** ascomata (arrowed, under stereoscope), **c.** stalked ascocarp, **d.** ascocarp, **e.** L.S. of ascocarp, **f.** asci, **g.** ascospores.

18–28 × 4–7 µm. Ascus apex non-amyloid, lacking apical ring or any discharge mechanism. Ascospores globose, smooth, light brown, aseptate, 2.5–4.5 µm diam., sometimes arranged in two rows.

Anamorph – Not seen.

Known distribution – known from Goa and Karnataka states of India.

Holotype – India, Goa, Canacona, Mashem, on leaves of *Garcinia indica*, 2 January 2005, P. Ashish, Herb. GUBH No. P166.



**Fig. 3.** Evolutionary relationships of *Caliciopsis indica* sp. nov. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The optimal tree with the sum of branch length = 1.44731930 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 909 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

**Table 1.** Comparison of *Caliciopsis indica* and similar species.

<b>Taxon</b>	<b>Ascocarps</b>	<b>Asci (<math>\mu\text{m}</math>)</b>	<b>Ascospores (<math>\mu\text{m}</math>)</b>	<b>Host</b>	<b>Distribution</b>	<b>Ref.</b>
<i>C. ellisii</i> Sacc.	1500 $\mu\text{m}$	15–20 $\times$ 8–11	6–7 $\times$ 3–3.5	<i>Populus</i> spp.	Idaho, Montana, Washington	1
<i>C. myrticola</i> Huguenin	500–620 $\mu\text{m}$	11.4–17.7 $\times$ 4.7–6.2	1.8–3.1 diam.	<i>Myrtus emarginata</i>	New Caledonia	1
<i>C. podocarpi</i> B. Huguenin	400–750 $\mu\text{m}$	11.4–17.7 $\times$ 4.7–6.2	3.1–3.6 $\times$ 2.6–3.6	<i>Podocarpus minor</i>	New Caledonia	1
<i>C. rapanae</i> B. Huguenin	up to 2000 $\mu\text{m}$	11.4–17.7 $\times$ 4.7–6.2	5.5–7.8 diam.	<i>Rapanea lanceolata</i>	New Caledonia	1
<i>C. veillonii</i> B. Huguenin	800–1500 $\mu\text{m}$	11.4–17.7 $\times$ 4.7–6.2	3.6–4.2 $\times$ 3.1–3.6	Undetermined host	New Caledonia	1
<i>C. xanthostemonis</i> B. Huguenin	337–411 $\mu\text{m}$	11.4–17.7 $\times$ 4.7–6.2	3.1–4.7 diam.	<i>Xanthostemonis baudouinii</i>	New Caledonia	1
<i>C. indica</i> sp. nov.	205–300 $\mu\text{m}$	18–28 $\times$ 4–7	2.5–4.5 diam.	<i>Garcinia indica</i>	India	2

1 = Benny et al. 1985; 2 = this paper

Additional specimens examined: (i) India, Karnataka, Uttara Kannada, Ankola, on leaves of *G. indica*, 22 October 2005, D.J. Bhat, Herb. GUBH No. P166; (ii) India, Karnataka, Dakshina Kannada, Uppinangadi, on leaves of *G. indica*, 12 November 2005, D.J. Bhat, Herb. GUBH No. P166. Culture, derived from holotype, No.: GUFCC 4947 = MTCC 9674.

Note – The genus *Caliciopsis* Peck, typified by *C. pinea* Peck, is characterized by ostiolate ascomata, centrum containing thin-walled, pseudoparenchymatous, hyaline tissue, bitunicate, pedicellate asci and smooth or minutely verrucose ascospores (Benny et al. 1985). *Caliciopsis indica* may be compared with *C. myrticola* (Huguenin 1969) in view of morphologically similar ascocarps, asci and ascospores. However, in *C. myrticola* ascomata are 500–620 µm long, asci are 11.4–17.7 × 4.7–6.2 µm and ascospores are 1.8–3.1 µm in diam. whereas in *C. indica* ascomata are 205–300 µm long, asci measure 18–28 × 4–7 µm and ascospores are 2.5–4.5 in diam. (Huguenin 1969). *C. myrticola* was isolated from *Myrtus emarginata* (Myrtaceae). Some other species in the genus have ascomata, asci or ascospores that are similar in size (Table 1). Phylogenetic analysis of partial 28S rRNA gene sequence data has confirmed its placement within the Coryneliaceae (Coryneliales, Eurotiomycetes) as shown in Fig. 3. The ITS/5.8S rRNA gene sequence, however, did not provide any clarity on the species delineation due to lack of reference sequences in GenBank (data not shown).

### Acknowledgements

DJB thanks the University Grants Commission, New Delhi, for a SAP assistance to the Department of Botany, the Council of Scientific & Industrial Research and Ministry of Environment and Forests, Government of India, New Delhi, for financial support in the form of research grants during the tenure of which this work was carried out. PJ thanks the CSIR, Government of India, for a research fellowship.

### References

- Benny GL, Samuelson DA, Kimbrough JW 1985 – Studies on the Coryneliales. IV. *Caliciopsis*, *Coryneliopsis*, and *Coryneliopsis*. Botanical Gazette 146, 437–448.
- Bhat DJ, Kamat N, Shirodkar A 2006 – In: Compendium and Proceedings of 2<sup>nd</sup> National Seminar on Kokum (*Garcinia indica* Choisy) (eds DJ Bhat, N Kamat, A Shirodkar). Western Ghats Kokum Foundation, Goa, India. 1–123.
- Choi YW, Hyde KD, Ho WH 1999 – Single spore isolation of fungi. Fungal Diversity 1, 29–38.
- Felsenstein J 1985 – Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783–791.
- Huguenin B 1969 – Micromycetes du Pacifique sud (Huitieme contribution) Ascomycetes de nouvelle-caledonie (II). Cahiers du Pacifique 13, 295–308.
- Kimura M 1980 – A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16, 111–120.
- Korikanthimath VS, Desai AR 2005 – Status of Kokum (*Garcinia indica* Choisy) in Goa. In: Preview of 2<sup>nd</sup> National Seminar on Kokum (*Garcinia indica* Choisy) (eds DJ Bhat, PG Kakodkar, A Shirodkar, N Kamat). Western Ghats Kokum Foundation & Goa University, Goa, India, 7–16.
- Pratibha J 2006 – Studies on foliicolous fungi from Goa and neighbouring areas of Maharashtra and Karnataka, India. Goa University. Ph.D. Thesis, 1–211.
- Pratibha J, Bhat DJ 2005 – *Bahusutrabeeja manoharacharii* sp. nov., a foliicolous hyphomycete from the forests of Western Ghats, India. In: Emerging Trends in Mycology, Plant Pathology and Microbial Biotechnology (eds G Bagyanarayana, B Bhandariah, IK Kunwar). BS Publication, 1–5.
- Pratibha J, Hyde KD, Bhat DJ 2004 – *Ceeveesubramaniomyces*, a new dematiaceous hyphomycete genus from India. Kavaka 32, 21–26.
- Saitou N, Nei M 1987 – The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.

- Shenoy BD, Jeewon R, Wu WP, Bhat DJ, Hyde KD 2006 – Ribosomal and RPB2 DNA sequence analyses suggest that *Sporidesmium* and morphologically similar genera are polyphyletic. *Mycological Research* 110, 916–928.
- Tamura K, Dudley J, Nei M, Kumar S 2007 – MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24, 1596–1599.
- White TJ, Bruns TD, Lee S, Taylor JW 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (eds MA Innis, DH Gelfand, J Sninsky, TJ White). Academic Press, San Diego, 315–322.