Nectria-related fungi causing dieback and canker diseases in China, with Neothyronectria citri sp. nov. described

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Abstract
To clarify phylogenetic relationships amongst Nectria, Neothyronectria and Thyronectria in Nectriaceae, we examined detailed morphological characters and performed phylogenetic analyses of a concatenated dataset, based on the ITS, LSU, tef1 and tub2 DNA sequences of fungal specimens in China. Four species of nectria-related fungi were identified, i.e. Nectria dematiosa, N. pseudotrichia, Neothyronectria citri and Thyronectria pinicola. The newly described species, Neothyronectria citri, is characterised by its ascomatal wall with bright yellow scurf, unitunicate asci, each with 4-spored and ascospores allantoid to short-cylindrical, uniseriate, muriform, hyaline to slightly yellowish-brown. This species has affinities with other one known species of Neothyronectria and can be distinguished by molecular data.

Keywords
DNA phylogeny, Nectriaceae, Systematic, Taxonomy

Introduction

Nectriaceae Tul. & C. Tul., typified by the genus Nectria (Fr.) Fr., was established by Tulasne and Tulasne (1865) to include nectria-related fungi having brightly pigmented ascomata with fusiform to allantoid ascospores and globose to fusiform phialidic conidia (Rossman et al. 1999, 2013, Rossman 2000, Lombard et al. 2015, Maharachchikumbura et al. 2015, Huang et al. 2018, Yang et al. 2018). Members of the family are unified...
by phenotypic characters such as uniloculate ascomata that are yellow, orange-red to purple and phialidic asexual morphs. Lombard et al. (2015) defined the generic concepts in *Nectriaceae*, based on a multi-gene phylogenetic analysis and resolved 47 genera supported by morphological observations. Since then, *Neothyronectria* was proposed as a new genus to accommodate the species, *Neothyronectria sophorae*, which is known only from the pycnidial asexual morph (Crous et al. 2016) and *Cosmosporella* was proposed as a new genus (Huang et al. 2018), thus 49 genera are now accepted in the *Nectriaceae*.

*Nectria*, typified by *N. cinnabarina* (Tode: Fr.) Fr., was initially established by Fries (1849). Some species of *Nectria* are weak parasites of woody plants (Samuels et al. 2009, Hirooka et al. 2011). Hirooka et al. (2012) reviewed the genus, based on the type and additional herbarium specimens, and accepted 29 species. They also monographed the genus *Thyronectria* as *Pleonectria* but because *Thyronectria* (1875) is older, it has priority over *Pleonectria* (1876) as explained by Jaklitsch and Voglmayr (2014). Many members of *Nectria* and *Thyronectria* occur on dead corticated twigs or branches of woody plants worldwide mainly in temperate and subtropical regions (Hirooka et al. 2012, Jaklitsch and Voglmayr 2014, Zeng and Zhuang 2016). To date, 42 species of *Thyronectria* have been accepted (Jaklitsch and Voglmayr 2014, Voglmayr et al. 2016, Zeng and Zhuang 2016, Lechat et al. 2018).

During trips to collect forest pathogens in China, several nectria-related fungi associated with canker or dieback diseases were collected. Based on a multi-locus phylogeny (ITS, LSU, *tef1* and *tub2*), we identified four nectria-related species in three genera of *Nectriaceae* and propose one new species in *Neothyronectria*.

**Materials and methods**

**Isolates**

Fresh specimens were collected from infected branches or twigs of diverse hosts from Beijing, Heilongjiang, Jiangxi, Shaanxi and Xinjiang provinces, China. Strains were isolated from fresh diseased branches and grown from ascospores or conidia by spreading the suspension on the surface of 1.8% potato dextrose agar (PDA), incubated at 25 °C for up to 24 h. Single germinating conidia were removed and transferred to fresh potato dextrose agar (PDA) plate. Specimens and isolates of the new species have been deposited in the Museum of Beijing Forestry University (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center (CFCC).

**Morphological analysis**

Morphological observations of the sexual and asexual morph in the natural environment were based on features of the fruiting bodies produced on infected plant tissues and micromorphology, supplemented by cultural characteristics. Gross morphology of fruiting bodies was recorded using a Leica stereomicroscope (M205 FA). Perithecia,
Nectria-related fungi causing dieback and canker diseases in China... pycnidia, synnemata and stromata were observed and described. To test ascomatal wall reactions, 3% KOH and 100% lactic acid (LA) were used. The micromorphological characteristics were examined by mounting fungal structures in clear lactic acid and 30 measurements at 1000× magnification were determined for each isolate using a Leica compound microscope (DM 2500) with differential interference contrast (DIC) optics. Colony characters and pigment production on PDA were noted after 10 d. Colony colours were described according to Rayner (1970). Longitudinal descriptions, nomenclature and illustrations of taxonomic novelties are deposited in MycoBank (http://www.MycoBank.org; Crous et al. 2004).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from colonies grown on cellophane-covered PDA, using a modified CTAB [cetyltrimethylammonium bromide] method (Doyle and Doyle 1990, Zhang et al. 2010). For PCR amplifications of phylogenetic markers, four different primer pairs were used (Table 1). PCR amplification products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyzer with a BigDye Terminater Kit v.3.1 (Invitrogen, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analyses

The quality of our amplified nucleotide sequences was checked and combined by SeqMan v.7.1.0 and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI), according to recent publications of the family Nectriaceae (Jaklitsch and Voglmayr 2014, Lombard et al. 2015, Crous et al. 2016, Yang et al. 2018). Sequences were aligned using MAFFT v. 7.310 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2016) and manually corrected using Bioedit 7.0.9.0 (Hall 1999). Phylogenetic analyses of the combined gene regions were performed using Maximum Parsimony (MP), Maximum-Likelihood (ML) and Bayesian Inference (BI) methods. The data were edited in AliView version: 1.19-beta1k and the evolutionary model obtained using MrModeltest v. 2.3 (Nylander et al. 2008) under the Akaike

| Gene | PCR primers (forward/reverse) | PCR: thermal cycles: (Annealing temp. in bold) | References of primers used |
|------|--------------------------------|------------------------------------------------|-----------------------------|
| ITS  | ITS1/ITS4                      | (95 °C: 30 s, 51 °C: 30 s, 72 °C: 1 min) × 35 cycles | White et al. 1990           |
| LSU  | LROR/ LR5                      | (95 °C: 45 s, 55 °C: 45 s, 72 °C: 1 min) × 35 cycles | Vilgalys and Hester 1990, Rehner and Samuels 1994 |
| tef1 | EF1-728F and EF-1567R          | (95 °C: 15 s, 55 °C: 20 s, 72 °C: 1 min) × 35 cycles | Carbone and Kohn 1999, Rehner 2001 |
| tub2 | T1/T2                          | (95 °C: 30 s, 55 °C: 30 s, 72 °C: 1 min) × 35 cycles | O'Donnell and Cigelnik 1997 |
Information Criterion (AIC) performed in PAUP v. 4.0b10. The MP analysis was performed by a heuristic search option of 1000 random-addition-schemes with a tree bisection and reconnection (TBR) algorithm. Maxtrees were set to 5000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). ML was performed using RAxML-HPC v.8 on XSEDE in CIPRES Science Gateway (Miller et al. 2010, 2015, Stamatakis 2014) with 1000 rapid bootstrap replicates using the GTR+I+G model of nucleotide substitution. BI was implemented by MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003) with GTR+I+G as the best-fit model. Posterior Probabilities (PP) were estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Two MCMC chains, started from random trees for 1,000,000 generations and trees, were sampled every 100th generation, resulting in a total of 10,000 trees. The first 25% of trees were discarded as the burn-in phase of each analysis. Branches with significant Bayesian Posterior Probabilities (BPP) were estimated in the remaining 7500 trees. Phylogenetic trees were viewed with FigTree v.1.3.1 (Rambaut and Drummond 2010) and processed by Adobe Illustrator CS5. Alignment and trees were deposited in TreeBASE (submission ID: 24366). The nucleotide sequence data of the new taxon have been deposited in GenBank (Table 1).

**Results**

**Phylogenetic analyses**

To reveal the phylogenetic position amongst *Nectria*, *Neothyronectria* and *Thyronectria* in *Nectriaceae*, a phylogenetic analysis was performed with combined ITS, LSU, *tef1* and *tub2* sequence data. Sequences of representative species were selected from NCBI (Jaklitsch and Voglmayr 2014, Crous et al. 2016, Yang et al. 2018). The ITS, LSU, *tef1*, *tub2* and combined data matrices contained 545, 781, 1033, 643 and 3010 characters with gaps, respectively. The alignment comprised 59 strains and *Emericellopsis glabra* (CBS 125295), *Hydropisphaera fungicola* (CSB 122304), *Nectriopsis exigua* (CBS 126110) and *Verrucostoma freycinetiae* (MAFF 240100) were selected as the outgroups.

The concatenated sequence alignment contained 932 parsimony-informative characters, 259 were variable and parsimony uninformative and 1819 were constant. The parsimony analysis yielded the maximum of 10 equally most parsimonious trees (TL = 5493 steps; CI = 0.386; RI = 0.685; RC = 0.264; HI = 0.614).

The phylogeny, resulting from the MP analysis of combined gene sequence data, is shown in Fig. 1. Overall, the topologies obtained from the different phylogenetic analyses were mostly similar and the best scoring MP tree is illustrated here. The MP and ML bootstrap support values above 50% are shown at the first and second position, respectively. Branches with significant BPP (≥ 0.95) in Bayesian analyses were thickened in the phylogenetic tree.
Table 2. Strains and GenBank accession numbers of the isolates used in this study.

| Species | Isolate No. | Substrate/Host | Country | GenBank Accession No. |
|---------|-------------|----------------|---------|----------------------|
|         |             |                |         | ITS | LSU | tef1 | tub2 |
| Allantonectria miltina | CBS 121121 | Agave americana | Italy | HM484547 | HM484572 | HM484524 | HM484609 |
| Emericellopsis glabra | CBS 125295 | Soil | Mexico | HM484860 | GQ505993 | HM484843 | HM484879 |
| Hydropisphaera fungicola | CBS 122304 | Decaying leaves on Populus trichocarpa | USA | HM484863 | GQ505995 | HM484845 | HM484877 |
| N. antarctica | CBS 115033 | Berberis aquifolium | USA | HM484556 | HM484560 | HM484516 | HM484601 |
| N. asiatica | MAFF 241439 | Bark of dead wood | Japan | HM484701 | HM484563 | – | HM484604 |
| N. aurantiaca | CBS 308.34 | Ulmus sp. | UK | JF832628 | JF832682 | JF832519 | JF832886 |
| N. balansae | CBS 125156 | Dead twigs of Acer species | USA | HM484548 | HM484562 | HM484527 | HM484606 |
| N. balansae | CBS 129349 | Twigs | China | JF832653 | JF832711 | JF832522 | JF832908 |
| N. berberidicola | CBS 128669 | Berberis vulgaris | France | JF832662 | JF832712 | JF832538 | JF832887 |
| N. cinnabarina | CBS 125165 | Dead twigs of Aesculus species | USA | HM484545 | HM484562 | HM484527 | HM484606 |
| N. dematiosa Subclade A | CBS 126570 | Bark | USA | HM484557 | HM484561 | HM484534 | HM484603 |
| N. dematiosa Subclade A | CFCC 53585 | Tilia mandshurica | China | MK861084 | MK861075 | MK902792 | MK902801 |
| N. dematiosa Subclade A | CFCC 53586 | Betula platyphylla | China | MK861085 | MK861076 | MK902793 | MK902802 |
| N. dematiosa Subclade B | CBS 125125 | Dead twigs of Acer macrophyllum | Canada | HM484676 | HM484717 | HM484645 | HM484797 |
| N. eustromatica | CBS 121896 | – | – | – | – | – | – |
| N. eustromatica | CBS 125578 | – | – | – | – | – | – |
| N. magnispora | CBS 123962 | Twigs | Japan | JF832663 | JF832683 | JF832539 | JF832896 |
| N. mariae | CBS 125294 | Buxus sempervirens | UK | JF832629 | JF832684 | JF832542 | JF832899 |
| N. nigrescens | CBS 125148 | Dead twigs of dicotyledonous tree | USA | HM484707 | HM484720 | HM484672 | HM484806 |
| N. nigrescens | CBS 128988 | Elaeagnus angustifolia | USA | JF832630 | JF832687 | – | JF832888 |
| N. nigrescens | CBS 129808 | Ulmus pumila | USA | JF832632 | JF832690 | – | JF832894 |
| N. polythalama | CBS 128672 | Twigs | New Zealand | JF832638 | JF832695 | JF832523 | JF832900 |
| N. pseudocinnabarina | CBS 129366 | Dead wood | Venezuela | JF832642 | JF832697 | JF832533 | – |
| N. pseudotrichia | CBS 551.84 | Bark | Japan | HM484554 | GQ506000 | HM484853 | HM484602 |
| N. pseudotrichia | MAFF 241452 | Bark | Japan | JF832649 | JF832706 | JF832531 | JF832903 |
| N. pseudotrichia | G.J.S. 09-1329 | Dead wood | Venezuela | JF832647 | JF832702 | JF832530 | JF832902 |
| N. pseudotrichia | CFCC 53587 | Robinia sp. | China | MK861086 | MK861077 | MK902794 | MK902803 |
| N. pseudotrichia | CFCC 53588 | Cinnamomum porrectum | China | MK861087 | MK861078 | MK902795 | MK902804 |
| N. pseudotrichia | CFCC 53589 | Rubus corchorifolius | China | MK861088 | MK861079 | MK902796 | MK902805 |
| N. sordida | CBS 125119 | Living woody vine | French | HM484857 | HM484868 | HM484848 | HM484874 |
| N. trisepata | HAMS 252485 | On rotten twig | China | KM026503 | KM026504 | KM026506 | KM026501 |
| N. ulmicola | CFCC 52117 | Ulmus davidioida var. japonica | China | MG231959 | MG231980 | MG232022 | MG232043 |
| N. ulmicola | CFCC 52118 | Ulmus davidioida var. japonica | China | MG231960 | MG231981 | MG232023 | MG232044 |
| Nectriopsis exigua | CBS 126110 | Myxomycete | Puerto Rico | HM484865 | GQ506014 | HM484852 | HM484883 |
| Neothyronectria citri | CFCC 53590 | Citrus maxima cv. Shatian | China | MK861080 | MK861071 | MK902788 | MK902797 |
| N. citri | CFCC 53591 | Citrus maxima cv. Shatian | China | MK861081 | MK861072 | MK902788 | MK902798 |
| N. sophorae | CBS 142094 | Sophora microphylla | Zew Zealand | KY173570 | KY173559 | – | KY173619 |
| Thyronectria aquifolii | CBS 307.34 | Ilex aquifolium | UK | JF832597 | JF832718 | JF832548 | JF832842 |
Figure 1. Maximum parsimony phylogenetic tree generated from analysis of a combined ITS, LSU, tef1 and tub2 sequence dataset for 59 taxa of *Allantonectria*, *Nectria*, *Neothyronectria* and *Thyronectria*. *Emeri-cellopsis glabra* (CBS 125295), *Hydropisphaera fungicola* (CSB 122304), *Nectriopsis exigua* (CBS 126110) and *Verrucostoma freycinetiae* (MAFF 240100) as outgroup taxa. Values above the branches indicate maximum parsimony and maximum likelihood bootstrap (left, MP BP ≥ 50%; right, ML BP ≥ 50%). The branches with significant BIPP values (≥ 0.95) in the BI analysis are thickened. Scale bar = 80 nucleotide substitutions. Strains in current study are in blue. Ex-type strains are indicated in bold.
Taxonomy

*Nectria* (Fr.) Fr., *Summa veg. Scand.*, Sectio Post. (Stockholm): 387, 1849

**Type species.** *Nectria cinnabarina* (Tode) Fr., *Summa veg. Scand.*, Sectio Post. (Stockholm): 388, 1849.

**Note.** Members of *Nectria* are typically weak parasites of woody plants and occur on hardwood trees and shrubs throughout the temperate zone of the northern hemisphere (Samuels et al. 2009, Hirooka et al. 2011). The genus *Nectria* is characterised by well-developed stromata, subglobose to globose, red to dark red, fleshy, soft-textured, uniloculate, warted perithecia that become cupulate when dry and are associated with coelomycetous asexual morphs. Ascii are unitunicate and clavate to cylindrical in shape. Ascospores are variable and usually broadly ellipsoid to long-fusiform, hyaline to yellow brown, smooth to striate and non- to multi-septate or muriform (Rossman et al. 1999, Hirooka et al. 2009, Maharachchikumbura et al. 2015).

*Nectria dematiosa* (Schwein.) Berk., *Grevillea* 4: 16, 1875

**Fig. 2**

**Description.** See Yang et al. (2018)

**Additional specimens examined.** CHINA. Heilongjiang Province, Liangshui Nature Reserve, 47°10’50.64”N, 128°53’41.03”E, on twigs or branches of *Tilia mandshurica* Rm.pr.et Maxim., 29 July 2016, Q. Yang (BJFC-S1400, living culture CFCC 53585); Xinjiang, 45°13’07.97”N, 81°46’24.71”E, on twigs or branches of *Betula platyphylla* Suk., 18 July 2017, C.M. Tian (BJFC-S1767, living culture CFCC 53586).

**Note.** *Nectria dematiosa* has a broad host range and is widely distributed in China, occurring as the most commonly *Nectria* species (Yang et al. 2018). This study is the first report of *N. dematiosa* from *Betula platyphylla* and *Tilia mandshurica*.

*Nectria pseudotrichia* Berk. & M.A. Curtis, *J. Acad. Nat. Sci. Philadelphia* 2, 2: 289. 1853

**Fig. 3**

**Description.** See Yang et al. (2018)

**Additional specimens examined.** CHINA. Shaanxi Province, Ankang City, 32°40’32.85”N, 109°18’57.38”E, on twigs or branches of *Robinia* sp., 29 July 2016, N. Jiang (BJFC-S1403, living culture CFCC 53587); Jiangxi Province, Ganzhou City, 24°40’51.80”N, 115°31’49.99”E, on twigs or branches of *Cinnamomum porrectum* (Roxb.) Kosterm., 12 May 2018, Q. Yang (BJFC-S1768, living culture CFCC 53588); Jiangxi Province, Ganzhou City, 24°59’44.81”N, 115°30’58.85”E, on twigs or branches of *Rubus corchorifolius* Linn. f., 12 May 2018, Q. Yang (BJFC-S1769, living culture CFCC 53589).
Note. *Nectria pseudotrichia* is one of the common tropical fungi in the genus *Nectria* and is distinguished in the genus by having muriform ascospores and a synnematous asexual morph.

**Neothyronectria** Crous & Thangavel, Persoonia 37: 329, 2016.

**Type species.** *Neothyronectria sophorae* Crous & Thangavel, Persoonia 37: 329, 2016.

Note. The genus *Neothyronectria* was described by Crous & Thangavel (2016) based on the only species, *N. sophorae*, which is known from a pycnidial asexual morph. *Neothyronectria* is characterised by pycnidial conidiomata that exude a creamy mucoid conidial mass and hyaline, ampulliform to subcylindrical conidia. In this study, we collected and illustrated here one additional taxon in *Neothyronectria*.

**Neothyronectria citri** C.M. Tian & Q. Yang, sp. nov.
MycoBank: MB830779
Figure 4

**Diagnosis.** *Neothyronectria citri* differs from its closest phylogenetic neighbour *Neothyronectria sophorae* in ITS, LSU and *tub2* loci, based on the alignments deposited in TreeBASE.

**Holotype.** CHINA. Jiangxi Province: Ganzhou city, 25°51'27.87"N, 114°58'18.95"E, on symptomatic branches of *Citrus maxima* (Burm.) Merr. cv. *Sha-tian* Yu, 11 May 2018, Q. Yang, Y.M. Liang & Y. Liu (holotype BJFC-S1770 designated here, ex-type culture CFCC 53590).

**Etymology.** Named after the host genus on which it was collected, *Citrus*.

**Description.** *Mycelium* not visible around ascomata or on the host. *Stromata* erumpent through epidermis, up to 0.6 mm high and 1 mm diam., pseudoparenchymatous, cells forming *textura angularis* to *t. globulosa*, intergrading with ascomatal wall. *Ascomata* superficial on well-developed stromata, scattered to aggregated in groups of 3–10, subglobose to globose, 200–270 μm diam., rarely slightly cupulate upon drying, sometimes with only a depressed apical region, yellowish-brown to grey, apical region slightly darker, no colour change in KOH or LA, sometimes surface scurfy or scaly, bright yellow to greenish-yellow. *Ascomatal surface cells* forming *textura globulosa* or *t. angularis*, sometimes including bright yellow scurf, 9–15 μm diam., walls pigmented, uniformly about 1.5 μm thick. *Ascomatal wall* 27–46 μm thick, of two regions: outer region 22–35 μm thick, intergrading with stroma, cells forming *textura globulosa* or *t. angularis*, walls pigmented, about 1.5 μm thick; inner region 9–15 μm thick, of elongate, thin-walled, hyaline cells, forming *textura prismatica*. *Asci* clavate, unitunicate, 53.5–65 × 8.5–11 μm, with inconspicuous ring at apex, 4-spored. *Ascospores* allantoid to short-cylindrical, uniseriate, rounded at both
ends, (17–)18–21(–23.5) × 8–9(–10) μm (n = 20), muriform, hyaline to slightly yellowish-brown.

**Culture characters.** Cultures incubated on PDA at 25 °C in darkness. Colony originally flat with white aerial mycelium, becoming pale yellowish due to pigment formation, conidiomata absent.

**Additional specimen examined.** CHINA. Jiangxi Province: Ganzhou City, 25°51’27.87”N, 114°58’18.95”E, on symptomatic branches of *Citrus maxima* (Burm.)
Note. *Neothyronectria citri*, as described here, is known from an ascomatal sexual morph phylogenetically allied to species of *Allantonectria* and *Thyronectria* (Fig. 1). In this study, two strains representing *Neothyronectria citri* cluster in a well-supported clade and appear most closely related to *Neothyronectria sophorae*, which was isolated from *Sophora microphylla* in New Zealand (Crous et al. 2016). *Neothyronectria citri* can be distinguished, based on ITS, LSU and tub2 loci from *Neothyronectria sophorae* (16/464 in ITS, 9/772 in LSU and 60/494 in tub2).

**Figure 3.** *Nectria pseudotrichia* (CFCC 53587) A–B habit of conidiomata on branches C–D conidiophores E–F conidia. Scale bars: 1 mm (A–B); 10 μm (C–F).
Figure 4. Neothyronectria citri (CFCC 53590) A–B habit of conidiomata on branches C transverse section of conidioma D longitudinal section of conidioma E–F asci G–H ascospores. Scale bars: 500 μm (B–D); 10 μm (E–H).

Thyronectria Sacc., Grevillea 4: 21, 1875.

Type species. Thyronectria rhodochlora (Mont.) Seeler, J. Arnold Arbor. 21: 455, 1940.

Note. Thyronectria Sacc. was established by Saccardo (1875) to include nectria-like fungi with immersed ascomata and muriform ascospores and characterised by well-
developed erumpent stromata which are often covered with yellow-green amorphous scurf and ascospores that sometimes bud in the ascus to produce ascoconidia (Jaklitsch and Voglmayr 2014, Lombard et al. 2015). Members of the genus occur on dead corticated twigs or branches of woody plants worldwide mainly in temperate and subtropical regions (Hirooka et al. 2012, Jaklitsch and Voglmayr 2014).

*Thyronectria pinicola* (Kirschst.) Jaklitsch & Voglmayr, *Persoonia* 33: 203, 2014.

Figure 5

**Basionym.** *Pleonectria pinicola* Kirschst., *Abh. Bot. Ver. Prov. Brandenburg* 48: 59, 1906.

**Description.** Stromata erumpent through epidermis, orange to red. Pycnidia solitary or aggregated in groups of 3–6, superficial on stroma or rarely immersed at base, subglobose, smooth to slightly roughened, cerebriformis or slightly cupulate upon drying, 225–400 μm high, 240–440 μm diam., red to bay, KOH+ slightly darker, LA+ slightly yellow. Pycnidial wall 16–40 μm thick, of two regions: outer region 11–15 μm thick, intergrading with stroma, cells forming *textura globulosa* or *t. angularis*, walls pigmented, about 1.5 μm thick; inner region 10–24 μm thick, of elongate, thin-walled, hyaline cells, forming *textura prismatica*. Conidiophores densely branched, generally with 1–3 branches, 8.5–24 μm long, 1.3–1.5 μm wide. Conidiogenous cells cylindrical monophialides on aerial, submerged or repent hyphae. Conidia formed abundantly on slimy heads, ellipsoidal to oblong, hyaline, straight, rounded at both ends, non-septate, (2–)3–3.5 × 0.7–1.0 μm (n = 20), smooth-walled.

**Culture characters.** Cultures incubated on PDA at 25 °C in darkness. Colony surface cottony with aerial mycelium, becoming yellowish-brown due to pigment formation, small reddish-brown sporodochial conidial masses produced after 3–4 wk.

**Specimens examined.** CHINA. Beijing: Chaoyang District, 40°00’35.31”N, 116°47’55.32”E, on symptomatic branches of *Pinus sylvestris* Linn. var. mongolica Litv., 11 June 2018, Q. Yang & N. Jiang (BJFC-S1773, living culture CFCC 53593 and CFCC 53594).

**Note.** The hosts of *Thyronectria pinicola*, synonymised with *Pleonectria pinicola*, are restricted to *Pinus*. Members of the genus distributed in Asia (China, Japan, Pakistan), Australia, Europe (Germany, Russia), North America (USA) and South America (Chile) (Jaklitsch and Voglmayr 2014). The asexual morph of *T. pinicola* in the natural environment has long, sterile hyphae extending from the hymenium and abundant conidiophores (Figs 4E–G). In the present study, two isolates from twigs of *Pinus sylvestris* var. *mongolica* were congruent with *T. pinicola*, based on morphology and DNA sequences data (Fig. 1). We therefore describe *T. pinicola* as a known species for this clade.
Figure 5. *Thyronectria pinicola* (CFCC 53593) A–C habit of conidiomata on branches D longitudinal section of conidioma E–G conidiogenous cells with conidia H conidia I–J culture on PDA and conidiomata. Scale bars: 1 mm (B); 500 μm (C–D); 10 μm (E–H).
Discussion

In this investigation of nectria-related fungi in China, we identified four species in three genera (Nectria, Neothyronectria and Thyronectria) of Nectriaceae, based on four combined loci (ITS, LSU, tef1 and tub2), as well as morphological characters. It includes Nectria dematiosa, N. pseudotrichia, and Thyronectria pinicola as well as one new species named Neothyronectria citri. The new species is characterised by well-developed erumpent stromata that are often covered with yellow-green amorphous scurf; asci unitunicate, clavate, with inconspicuous ring at apex, each with 4-spored; ascospores allantoid to short-cylindrical, uniseriate, muriform, hyaline to slightly yellowish.

Species revised by Rossman et al. (1999) in Nectria were monographed by Hirooka et al. (2012), who recognised three genera, i.e. Allantonectria, Nectria and Pleonectria. Allantonectria, based on Allantonectria miltina, was recognised as a monotypic genus with small, aseptate ascospores, trichoderma-like conidiophores and occurring on monocotyledonous plants. The genus Thyronectria (as Pleonectria) is characterised by having ascomata with bright yellow scurf, ascospores that often bud to produce ascoconidia inside or outside of the asci and/or a pycnidial anamorph (Hirooka et al. 2012). Based on the lack of bright yellowish scurf on the ascomata, the genus Nectria is easily distinguished from Allantonectria and Thyronectria. In this study, Neothyronectria citri was identified as a new species in Neothyronectria, which was typified by Neothyronectria sophorae having ampulliform to subcylindrical conidia (Crous et al. 2016). Unlike species of Thyronectria, Neothyronectria did not produce ascoconidia but they have bright yellow scurf on the ascomatal wall.

In the taxonomy of hypocrealean fungi, the reaction of the perithecial wall to KOH is considered as an important character (Rossman et al. 1999, Zeng and Zhuang 2016). Most species of Allantonectria and Thyronectria have perithecial colour turning darker to blood-red or purple in KOH. However, some species in Thyronectria display a weak or negative reaction to KOH, which might be influenced by the presence of scurf covering the perithecia or their dark-coloured ascomata (Hirooka et al. 2012, Jaklitsch and Voglmayr 2014, Zeng and Zhuang 2016). In our study, the dark perithecial walls of Neothyronectria citri do not change colour in KOH but the major features, such as well-developed stromata and ascomata with bright yellow scurf, as well as the molecular data, also provide strong evidence that it belongs to Neothyronectria.

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