One new species and two new records of Xylarialean fungi from Andaman Islands, India

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Abstract

Information on the molecular diversity of the Xylarialean fungi from the Andaman and Nicobar Islands is scarce. Xylarialean fungi are widely distributed in India, and studies revealed that two new records Hypomontagnella spongiphila and Nemania bipapillata, one new species Neoanthostomella samachedabeejae. In the phylogenetic tree generated by the ITS sequence, results were showing that three species good bootstrap support. In addition, the new species is compared with existing species in detail in the table. Microscopic and molecular studies support all these species.

Key words – Ascomycota – Hypoxylaceae – Taxonomy – Xylariaceae

Introduction

The fungi had poorly investigated from the Andaman and Nicobar Islands (A and N), India, until 2014 (Singh 1969, 1970, 1973, Upreti & Singh 1987, Upreti 1990, 2014, Bhat 1993, Chinnaraj 1993, Jagadeesh Ram 2014, Jagadeesh Ram & Sinha 2016). Niranjan & Sarma (2018a) have reported the complete check list of fungi from the Islands and compiled 446 fungi. Among 446, most of the ascomycetes are inhabit in terrestrial plant leaves and marine fungi (Chinnaraj 1993, Hosagoudar 2013, Jagadeesh Ram & Sinha 2016, Niranjan & Sarma 2018a, b). The examination of dead and decomposing twigs on the forest floor has resulted in discovering new species and records of Xylariales.

Xylariaceae has been one of the dominant family found in the Indian subcontinent for which many taxa have been described (Thind & Wairatch 1969, 1976, Karun & Sridhar 2015, Dargan 2016, Nandan Patel & Krishnappa 2017, Debnath et al. 2018). Most of the exploration of Xylariaceae species has been compiled from Southern India by Pande (2008) followed by Karun & Sridhar (2015) reported 24 Xylaria species, similarly in northern part covered by Koyani et al. (2016) reported over 19 species of Xylariaceae from Gujarat state, India. From the Andaman Islands, 8 species have been reported, including two new Rosellinia species (Niranjan & Sarma 2018a, c) and remaining new records. The recent treatments on Xylariaceae have been published recently (Senanayake et al. 2015, Daranagama et al. 2018). Xylariaceae consists of more than 85 genera and 1300 species Koyani et al. (2016) and revised to 87 genera (Maharachchikumbura et al.
Later on phylogenetic intervention delimitate to describe the new combinations leads to reduce 37 genera (Daranagama et al. 2018 and Wendt et al. 2018).

The recent revision of Xylariales order (Hyde et al. 2020) recognized 14 families (including Myelospermataceae under Xylariales incertae sedis) and the dominant families Xylariaceae and Hypoxylaceae consists of 32 and 19 genera respectively. The recent studies eructed new genera Neoxylaria (Konta et al. 2020) added and detailed notes on Xylariaceae.

**Materials & Methods**

The morphological studies performed based on Niranjan & Sarma (2018b). Morphological identification was performed by referring to various monographs and individual publications, including Pande (2008), Maharachchikumbura et al. (2016) and Hyde et al. (2020). Herbarium samples have been deposited at the Agharkar Research Institute (ARI) of the Ajrekar Mycological Herbarium (AMH), Pune, India. Cultures are maintained in Fungal Biotechnology Laboratory, Department of Biotechnology, Pondicherry University. GenBank accession numbers are available at https://submit.ncbi.nlm.nih.gov/subs/. The individual ITS sequences obtained were submitted to the NCBI Blast search tool to reveal closely related matches on GenBank. Multiple sequence alignments were performed in online software (http://mafft.cbrc.jp/alignment/server/index.html, Katoh & Standley 2013). All the phylogenetic data sets used in this study are mentioned in Table 1.

**DNA extraction, amplification and sequencing**

The isolation of individual spores was performed as described by Choi et al. (1999). Three pure axenic cultures in malt extract agar (MEA) were grown for a week at 28°C, DNA extraction performed by manufacturer protocol (Thermo Scientific, USA). The internal transcribed spacer (ITS) was chosen for phylogenetic analysis. This region was amplified by PCR using the pair of primers ITS1 and ITS4. The polymerase chain reaction (PCR) was performed with a total volume of 25μL, 12 μL of Taq DNA Polymerase 2X Master Mix RED 1.5 mM MgCl2, 1 μL of each primer (10 μM), 1 μL (10–50 ng) genomic DNA and remaining volume makeup with nuclease free water. PCR amplification conditions were set as follows, an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 90 seconds, primer annealing 52°C for ITS, primer extension at 72°C for 1 min and a final extension step at 72°C for 10 min. PCR amplification was checked on 0.8% Agarose gel stained with ethidium bromide. The PCR products were purified by using a Gene JET PCR purification kit (Thermofisher, Lithuania) by following the manufacturer’s protocol. Then the PCR products were sent outsourcing (Agri Genome, Kochi, India) for the sequencing.

**Phylogenetic analysis**

The phylogeny was constructed using aligned sequence data performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian criteria (MB). The maximum likelihood was represented by using the randomized accelerated maximum likelihood (RAxML). The RAxML-HPC2 in XSEDE (version 8.2.8) (Stamatakis 2014) on the CIPRES Science Gateway platform (Miller et al. 2010) using the GTR + I + G evolution model. The phylograms were visualized with the FigTree v. 1.4.0 program (Rambaut 2012) and were reorganized in Microsoft Power Point (v. 2007) and Adobe Photoshop (v. 7.0, Adobe®, San José, CA).

MP was achieved with PAUP v. 4.0b10 (Swofford 2002), with the following parameters, as disordered characters of equal weight, random addition of taxa, branch exchange with the bisection tree reconnection algorithm (TBR), the branch reduce if the maximum branch length was zero. Alignment gaps were treated as missing characters in the combined dataset analysis, where they occurred in relatively conserved regions. The trees were deduced using the heuristic search option with 1000 additions of random sequences, with a maximum of trees set to 1000. Descriptive tree statistics for parsimony. The length of the trees (TL), the consistency index (CI), the retention index (RI), the relative consistency index (RC) and the homoplasy index (HI) were calculated for the
trees generated according to different optimization criteria. Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine if the trees were significantly different.

MB analysis was performed with MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001) to assess the subsequent Bayesian probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by sampling Markov Chain Monte Carlo (BMCMC). GTR + I + G was used in the command. Six simultaneous Markov chains were run over 5000000 generations, and tree samples were taken every 1000 generations. The distribution of recording probability scores was examined to determine the stationary phase for each search and to decide whether additional trials were needed to reach convergence, using the Tracer 1.5 program (Rambaut & Drummond 2007). The first 20% of the trees generated were discarded, and the remaining 80% was used to calculate the subsequent probabilities of the majority rule consensus tree. A BYPP greater than 0.60 is indicated above each node. We consider bootstrap support >75 as strong support, between 50 and 75 as moderate support and less than 50 as minimum support.

Results

Molecular phylogenetic analyses

In the phylogeny of Hypomontagnella spongiphila (NFCC-4378) constructed using closely related taxa Annulohyphoxylon, Hypomontagnella and Daldinia addition to Xylaria hypoxylon (CBS 122620) selected as the outgroup. RAxML analysis yielded a minimum scoring tree with a final ML optimization likelihood value of -12664.883372. The matrix had 427 distinct alignment patterns with 9.62% of indeterminate characters or gaps. The estimated base frequencies were as follows A = 0.251363, C = 0.248286, G = 0.231114, T = 0.269223, substitution rate AC = 1.310818, AG = 2.3338671, AT = 1.597524, CG = 0.974316, CT = 3.212431, GT = 1.000000. Proportion of invariable sites I= 0.298569, gamma distribution shape parameter α = 0.693555. The maximum parsimonious dataset is 686 characters, including 273 constants, 297 informative parsimony and 116 uninformative parsimony. The parsimonious analysis of the data matrix resulted in a thousand equally parsimonious trees with a length of 2939 steps (CI = 0.269, RI = 0.397, RC = 0.107, HI = 0.731) in the first tree. The phylogenetic trees result from ML, a distinct complex of Hypomontagnella was observed within the Hypoxylon sequences similar and consistent with previous studies (Wibberg et al. 2020). Phylogenetic analysis has shown that our H. spongiphila NFCC4378_MT644605 nested with H. spongiphila CCL_KY744359 with a 64% strong bootstrap support in MP.

In the Nemania bipapillata NFCC-4519 phylogenetic analysis, presumed by morphology assumption it closely related to Nemania, therefore we selected the Nemania and allied genera sequences were selected in addition, Botryosphaeria dothidea BD080705002 as outgroup. RAxML analysis yielded a minimum scoring tree with a final ML optimization likelihood value of -4780.691359. The matrix had 288 distinct alignment patterns with 10.53% of undetermined characters or gaps. Estimated base frequencies were as follows, A = 0.242739, C = 0.255614, G = 0.239944, T = 0.261703, substitution rates AC = 1.203682, AG = 2.460070, AT = 1.000000, CG = 0.597153, CT = 3.829878, GT = 1.000000. Proportion of invariable sites I= 0.258137, gamma distribution shape parameter α = 0.532472. The maximum parsimonious (MP) dataset consists of 509 characters, of which 249 were constant, 203 parsimony–informative and 57 parsimony–uninformative. The parsimony analysis of the data matrix resulted in one thousand equally parsimonious trees with a length of 949 steps (CI = 0.496, RI = 0.755, RC = 0.375, HI = 0.504) in the first tree. The overall topology of the phylogenetic trees resulted from ML, MP, similar and incongruent in with earlier studies (Hyde et al. 2020). The phylogenetic analysis showed that our Nemania bipapillata NFCC-4519 has sister-cladding with Nemania bipapillata JQ862661 with strong bootstrap support 100% in MP.

The phylogenetic tree of Neoanthostomella samachedabeejae NFCC-4517, constructed by ITS sequence taxa from Xylariaceae while Sordaria tomentoalba_CBS56972_MH860578 served as the outgroup taxon. RAxML analysis yielded a minimum scoring tree with a final ML
optimization likelihood value of -5572.802237. The matrix had 238 distinct alignment patterns with 1.47% of indeterminate characters or gaps. The estimated base frequencies were as follows: A = 0.254423, C = 0.248620, G = 0.223137, T = 0.273819, substitution rate AC = 2.151608, AG = 2.985945, AT = 2.416015, CG = 1.070409, CT = 6.021074, GT = 1.000000. Proportion of invariable sites I = 0.242763, gamma distribution shape parameter α = 0.566179. The maximum economical (MP) data set consists of 397 characters, of which 169 were constant, 186 parsimony-informative and 42 parsimony-non-informative. The parsimony analysis of the data matrix showed a thousand equally parsimonious trees with a length of 1148 steps in the first tree (CI = 0.349, RI = 0.642, RC = 0.224, HI = 0.651). The phylogenetic analysis of N. samachedabeejae NFCC4517_MT644606 has a branch cladding with N. viticola MFLUCC160243_NR16551 with low bootstrap support of 61% in MP. The phylogenetic tree similar results Daranagama et al. (2016) and one new combination Pseudoanthostomella thailandica (= Anthostomella thailandica) confirmed.

**Taxonomy**

*Hypomontagnella spongiphila* Kuhnert 2020

Saprobic on un-identified twig. Teleomorph – Stromata superficial, black thick woody to carbonaceous, covered the ascomata except in apical papilla. Ascomata perithecial, grouped in stromata, globose, bipartite, semi-immersed in stromata up to 1/3 portion, papilla below the stromata, grey purple colour in 10% KOH. Peridium consists of outer dark brown to inner hyaline cell layers. Hamathecium paraphyses, numerous, septate, branched, longer than asci uneven width 1–5 μm. Asci 71–105 × 5–5.5 μm ($\bar{x}$ = 82 × 5, n = 25), unitunicate, cylindrical, rounded end with apical chamber without apical ring, long pedicel, smooth-walled. Ascospores 9.5–11 × 5–5.5 μm ($\bar{x}$ = 11 × 5, n = 25), 8-spored, hyaline to purple to brown at maturity, rarely overlapping, asperate, straight germ slit with central globose guttulate, ellipsoid, inequilateral, obtuse end. Anamorph – Undetermined.

Known distribution – French Polynesia and India.

Material examined – INDIA, Andaman and Nicobar Islands, South Andaman, Chidiya Tapu, View Point Area (11°29’22”N 92°42’36.6”E). Isolated on un-identified twig, 8 January 2017, M. Niranjan and V.V. Sarma (PUFN 17449). Submitted in Ajrekar Mycological Herbarium-AMH (AMH-9985) and Ex-type living culture (NFCC-4378).

Notes – Hypomontagnella was described by Lambert et al. (2019) based on morphology, chemotaxonomy and molecular phylogeny. Hypomontagnella consists of perispores and not find in *Annulohypoxylon* and *Jackrogersella* similarly distinct from *Hypoxylon* in having annulate disc with papillate ostioles and lack of stromatal granules. Presently Hypomontagnella dwell six species *H. austrobahiensis*, *H. barbarensis*, *H. monticulosa*, *H. rubigineoareolata*, *H. spongiphila*, *H. submonticulosa*. The present collection of *Hypomontagnella spongiphila* shares morphological similarities with the genus. The holotype (H. spongiphila) was collected from the marine source (Wibberg et al. 2020) that lack of teleomorph and anamorph, whereas the current specimen was collected from the terrestrial habitat but it is sharing sequence similarities (Leman-Loubiere et al. 2017, Lambert et al. 2019. Therefore, *H. spongiphila* can be survived in marine, and terrestrial conditions and our collection also can improve the species knowledge by adding the morphological data. Herein, our collection is provided as a new record in India.

*Nemania bipapillata* (Berk. and M.A. Curtis) Pouzar, Ceská Mykologie 39 (1): 24 (1985)

Saprobic on an unidentified twig. Teleomorph – Ascostromata, superficial, carbonaceous, central ostiolated, dark brown papilla, surface wave like circles, thick at base and thin towards to apex, perithecial ascomata, mostly single, rarely 2-3 covered by stromata. Peridium composed of two layers, outer thick brown and inner pale brown textura prismatica cells. Hamathecium paraphyses upto 7 μm wide, filamentous, unbranched, guttulate, longer than the asci, wide at base

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and arrow towards apex. *Asci* 160–215 × 10–13 μm (\(\bar{x} = 185 \times 12, n = 25\)), spore bearing part 65–89 length (\(\bar{x} = 74\)) unitunicate, 8–spored, cylindrical, apical ring 2.3–2.8 × 2–2.2 μm (\(\bar{x} = 2.5, n = 25\)), J+ in Lougals solution, long pedicel, persistent. *Ascospores* 11–13 × 4.6–7.8 μm (\(\bar{x} = 12 \times 5, n = 25\)), hyaline to pale brown, becoming dark brown at maturity, in-equilateral ends, straight germ slit, smooth-walled. Anamorph – Undetermined.

**Fig. 1** – Phylogram generated from maximum parsimony by using the various sequences belongs to the Xylariaceae. The best scores generated using the maximum likelihood (ML) and parsimony.
(MP) as 84/64 bootstrap values are given at the above nodes. Newly generated sequences of *Hypomontagnella spongiphila* in **bold**. The tree was rooted with *Xylaria hypoxylon* as outgroup.

**Fig. 2** – Phylogram of the taxon *Nemia* generated by maximum parsimony based on ITS dataset. Newly generated sequences of *Nemia bipapillata* is in **bold**. The tree was rooted with *Botryosphaeria dothidea* as an outgroup.

Known distribution – Australia, Belgium, Brazil, Bhutan, Brunei Darussalam, Canada, Congo, Costa Rica, Colombia, Cuba, Denmark, French Guiana, Gabon, Guyana, Hong Kong, Japan, India,
Mexico, New Zealand, Thailand, Taiwan, Trinidad and Tobago, Panama, Pakistan, Philippines, Paraguay, South Africa, Sierra Leone, Switzerland, UK, USA.

Material examined – INDIA, Andaman and Nicobar Islands, South Andaman, Chidiya Tapu, Near Viewpoint (11°30'44"N 92°42'34"E), Recorded on Paraishia insignis decaying twig, 20 May 2018, M. Niranjan and V.V. Sarma (PUFN 18745) AMH 10065. Ex-type living culture NFCC-4519.

Fig. 3 – Phylogram of Neoanthostomella samachedabeejae generated by maximum parsimony with Sordaria tomentoalba as outgroup. A newly generated sequence is in red bold.
**Fig. 4** – *Hypomontagnella spongiphila* (NFCC-4378). a, b Stromata. c Stromata grey purple in 10% KOH. d Paraphyses. e-g Asci. h Peridium. i-n Ascospores. Scale bars: h = 50 µm, d-g = 20 µm. i-n = 10 µm.

Notes – *Nemania* was introduced by Pouzar (1985) to fill distinct species from the genus *Hypoxylon*, are found as saprophytic and endophytic (Medina et al. 2019). The present record of *N. bipapillata* sharing the similarities with the original description in having the hemispherical stromata, pale brown flattened cells of peridium, cylindrical asci, and brown ascospores. The present taxon distinct in having the superficial ascostromata, *textura prismaticca* and *textura angularis* of peridium, whereas previous one does not. The ascospores of current specimen having slightly smaller ascospores (11–13 × 4.6–7.8 vs. 10–15 × 5–6.5), lack of sheath and pod like appendages compared to original description (Smith & Hyde 2001, Ju & Rogers 2002).

*Neoanthostomella samachedabeejae* M. Niranjan and V.V. Sarma sp. nov.

Index Fungorum number: IF558023

Etymology – The ascospores have a straight longitudinal germ slit (sama = straight, cheda = slit, bija = spores).
Saprobic on an unknown decaying twig. Teleomorph – *Pseudostromata* aggregated rarely clustered, brown, lignicolous. Ascomata 260–290 × 170–250 μm, perithecial, globose, scattered, immersed, coriaceous, individual central ostiolated, erumpent neck, central empty canal. *Peridium* 17.2 μm wide, consist of brown to hyaline textural subglobosa and textura angularis cell layers. *Hamathecium* paraphyses 2–2.5 μm wide, septate, unbranched, guttulate, thin towards apex. *Asci* 58–94 × 6–7.5 μm (\(\bar{x} = 76 \times 7, n=25\)), unitunicate, cylindrical, without apical ring but it consists of apical thickening, rounded apex, medium pedicel, smooth-walled. *Ascospores* 9–12 × 4–6 μm (\(\bar{x} = 11 \times 5, n=25\)), ovoid to oblong, initially hyaline becoming brown at maturity, arranged in an overlapping uniseriate, straight germ slit, smaller than the spore length, obtuse ends, smooth walled. Anamorph – Undetermined.

**Fig. 5** – *Nemania bipapillata* (NFCC-4519). a, b Ascostromata. c Vertical section of ascostroma.
d, e Paraphyses. f-h Asci. i *textura porrecta*. j *textura prismatica*. k-m Ascospores. n, o Pure culture on malt extract agar. Scale bars: d-h = 50 μm, i-m = 10 μm.

**Fig. 6** – *Neoanthostomella samachedabeejae* (NFCC-4517, holotype). a, b Ascomata. c, d Vertical section of ascomata. e-g Asci h Peridium. h-k Asci. i Periphyses. i, j Pure culture on malt extract agar. k-m Ascospores. Scale bars: c = 200 μm, d, h = 50 μm, e-g, k-m = 10 μm.

Known distribution – INDIA (Present study).

Material examined – India, Andaman and Nicobar Islands, South Andaman, Chidiya Tapu, View Point (11°30’49”N 92°42’38”E), an unknown decaying twig, 20 May 2018, M. Niranjan and V.V. Sarma (PUFNI 18753). The herbarium AMH 10058, Ex-type living culture NFCC-4517.

Notes – New records and species of *Anthostomella* and allied genera have been reported recently (Lu et al. 1999, Rappaz 1995, Lu & Hyde 2000, Crous et al. 2015, Daranagama et al. 2016, Voglmayr et al. 2018. Mostly they are collected in forests as saprobiic and endophytic forms. Molecular distinct *Anthostomella* species were transferred into the newly described genera such as *Alloanthostomella*, *Anthostomelloides*, *Neoanthostomella* and *Pseudoanthostomella* (Daranagama et al. 2016) along with a key provided to *Anthostomella*-like genera. The genus *Neoanthostomella* was described by Dai et al. (2017) with *N. pseudostromatica* type species. Later one more species were added *N. viticola* (Daranagama et al. 2016). The morphological description pseudostromata solitary to gregarious. Ascomata gregarious, growing together in a single pseudostroma, periphysate, ostiolate carbonaceous neck. Peridium brown to hyaline cells of *textura angularis*
layers. Ascospores ellipsoid, aseptate, dark brown, guttulate, full length germ slit. *Neoanthostomella samachedabeejae* is distinct from the existing species (Table 1). Pseudostromata of *N. samachedabeejae* consists of aggregated ascomata whereas solitary to gregarious in *N. pseudostromatia* and clustered in *N. viticola*. The ascomata slightly larger than *N. viticola*, except *N. pseudostromatia* has wider ascomata, the peridium of *N. samachedabeejae* composed of both textura sub-globosa and textura angularis while *N. pseudostromatia* and *N. viticola* composed of only textura angularis. *N. samachedabeejae* asci are smaller than the earlier species. Still, the pedicle is medium length and commonly presence of apical thickening. The Ascospores of *N. pseudostromatia* have larger than *N. samachedabeejae* and *N. viticola* and the germ slit found in all the species of *Neoanthostomella* except *N. viticola*. Thick mucilaginous sheath found in *N. pseudostromatia*, thick or indistinct in *N. samachedabeejae* but not found any sheath in *N. viticola*. In the phylogenetic tree *N. samachedabeejae* showing distinct clade; therefore morphology and molecular studies were delineate the *N. samachedabeejae* as new species.

**Pseudoanthostomella thailandica** (Daranag and Hyde) M. Niranjan and V.V. Sarma, comb. nov.

Index Fungorum: IF552287

Basionym – *Anthostomella thailandica* Daranag and K.D. Hyde 2016.

### Table 1 Morphological difference of *Neoanthostomella* species.

| Fungi             | Pseudostromata | Ascomata (μm) | Peridium | Asci (μm) | Ascospores (μm) |
|-------------------|----------------|---------------|----------|-----------|-----------------|
| *N. pseudostromatia* | solitary to gregarious | 160–280 ×150–300, globose to sub-globose, periphysate ostiolar neck | Composed of textura angularis | 75–110×8.5–13.5, short furcate pedicel. No apical thickening | 11.5–15×4–5.5, dark brown, slightly pointed end, straight germ slit, thick mucilagenous sheath |
| *N. samachedabeejae* | Aggregated | 258–288 × 172–247, conical to column, brittle neck | Composed of textural subglobosa and textura angularis | 58.5–93.9 × 6.2–7.5, medium pedicile, apical thickening | 9.3–12.4 × 3.9–6.0, hyaline to brown, sharp and rounded end, full length germ slit, incipient sheath |
| *N. viticola* | Clustered, rarely solitary | 160-203 × 180–225, conical-irregular-shaped areas, ostiolar neck | Outward textura irregularis, inward textura angularis | 85-117 × 5-7, long pedicile, apical thickening | 5.7-11 × 3.4-4.8, light brown, broad ends, lack of germ slit |

### Discussion

In this study, the scope of the two new records *Hypomontagnella spongiphila*, *Nemania bipapillata* and a new species *Neoanthostomella samachedabeejae* belonging to Xylariales, collected from the of Andaman and Nicobar islands, India, was expanded. Three species were carried out by microscopic examination to confirm the closely related species and it further confirmed by molecular sequence data. Detailed descriptions of each species and notes on their geographic distribution are provided, along with molecular sequence data. Phylogenetic trees that have less than 60 bootstrap values were not included. Among the three presented in the document, *N. samachedabeejae* has become a new to science, the remaining two are new to the Andaman Islands and one new combination raised *Pseudoanthostomella thailandica* from basionym *Anthostomella thailandica*. The present study has generated the morphological and molecular data
of ITS for isolates from the Andaman Islands and provides an update of the molecular sequence data. These results emit light in subsequent studies of Xylariaceae fungi on the remaining islands.

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