Two new species of Geejayessia (Hypocreales) from Asia as evidenced by morphology and multi-gene analyses

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Abstract

Two new species of Geejayessia are introduced, based on materials collected from central China. Geejayessia clavata sp. nov. is characterised by gregarious, red brownish to dark red, oval-subglobose to globose perithecia that are formed on a basal stroma; (4–7-)8-spored cylindrical asci; ellipsoidal or rarely broadly ellipsoidal, uniseptate, smooth or finely verruculose ascospores; clavate, asperate microconidia and absence of macroconidia. Geejayessia sinica sp. nov. is characterised by red to bright red, pyriform, subglobose to globose, perithecia on a basal stroma, collapsing laterally when dry; subcylindrical to clavate asci with a rounded apex; ellipsoidal, uniseptate ascospores; and falcate, multi-septate macroconidia with an arcuate tip. Morphological distinctions of the new species from the related fungi are discussed. This is the first report of Geejayessia from Asia.

Keywords

Cosmospora-like fungi, Nectriaceae, Systematic, Taxonomy

Introduction

Some fusarium-like species having gregarious, multicoloured, broadly ampulliform short-necked or broadly ellipsoidal perithecia were previously placed in Cosmospora Rabenh. and Nectria (Fr.) Fr. (Booth 1959; Samuels and Rogerson 1984; Nirenberg and Samuels 2000) until the genus Geejayessia Schroers, Gräfenhan & Seifert, typified by G. cicatricum (Berk.) Schroers, was introduced (Schroers et al. 2011). The genus is characterised...
by prosenchymatous stromata erumpent through substrates, caespitose, broadly pyri-
form, pale orange, brownish to reddish-orange, bright red to black perithecia, reacting
to potassium hydroxide (KOH) and lactic acid (LA); cylindrical or clavate asci with eight
ascospores; broadly ellipsoidal to ellipsoidal ascospores that are uniseptate, slightly con-
stricted at the septum, hyaline or pale brown to yellowish-brown, smooth or verruculose
at maturity; and multiseptate, slightly curved macroconidia with conspicuous pedicellate
foot cell (Schroers et al. 2011; Lombard et al. 2015). Members of Geejayessia exhibit host
specificity and mainly occur on Buxus spp., Celtis occidentalis and Staphylea trifolia and
were reported only from Europe, North America and Oceania (Samuels and Rogerson
1984; Nirenberg and Samuels 2000; Schroers et al. 2011).

In our examinations of nectriaceous collections from central China, two cosmospora-
like fungi were encountered. Judging by perithecial gross morphology, anatomic struc-
tures and culture characteristics, they represented two previously undescribed species of
Geejayessia. Their taxonomic placements were confirmed by multigene phylogenetic anal-
yses. Distinctions between the new species and their closely related fungi are discussed.

Materials and methods
Sampling and morphological studies

Specimens were collected from Shennongjia National Nature Reserve and Longyuwan
National Forest Park and were deposited in the Herbarium Mycologicum Academiae
Sinicae (HMAS). Methods used by Luo and Zhuang (2010) and Schroers et al. (2011)
were generally followed for morphological observations. The test for colour changes
of the perithecial wall was made with 3% KOH and 100% LA. To observe internal
and microscopic characteristics of the perithecial wall, longitudinal sections through
ascomata were made with a freezing microtome (YD-1508-III, Jinhua, China) at a
thickness of 6–8 μm. Microscopic examinations and measurements were taken from
longitudinal sections and squash mounts in lactophenol cotton blue solution using
an Olympus BH-2 microscope (Tokyo, Japan). Photographs were taken with a Leica
DFC450 digital camera (Wetzlar, Germany) attached to a Leica M125 stereomicro-
scope (Milton Keynes, UK) for gross morphology and a Zeiss AxioCam MRc 5 digital
camera (Jena, Germany) attached to a Zeiss Axio Imager A2 microscope (Göttingen,
Germany) for anatomical structures. Measurements of individual structures were based
on 30 units, except when otherwise noted. Cultures were obtained by single ascospore
isolation from fresh ascomata. To determine colony features, isolates were grown on
cornmeal dextrose agar [CMD, 4% (w/v) cornmeal + 2% (w/v) dextrose + 2% (w/v)
agar], potato dextrose agar [PDA, 20% (w/v) potato + 2% (w/v) dextrose + 2% (w/v)
agar] and synthetic nutrient-poor agar (SNA; Nirenberg 1976) in 90 mm plastic dishes
at 25 °C for 7 d. For the observation of conidiophores, macroconidia and microco-
nidia, cultures were grown on SNA at 25 °C with alternating periods of light/darkness
(12 h/12 h). Colony growth rates were measured after 7 d.
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### Table 1. List of species, herbarium/strain numbers and GenBank accession numbers of materials used in this study.

| Species                              | Herbarium/strain numbers | GenBank accession numbers |
|--------------------------------------|--------------------------|--------------------------|
|                                      |                          | acl1        | ITS          | rpb2          |
| *Albonectria albosuccinea* (Pat.) Rossman & Samuels | BBA 64502                | HQ897837    | HQ897788     | HQ897699      |
| *A. rigidiuscula* (Berk. & Broome) Rossman & Samuels       | CBS 122570               | HQ897896    | HQ897815     | HQ897760      |
| *Cyanonectria buxi* (Fuckel) Schroers, Gräfenhan & Seifert    | CBS 125554               | HM626629    | HM626660     | HM626688      |
| *C. cyanostoma* (Sacc. & Flageolet) Samuels & P. Chaverri  | CBS 101734               | HQ897895    | FJ474076     | HQ897759      |
| *Dialonectria epispheeria* (Tode) Cooke                       | CBS 125494               | HQ897892    | HQ897811     | HQ897756      |
| *D. ullevolea* Seifert & Gräfenhan                              | CBS 125493               | HQ897918    | KM231821     | HQ897782      |
| *Feutrium sambrucinum* Fuckel                                  | CBS 14695                | KM231015    | KM231813     | KM232381      |
| *F. subhamatum* Reinkeing                                      | BBA 62431                | HM897916    | HQ897830     | HQ897780      |
| *Fusioillia acelitera* (Tubaki, C. Boeth & T. Harada) Gräfenhan & Seifert | BBA 63789                | HQ897839    | HQ897790     | HQ897701      |
| *F. mattoi* (Hosoya & Tubaki) Gräfenhan & Seifert             | CBS 58178                | HQ897858    | KM231822     | HQ897720      |
| *Geejayessia atrofusca* (Schwein.) Schroers & Gräfenhan       | CBS 125505               | HM626628    | HM626659     | HM626682      |
| *G. celtidicola* Gräfenhan & Schroers                         | CBS 125502               | HM626625    | HM626657     | HM626685      |
| *G. cicatricum* (Berk.) Schroers                               | CBS 125552               | HQ728171    | HQ728145     | HQ728153      |
| *G. desmazieri* (De Not. & Bex.) Schroers                     | CBS 125507               | HM626633    | HM626651     | HM626675      |
| *G. davata Z.Q. Zeng & W.Y. Zhuang                            | HMAS 248725              | KY873305    | KY873307     | KY873309      |
| *G. sinica* Z.Q. Zeng & W.Y. Zhuang                           | HMA S248726              | KY873306    | KY873308     | KY873310      |
| *G. zealandica* ( Cooke) Schroers                              | CBS 11193                | HM626626    | HM626658     | HM626684      |
| *Macronectria leptophloeaeae* (Niesl) Gräfenhan & Schroers     | CBS 100001               | HQ897891    | HQ897810     | HQ897755      |
| *M. papulonectariae* (Scaveo) Gräfenhan & Seifert             | CBS 125495               | HQ897912    | HQ897826     | HQ897776      |
| *Microcera coccophila* Desm.                                   | CBS 31034                | HQ897843    | HQ897794     | HQ897705      |
| *M. diploa* (Berk. & M.A. Curtis) Gräfenhan & Seifert         | BBA 62173                | HQ897899    | HQ897817     | HQ897763      |
| *Nalanthamala psidii* (Sawada & Kuros.) Schroers & M.J. Wingl. | CBS 116952               | KM231073    | AY864836     | KM232401      |
| *Neocosmospora ramosa* (Bat. & H. Maia) Lombard & Crous         | CBS 50963                | KM231004    | KM231802     | KM232369      |
| *N. vasinfecta* E.F. Sm.                                       | CBS 32554                | KM231005    | KM231803     | KM232370      |
| *Seylonectria applanata* Höhn.                                 | CBS 125489               | HQ897875    | HQ897805     | HQ897739      |
| *S. partoni* (Grew.) Gräfenhan                                | DAOI 235818              | HQ897919    | HQ897831     | HQ897783      |
| *Thymoneura concentrica* (Mont. & Fr.) Voglmayr & Jaklitsch    | CBS 47469                | KM231080    | KM231835     | KM232408      |

* Numbers in bold indicate the newly provided sequences.

DNA extraction, PCR amplification and sequencing

The genomic DNA was extracted from fresh mycelium following the methods of Wang and Zhuang (2004). Three primer pairs, acl1-230up/acl1-1220low (Gräfenhan et al. 2011), ITS5/ITS4 (White et al. 1990) and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used to amplify the sequences or partial sequences of the larger subunit of the ATP citrate lyase (ACL1), the internal transcribed spacers with the 5.8S nuclear ribosomal DNA (ITS) and the second largest subunit of the RNA polymerase II (RPB2), respectively. PCR reactions were performed on an ABI 2720 Thermal Cycler (Applied Biosciences, Foster City, California, USA), based on the procedures detailed in Gräfenhan et al. (2011), White et al. (1990) and Liu et al. (1999). DNA sequencing was carried out in both directions on an ABI 3730XL DNA Sequencer (Applied Biosciences).
Sequence alignment and phylogenetic analyses

Newly generated sequences and those retrieved from GenBank are listed in Table 1. *Na-\textit{lanthamala psidii} (Sawada & Kuros.) Schroers & M.J. Wingf. and *\textit{Thyronectria concentrica} (Mont. & Fr.) Voglmayr & Jaklitsch were used as outgroup taxa. Sequences were assembled, aligned and the primer sequences were trimmed with BioEdit 7.0.5 (Hall 1999) and converted to NEXUS files by ClustalX 1.8 (Thompson et al. 1997). The partition homogeneity test of ACL1, ITS and RPB2 regions was performed with PAUP 4.0b10 (Swofford 2002). To confirm the phylogenetic positions of the new species, sequences of ACL1, ITS and RPB2 were combined and analysed with Bayesian Inference (BI) and Maximum Parsimony (MP) analyses. The MP analysis was performed with PAUP 4.0b10 (Swofford 2002) using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR (tree bisection and reconnection) branch swapping. Topological confidence of resulted trees was tested by maximum parsimony bootstrap proportion (MPBP) with 1000 replications, each with 10 replicates of random addition of taxa. The BI analysis was conducted by MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a Markov chain Monte Carlo algorithm. Nucleotide substitution models were determined by MrModeltest 2.3 (Nylander 2004). GTR+I+G was shown to be the best-fit model for the combined sequences in the BI analysis. Four Markov chains were run simultaneously for 1,000,000 generations with the trees sampled every 100 generations. A 50% majority rule consensus tree was computed after excluding the first 2500 trees as ‘burn-in’. Bayesian inference posterior probability (BIPP) was determined from the remaining trees. Trees were examined in TreeView 1.6.6 (Page 1996). BIPP greater than 90% and MPBP greater than 50% are shown at the nodes.

Results

Sequence comparison and phylogenetic inference

The ACL1, ITS and RPB2 sequences of 25 taxa belonging to 10 genera having fusarium-like asexual states were analysed through the methods of BI and MP. The PHT ($P = 0.01$) indicated that the individual partitions were not highly incongruent (Cunningham 1997); the three loci were thus combined for phylogenetic analyses. The combined datasets include 2258 characters, of which 1085 were constant, 173 variable and parsimony-uninformative and 1000 parsimony-informative. The MP analysis resulted in a single most parsimonious tree (tree length = 4885, CI = 0.4491, HI = 0.5509, RI = 0.5638, RCI = 0.2532). The final matrix was deposited in TreeBASE with accession No. S20853. The BI tree generated is shown (Figure 1). The topology of the BI tree is similar to that of the MP tree. The 25 investigated species were grouped together (BIPP/MPBP = 100%/96%) and further segregated into two main clades (Figure 1). Species of *\textit{Geejayessia} clustered into one clade together
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Figure 1. A Bayesian Inference trees inferred from the combined ACL1, ITS and RPB2 sequences. BIPP (left) above 90% and MPBP (right) above 50% are indicated at nodes.

with Albonectria Rossman & Samuels, Cyanonectria Samuels & P. Chaverri, Fusarium and Neocosmospora E.F. Sm. (BIPP/MPBP = 100%/100%) and those of Dialonectria (Sacc.) Cooke, Fusicolla Bonord., Macroconia (Wollenw.) Gräfenhan, Seifert & Schroers, Microcera Desm. and Stylonectria Höhn. formed another clade (BIPP/MPBP = 100%/98%). HMAS 248725, HMAS 248726 and other representatives of Geejayessia formed a highly supported monophyletic group (BIPP/MPBP = 100%/100%), which confirmed their taxonomic positions in the genus.
Taxonomy

Geejayessia clavata Z.Q. Zeng & W.Y. Zhuang, sp. nov.
Fungal Names: FN570429
Figures 2, 3

Holotype. CHINA, Henan Province, Longyuwan, 33°40'45"N, 111°46'26"E, alt. 1500 m, on bark of Buxus sp., 17 September 2013, H.D. Zheng, Z.Q. Zeng & Z.X. Zhu 8728 (holotype: HMAS 275654), dried ex-type culture HMAS 248725.

Etymology. The specific epithet refers to the clavate microconidia.

Description. Mycelium not visible around ascomata or on host. Ascomata perithecial, crowded in group of 5 to 40, on basal stroma, oval, subglobose to globose,
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**Culture characteristics.** Colony on PDA 48 mm diam. after 7 d at 25 °C, surface cottony, aerial mycelium white, producing vinaceous pigment in medium. Colony on SNA 30 mm diam. after 7 d at 25 °C, surface slightly floccose, with sparse whitish aerial mycelium. Colony on CMD 56 mm diam. after 7 d at 25 °C, surface floccose, with sparse whitish aerial mycelium, producing vinaceous pigment in medium. Conidiophores with short simple branches. Conidiogenous cells monophialidic, cylindrical, tapering toward the tip, 12–63 × 1.5–3.5 μm. Conidia clavate, not in chains, hyaline, aseptate, 4–7 × 0.8–2 μm (n = 60). Macroconidia and chlamydospores not observed.

![Figure 3. *Geejayessia clavata* asexual state (HMAS 248725): a–c colony on PDA (a) SNA (b) and CMD (c) d–j conidiophores, phialides and/or microconidia on SNA. Scale bar: 10 μm (d–j).](image-url)
Notes. Attempts were made to obtain macroconidia of the fungus in culture, but failed. Although the falcate macroconidia are lacking, the major phenotypic features of the fungus, such as occurrence on bark of Buxus sp., perithecia broadly ampulliform with a short neck, asci cylindrical with a rounded apex, ellipsoidal ascospores unisep-tate and conidiophores monopha-lidic, fit well with the generic concept of Geejayessia. The molecular data confirm the taxonomic placement and indicate its close relationship with G. atrofusca (Figure 1, BIPP/MPBP = 100%/89%). Geejayessia atrofusca differs significantly in dark brown to black ascomata that do not change colour in KOH or LA, wider asci [(7.5-)9.8-13.3(-15) μm wide] and longer ascospores [(10-)11.2-14.2(-17.0) μm long]. Its microconidia are oblong to slightly curved and falcate but not clavate and are longer and wider (Samuels and Rogerson 1984).

Geejayessia sinica Z.Q. Zeng & W.Y. Zhuang, sp. nov.
Fungal Names: FN570430
Figures 4, 5

Type. CHINA, Hubei Province, Shennongjia, 31°29′17″N, 110°20′58″E, alt. 2800 m, on bark of Buxus sp., 15 September 2014, Z.Q. Zeng, H.D. Zheng, W.T. Qin & K. Chen 9606 (holotype: HMAS 254520), dried ex-type culture HMAS 248726.

Etymology. Specific epithet refers to the type locality China.

Description. Mycelium not visible around ascomata or on host. Ascomata peri-thecial, solitary or in groups of 5 to 40, with a basal stroma, pyriform or subglobose to globose, smooth, collapsing laterally when dry, red to bright red with a dark red ostiolar region, turning dark purple red in KOH and light yellow in LA, 255–343 × 176–314 μm (n = 14). Perithecial wall of a single layer, 18–38 μm thick, of textura prismatica, cells 8–23 × 2–6 μm, walls 1.2–1.5 μm thick. Asci subcylindrical to clavate, with a rounded apex, 6(−8)-spored, 88–123 × 7–10(−12.5) μm. Ascospores ellipsoidal, hyaline or pale brown, smooth or finely warty, bicellular, slightly constricted at septum, obliquely uniseriate, 10–18(−20) × 5–7.5 μm.

Culture characteristics. Colony on PDA 42 mm diam. after 7 d at 25 °C, surface cottony, with whitish aerial mycelium, forming concentric rings, with pale vinaceous pigment produced in medium. Colony on SNA 26 mm diam. after 7 d at 25 °C, surface slightly velvet, with sparse whitish aerial mycelium. Colony on CMD 40 mm diam. after 7 d at 25 °C, surface radial, slightly floccose, with sparse whitish aerial mycelium. Conidiophores with short simple branches. Conidiogenous cells monopha-lidic, cylindrical, slightly tapering toward the tip, indefinite in length. Macroconidia falcate, with an arcuate tip and a pedicellate foot cell, hyaline, (3–4–)5-septate, 3-sep-tate: 30–53 × 4–5 μm, 4-septate: 50–60 × 4.5–5.2 μm, 5-septate: 53–80 × 4.6–5.3 μm. Microconidia and chlamydospores not observed.

Notes. Geejayessia sinica is phylogenetically related to and morphologically similar to G. cicatricum and G. desmazieri in perithecial gross morphology, subcylindrical to
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Clavate asci, ellipsoidal to broadly ellipsoidal, uniseptate ascospores, falcate macroconidia (Schroers et al. 2011). *Geejayessia cicatricum* differs from *G. sinica* in having smaller perithecia (160–260 × 125–250 μm) and ascospores [(9.5–)11.5–13(–14.5) × (4.5–)5.0–6(–6.5) μm], thinner perithecial wall [(12–)13.5–18(–21) μm thick), shorter asci [(65.5–)73–92.5(–103) μm long), macroconidia with more septa [(2–)5–7(–8) and slow growth on PDA (15–20 mm diam. after 7 d at 25 °C) (Schroers et al. 2011). *Geejayessia desmazieri* is distinguished by shorter asci [(75.5–)85(–100) μm long), smaller ascospores [(9.5–)11–12.5(–15) × (4.5–)5.5–6(–7) μm] and slow growth on PDA (20 mm diam. after 7 d at 25 °C) (Schroers et al. 2011). The ITS sequence of *G. sinica* differs from that of the other two species by 29 bp and 29 bp divergences in total length of 521 bp. The protein-encoding gene sequences of *G. sinica* differ from those of *G. cicatricum* (*G. desmazieri*) by 59 (66) bp differences of 815 bp long ACL1 fragment and 34 (35) bp differences of the 672 bp long RPB2 region.

**Figure 4.** *Geejayessia sinica* sexual state (holotype, HMAS 254520): a–c ascomata on natural substrate d–f colour of perithecium in water (d), 3% KOH (e) and 100% lactic acid (f) g median section through perithecium h–j asci with ascospores k–m ascospores. Scale bars: 1 mm (a–c); 100 μm (d–f); 50 μm (g); 10 μm (h–m).
Schroers et al. (2011) recognised five species of *Geejayessia*. *Geejayessia montana* Lechat & J. Fourn was recently described and its placement was supported by morphological characteristics of both sexual and asexual states, as well as analysis of ITS sequences (Lechat and Fournier 2017). Meanwhile, a new combination, *G. hispanica* (Lechat & Priou) Lechat & J. Fourn was proposed based on the ITS sequence of *Geejayessia* sp. BRFM 1015 (GenBank accession no. JX082350) (Lechat and Fournier 2017). However, ‘*Geejayessia hispanica*’ grows on *Phoenix canariensis* rather than *Buxus*, *Celtis* or *Staphylea*, which deviates from the original generic concept of the genus (Schroers et al. 2011). This fungus was treated as *Cosmospora hispanica* Lechat & Priou in the present study. *Cosmospora matuoi* Hosoya & Tubaki was also combined with *Geejayessia* as *G. matuoi* (Hosoya & Tubaki) Lechat & Rossman (Lechat and Rossman 2017). Nevertheless, Gräfenhan et al. (2011) and Lombard et al. (2015) treated *Cosmospora matuoi* as a member of *Fusicolla*, which is followed in this study. To clarify the taxonomic positions of ‘*G. hispanica*’ and ‘*G. matuoi*’, more evidence is certainly required.

**Discussion**

*Figure 5. Geejayessia sinica* asexual state (HMAS 248726): a–c colony on PDA (a), SNA (b) and CMD (c) d conidiophores, conidiogenous cells and macroconidia on SNA e–l Macroconidia on SNA. Scale bars: 50 μm (d–h); 10 μm (i–l).*
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According to the International Code of Nomenclature for algae, fungi and plants (McNeill et al. 2012), the name *Fusarium* is accepted as the correct generic name for fungi with *Gibberella* Sacc. sexual states (Rossman et al. 2013). The asexual states of other genera are marked as fusarium-like (Lombard et al. 2015). In the present study, the phylogeny, based on analyses of the combined ACL1, ITS and RPB2 sequences, recognised nine clades amongst the investigated taxa which are in accordance with the genera *Albonectria*, *Cyanonectria*, *Dialonectria*, *Fusicolla*, *Geejayessia*, *Macroconia*, *Microcera*, *Neocosmospora* and *Stylonectria*. This result is basically consistent with that by Schroers et al. (2011).

Joining the two new species to the *Geejayessia* clade, the tree topology (Figure 1) remains basically the same as that revealed by Schroers et al. (2011). Our result showed *G. clavata* and *G. atrofusca* both forming microconidia in culture, grouped together with relatively high statistical supports (Figure 1, BIPP/MPBP = 100%/89%). *Geejayessia sinica*, *G. cicatricum* and *G. desmazieri*, as sister-groups, are poorly supported (BIPP/MPBP less than 50%).

Host specificity has been shown in some fungi of Nectriaceae; for example, *Thyronectria aurigera* (Berk. & Ravenel) Jaklitsch & Voglmayr occurs only on Oleaceae, *T. berolinensis* (Sacc.) Seaver on *Ribes* and *T. aquifolii* (Fr.) Jaklitsch & Voglmayr on *Ilex aquifolium* (Jaklitsch and Voglmayr 2014; Zeng and Zhuang 2016). Species of *Geejayessia* are also host-specific. As known currently, *G. clavata*, *G. sinica*, *G. cicatricum* and *G. desmazieri* occur only on *Buxus* spp., *G. celtidicola* only on *Celtis occidentalis* and *G. atrofusca* only on *Staphylea trifolia* (Schroers et al. 2011).

The genus *Geejayessia* was previously known from Europe, North America and Oceania (Samuels and Rogerson 1984; Nirenberg and Samuels 2000; Schroers et al. 2011). The new species discovered from central China extends the distribution of the genus to Asia.

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