A morphological and phylogenetic revision of the Nectria cinnabarina species complex

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Abstract: The genus Nectria is typified by N. cinnabarina, a wood-inhabiting fungus common in temperate regions of the Northern Hemisphere. To determine the diversity within N. cinnabarina, specimens and cultures from Asia, Europe, and North America were obtained and examined. Their phylogeny was determined using sequences of multiple loci, specifically act, ITS, LSU, rpb1, tef1, and tub. Based on these observations, four species are recognised within the N. cinnabarina complex. Each species is delimited based on DNA sequence analyses and described and illustrated from specimens and cultures. The basionym for N. cinnabarina, Sphaeria cinnabarina, is lectotypified based on an illustration that is part of the protologue, and an epitype specimen is designated. Mature conidia of N. asiatica, N. cinnabarina, and N. nigrescens but not N. dematiosa bud when the mature conidia are crowded. On PDA the optimal temperature for growth for N. dematiosa is 20 °C, while for the other three species it is 25 °C. Based on our phylogenetic analyses, three subclades are evident within N. dematiosa. Although subtle culture and geographical differences exist, these subclades are not recognised as distinct species because the number of samples is small and the few specimens are insufficient to determine if morphological differences exist in the natural environment.

Key words: Ascomycota, Hypocreales, molecular systematics, Nectriaceae, plant pathogen, type species.

Taxonomic novelty: Nectria asiatica Hirooka, Rossman & P. Chaverri, sp. nov.

INTRODUCTION

Nectria cinnabarina is the type species of the genus Nectria (Hypocreales, Nectriaceae). This species is characterised by red, globose, fleshy, warted perithecia that often become cupulate upon drying, 0–3-septate ascospores, and an anamorph referred to as Tubercularia vulgaris (Rossman et al. 1999). Nectria cinnabarina is a relatively common species that occurs on a range of hardwood trees and woody shrubs throughout the temperate regions of the Northern Hemisphere. It is occasionally considered to be a plant pathogen causing a disease on apple and other hardwood trees known as "coral spot" because of the pinkish sporodochia of its Tubercularia anamorph (Sinclair & Lyon 2005).

Nectria cinnabarina was originally described as Sphaeria cinnabarina by Tode (1791). When Fries (1849) sanctioned Sphaeria cinnabarina, he transferred this name to Nectria. Nectria cinnabarina was designated the lectotype species of the genus by Clements & Shear (1931). Nectria was conserved with this type species over Ephedrosphaera and Hydrapisphaera (Cannon & Hawksworth 1983).

In studying the species of Nectria in the UK, Booth (1959) emphasised perithecial wall structure when he referred the large genus into groups. He included three species in what he referred to as the Nectria cinnabarina group: N. cinnabarina, N. aurantiaca, and N. ralfsii. When Rossman (1989) and Rossman et al. (1999) restricted Nectria s. str. to species congreneric with N. cinnabarina, they included N. aurantiaca and other species with a similar perithecial wall structure in Nectria s. str. Nectria ralfsii is now regarded a species of Bionectria, B. ralfsii (Schroers 2001).

Because of its morphological heterogeneity, 20 varieties and forms of Nectria cinnabarina exist as well as numerous synonyms. Wollenweber (1926, 1930) recognised three varieties of N. cinnabarina. Nectria cinnabarina var. minor was distinguished from the type variety by its smaller ascospores and conidia, while Nectria cinnabarina var. dendroidea has remarkably long, stipitate sporodochia. Nectria cinnabarina var. ribis (≡ N. ribis) was said to have larger ascospores and conidia than the other two varieties. Jørgensen (1952) published a monograph on N. cinnabarina and suggested that Nectria ribis was a "nomen confusum", being a mixture of N. cinnabarina and N. berolinensis. Despite detailed observations, he did not find differences among specimens of N. cinnabarina; however, he noted differences between specimens on non-Ribes hosts and those on Ribes that he recognised as N. cinnabarina var. ribis.

Tubercularia (Tode 1790) includes anamorphs of several species in the Nectria cinnabarina group (Booth 1959, Rossman 1983). Tubercularia, conserved based on T. vulgaris, was segregated from fungi with black sporodochia by Fries (1832). Saccardo (1886) divided species of Tubercularia into four groups based on differences in substrate; however, his taxonomic concept was revised by Pauletto (1887) who emphasised the acropleurogenously developing phialides. Petch (1940) organised and revised the British records of Tubercularia. Seifert (1985) provided a thorough account of Tubercularia accepting eight species including T. vulgaris with many synonyms.
Although Tode (1790, 1791) described and illustrated both Sphaeria cinnabarina and Tubercularia vulgaris, he did not recognise their relationship as states of one species. Later, Fries (1828) determined that these were the sexual and asexual states of the same species. Modern authors have confirmed that N. cinnabarina and T. vulgaris are manifestations of the same species (Seifert 1985, Rossman 1989).

Nectria cinnabarina is commonly regarded as a saprobe; as mentioned above, it sometimes causes cankers on hardwood trees and woody shrubs. The parasitic occurrence of N. cinnabarina was first reported by Mayr (1883), who considered this species to be parasitic on Acer, Aesculus, Prunus, Robinia, Spiraea, Tilia, and Ulmus. Many hardwood trees and woody shrubs around the world have been reported as hosts for N. cinnabarina (Sinclair & Lyon 2005). Jegensen (1952) demonstrated that N. cinnabarina was a facultative parasite and saprobe, but could not differentiate pathogenic races. He mentioned the following genera as the most common hosts of N. cinnabarina in Denmark: Acer, Aesculus, Carpinus, Fagus, Fraxinus, Malus, Prunus, Ribes, Tilia, and Ulmus. Similarly the anamorph has been commonly reported on woody substrates in many plant families (Seifert 1985).

Based on our hypothesis that Nectria cinnabarina is a heterogeneous and might comprise several species, detailed morphological and molecular phylogenetic analyses of this species were undertaken. Many isolates of freshly collected and herbarium specimens from around the world were analysed to define phylogenetic species within the N. cinnabarina species complex (NCSC). Each species is described and illustrated and a key is provided.

Table 1. Isolates and accession numbers used in the phylogenetic analyses.

| Species          | Isolate No. | Herbarium No. | Substrate/Host | Country | GenBank Accession No. |
|------------------|-------------|---------------|----------------|---------|-----------------------|
| Cosmospora coccinea | A.R. 2741, CBS 114050 | BPI 802729 | Inonotus nodulosus | Germany | GQ505967 * GQ505990 * HM484537 GQ506202 * HM484515 HM484589 |
| Cyanonectria cyanostoma | G.J.S. 98-127, CBS 101734 | BPI 743037 | Buxaceae | France | GQ505961 * HM484558 FJ474081 * GQ506017 * HM484535 HM484611 |
| Nectria antarctica | A.R. 2767, CBS 115033, ATCC 204178 | BPI 746217 | Dead stem of Mahonia aquifolium | USA | HM484501 HM484556 HM484560 HM484575 HM484516 HM484601 |
| Nectria aquifoli | A.R. 4108, CBS 125147 | BPI 880698 | ilex aquifolium | UK | HM484406 HM484538 HM484565 HM484579 HM484522 HM484590 |
| Nectria asiatica | MAFF 241408 | BPI 879980 | Dead wood | Japan | – HM484503 HM484744 HM484790 – HM484815 |
| A.R. 4639, CBS 126568 | MAFF 241401 | BPI 879978 | Dead wood | China | – HM484713 HM484727 HM484787 – HM484811 |
| MAFF 241435 | BPI 879973 | Dead wood | Japan | – HM484624 HM484716 HM484747 HM484798 – HM484817 |
| MAFF 241399 | BPI 879976 | Prunus sp. | Japan | – HM484715 HM484751 HM484791 – HM484813 |
| MAFF 241448 | BPI 879974 | Dead twig | Japan | – HM484626 – HM484728 HM484793 – HM484809 |
| MAFF 241398 | BPI 879975 | Dead wood of Zelkova serrata | Japan | – HM484508 HM484702 HM484738 HM484792 – HM484812 |
| MAFF 241439 | BPI 879972 | Bark of dead wood | Japan | – HM484505 HM484701 HM484563 – HM484604 |
| MAFF 241405 | BPI 879979 | Dead twig of Prunus sp. | Japan | – HM484708 HM484748 HM484789 – HM484814 |
| MAFF 241400 | BPI 879977 | Dead stem of Sorbus commixta | Japan | – HM484623 HM484705 HM484743 HM484796 – HM484818 |
| Nectria aurigera | A.R. 3717, CBS 109874 | BPI 841465 | Twigs dead, Fraxinus excelsior | France | HM484511 HM484551 HM484573 HM484586 HM484521 HM484600 |
| Nectria austroamericana | A.R. 2808, CBS 126114 | BPI 746395 | Gleditsia triscanths | USA | GQ505960 * HM484555 GQ505988 * GQ506016 * HM484520 HM484597 |
| Species          | Isolate No.  | Herbarium No. | Substrate/Host | Country      | GenBank Accession No. |
|------------------|--------------|---------------|----------------|--------------|-----------------------|
| *Nectria balansae* | A.R. 4446, CBS 123351 | BPI 879477 | Cornonia sp. | France | GQ505977 * | HM484552 | GQ505996 * | GQ506026 * | HM484525 | HM484607 |
| *Nectria balansae* | A.R. 4478, CBS 125166 | BPI 746346 | Branches standing. Ribes rubrum | Austria | HM484510 | HM484543 | HM484568 | HM484583 | HM484517 | HM484594 |
| *Nectria cinnabarina* | A.R. 4327, CBS 125154 | G.J.S. 91-111, CBS 713.97 | Acer sp. | Canada | HM484642 | HM484688 | HM484733 | HM484778 | HM484666 | HM484824 |
| *Nectria cinnabarina* | A.R. 4340, CBS 125156 | BPI 878335 | Acer saccharum | Canada | HM484636 | HM484687 | HM484741 | HM484780 | HM484667 | HM484822 |
| *Nectria cinnabarina* | A.R. 4341, CBS 125157 | BPI 878311 | Acer pseudoplatanus | Canada | HM484643 | HM484694 | HM484723 | HM484766 | HM484670 | HM484833 |
| *Nectria cinnabarina* | A.R. 4379, CBS 125158 | BPI 878313 | Fagus sp. | USA | HM484660 | HM484696 | HM484739 | HM484772 | HM484668 | HM484830 |
| *Nectria cinnabarina* | A.R. 4337, CBS 127668 | BPI 878312 | Acer pseudoplatanus | Denmark | HM484631 | HM484690 | HM484726 | HM484775 | HM484659 | HM484826 |
| *Nectria cinnabarina* | A.R. 4477, CBS 125165 | BPI 879961 | Twigs of Aesculus sp. | France | HM484503 | HM484548 | HM484562 | HM484577 | HM484527 | HM484606 |
| *Nectria cinnabarina* | A.R. 4496 | BPI 878878 | Populus tremula | Ukraine | HM484641 | HM484712 | HM484731 | HM484768 | HM484658 | HM484831 |
| *Nectria cinnabarina* | A.R. 4302, CBS 125150 | BPI 878317 | Acer pseudoplatanus | Germany | GQ505975 * | GQ505997 * | GQ506027 * | HM484663 | HM484832 |
| *Nectria coryli* | A.R. 4303, CBS 125151 | BPI 878316 | Twigs of Ulmus sp. | Netherlands | HM484628 | HM484692 | HM484755 | HM484699 | HM484656 | HM484834 |
| *Nectria coryli* | A.R. 4561, Y.H. 0815 | BPI 880697 | Twigs of Rhus cotinus | USA | HM484659 | HM484539 | HM484566 | HM484801 | HM484536 | HM484596 |
| *Nectria cucurbilula* | CBS 259.58 | | Pinus sylvestris | Netherlands | GQ505974 * | HM484541 | GQ505996 * | GQ506028 * | HM484530 | HM484592 |
| *Nectria dermatosus* | CBS 126570, G.J.S. 94-37 | BPI 749337 | Acer sp. | USA | HM484502 | HM484557 | HM484561 | HM484576 | HM484534 | HM484603 |
| *Nectria dermatosus* | A.R. 4328, CBS 125155 | CBS 279.48 | Acer pseudoplatanus | USA | HM484616 | HM484680 | HM484725 | HM484761 | HM484648 | HM484799 |
| *Nectria dermatosus* | CBS 126570, G.J.S. 94-37 | CBS 278.48 | Acer pseudoplatanus | Netherlands | GQ59974 | GQ505997 | GQ506028 | GQ506028 | GQ506028 | GQ506028 |
| *Nectria dermatosus* | A.R. 4300, CBS 125159 | BPI 878308 | Acer pseudoplatanus | Poland | HM484615 | HM484682 | HM484729 | HM484760 | HM484647 | HM484800 |
| *Nectria dermatosus* | A.R. 2702, CBS 125127 | BPI 802215 | Dead twig of Acer macrophyllum | Canada | HM484613 | HM484677 | HM484719 | HM484758 | HM484646 | HM484798 |
| *Nectria dermatosus* | A.R. 2702, CBS 125127 | BPI 879696 | Dead twig of Rosa sp. | Japan | HM484617 | HM484704 | HM484750 | HM484795 | HM484653 | HM484803 |
| *Nectria dermatosus* | A.R. 4638, CBS 127667 | BPI 879984 | Attached branches of Weigela coraeensis | Japan | HM484714 | HM484732 | HM484764 | HM484652 | HM484804 |
### Table 1. (Continued).

| Species                  | Isolate No. | Herbarium No. | Substrate/Host          | Country            | GenBank Accession No. |
|--------------------------|-------------|---------------|-------------------------|--------------------|-----------------------|
| *Nectria lamyi*          | A.R. 2779, CBS 115034 | BPI 746349 | Berberis vulgaris        | Austria            | HM484507, HM484544, HM484569, HM484582, HM484582, HM484593 |
| *Nectria milkina*        | A.R. 4391, CBS 121121 | BPI 878442 | Decaying leaves of Agave americana | Italy              | HM484514, HM484547, HM484572, HM484587, HM484524, HM484609 |
| *Nectria nigrescens*     | A.R. 4228, CBS 125148 | BPI 878455A | Dead twig of Acer sp.    | France             | HM484619, HM484711, HM484745, HM484785, HM484673, HM484808 |
|                          | A.R. 4211, CBS 125149 | BPI 871083 | Dead twig of dictyoldenous tree | USA             | HM484618, HM484707, HM484720, HM484781, HM484672, HM484806 |
|                          | A.R. 4475, CBS 125164 | BPI 878457 | Twigs of Fagus sylvestica | France             | HM484504, HM484550, HM484564, HM484578, HM484526, HM484605 |
|                          | AR 4565, CBS 127666 | BPI 879986 | Dead twig               | USA                | HM484620, HM484683, HM484730, HM484784, HM484674, HM484810 |
|                          | A.R. 4213, CBS 125149 | BPI 871084 | Dead twig of Betula lutea | USA              | HM484622, HM484679, HM484721, HM484782, HM484675, HM484819 |
|                          | A.R. 4394, CBS 125162 | BPI 878449 | Twigs of Celtis occidentalis | Canada         | HM484621, HM484678, HM484737, HM484783, –, HM484807 |
| *Nectria pseudocinnabarina* | A.R. 4548 | C.L.L. 8299 | Unknown                  | Mexican           | –, HM484553, HM484574, HM484588, HM484529, HM484608 |
| *Nectria pseudodubrichia* | CBS 551.84 | Unknown | Unknown                   | Japanese          | GQ505976 *, GQ484554, GQ506000 *, GQ506030 *, GQ484532, GQ484602 |
| *Nectria pyriformis*     | A.R. 2786, CBS 125131 | BPI 746398 | Acer campestre           | Austria            | HM484512, HM484545, HM484570, HM484584, HM484519, HM484598 |
| *Nectria sinopica*       | CBS 462.93 | CBS H-19479, CBS H-19465 | Hedera helix          | Netherlands        | GQ505973 *, GQ484542, GQ506001 *, GQ506031 *, GQ484531, GQ484595 |
| *Nectria zanthoxyli*     | A.R. 4280, CBS 126113 | BPI 878445 | Crataegus sp.            | France             | HM484513, HM484546, HM484571, HM484585, HM484523, HM484599 |
| *Theletonia westlandica* | G.J.S. 83-156, CBS 112264 | Dacrydium cupressinum | New Zealand            | –                  | GQ505959, GQ484559, GQ506087 *, GQ506015 *, GQ484533, GQ484610 |

A.R.: Amy Y. Rossman, USDA-ARS MD USA; ATCC: American Type Culture collection, Manassas, VA, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; C.L.L.: Christian Lechat, Ascofrance, Villiers en Bois, France; G.J.S.: Gary J. Samuels, USDA-ARS MD USA; A.R.: Amy Y. Rossman, USDA-ARS MD USA; ATCC: American Type Culture collection, Manassas, VA, USA; BPI: U.S. National Fungus Collections USDA-ARS MD USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; C.L.L.: Christian Lechat, Ascofrance, Villiers en Bois, France; G.J.S.: Gary J. Samuels, USDA-ARS MD USA; MAFF: MAFF Genebank, National Institute of Agrobiological Sciences, Ibaraki, Japan; Y.H.: Yuuri Hirooka, USDA-ARS MD USA.

Sequences obtained from GenBank.

Morphological observations

For morphological characterisation of the teleomorph, the macromorphology of the perithecia and stroma was observed and described as follows: distribution of perithecia on the host; perithecia shape, colour and reaction to 3 % w/v potassium hydroxide (KOH) and 100 % lactic acid (LA) using a stereoscope (Zeiss, STEMI SV11, Jena, Germany). To observe internal and microscopic characteristics, the perithecia and stroma were sectioned by hand and rehydrated in water, KOH, and LA. Characteristics of asci and ascospores were observed by rehydrating the perithecia in water, removing part of the centrum with a fine glass needle, and placing it onto a glass slide. Microscopic observations were made using a compound microscope (Zeiss, Axioskop 2 Plus, Jena, Germany). To determine growth rates, colony colour, and odour, isolates were grown on PDA in 9-cm plastic dishes at 25 °C for 7 d in the dark. For observation of sporulating structures, the cultures were grown on a low nutrient agar (SNA; Nirenberg 1976). Cultures on SNA were incubated at 25 °C with alternating 12 h/12 h fluorescent light/darkness for 2–3 wk. Young conidia are those that develop after one or two d on SNA while mature conidia are 4–5 d old. To stimulate budding, mature conidia produced on SNA were suspended in distilled water and then streaked on SNA. After 24 h, budding mature conidia and germ tubes were produced. Images were captured with a Nikon DXM1200 digital camera. Some composite images were made with Helicon Focus v. 4.21.5 Pro (Helicon Soft, www.heliconfocus.com). All recognition of colour such as perithecia, ascospores, conidia, and top and reverse colony colour were described according to Kmerup & Wanscher (1978).

Statistical analysis

Measurements of continuous characters such as length and width were made using Scion Image software beta v. 4.0.2 (Scion Corporation, Frederick, Maryland, USA) and are based on up to 50 measurements for structures in each isolate. For morphological structures, descriptive statistics (minimum, mean, median, maximum, and standard deviation) were computed and variation of morphological characters displayed graphically using mean values and their corresponding 95 % confidence intervals. All computations were performed using Systat 10 (Systat Software, San José, California, USA). Only isolates for which all data were available were included in the analysis. Ranges are reported as mean values ± one standard deviation; the number of items measured is given in parentheses together with maximum and minimum.

Cardinal temperatures

Disks of 5 mm diam were cut from the edge of young colonies and placed in the centre of PDA plates, then incubated at temperatures from 15 to 35 °C at 5 °C intervals in complete darkness. Diameters of the colonies on three plates for each isolate at each temperature were measured daily for 1 wk.
Table 2. Genes/loci used in the phylogenetic analyses for members of the genus Nectria. Information on the primers, base pairs, PCR protocols, and models of nucleotide substitution are indicated.

| Locus | Primers used (reference) | PCR protocol: Annealing temp. & cycles | Nucleotide substitution models | Included sites (# of excluded sites) | Phylogenetically informative sites (%) | Uninformative polymorphic sites | Invariable sites |
|-------|---------------------------|----------------------------------------|---------------------------------|-------------------------------------|--------------------------------------|-------------------------------|-----------------|
| Act   | Taclf, Talc2 (Samuels et al. 2006) | 65 °C, 30 s, 15´ | GTR+G | 613 (127) | 111 (18 %) | 43 | 459 |
| ITS   | IT55, IT54 (White et al. 1990) | 53 °C, 1 min, 35´ | TIM3+H+G | 539 (279) | 62 (12 %) | 52 | 425 |
| LSU   | LR5, LROR (Vilgalys n.d.) | 53 °C, 1 min, 35´ | TIM3+H+G | 807 (150) | 67 (8.3 %) | 39 | 701 |
| Rpb1  | cebp1a, rpb1c (Castlebury et al. 2004) | 50 °C, 2 min, 40´ | TIM2+H+G | 590 (540) | 233 (40 %) | 65 | 292 |
| Tefl  | tefl-728, tefl-1567 (Carbone & Kohn 1999, Rehner 2001) | 66 °C, 55 s, 9´ | GTR+H+G | 645 (261) | 142 (22 %) | 43 | 460 |
| Tub   | βtub-T1, βtub-T2 (O’Donnell & Cigelnik 1997) | 55 °C, 30 s, 35´ | TPM3uf+H+G | 479 (408) | 192 (40 %) | 32 | 255 |
| Total |                          |                               |                                 | 3673 | 807 (22 %) | 274 | 2592 |

Table 3. Genes/loci used in the phylogenetic analyses for members of Nectria cinnabarina species complex (NCSC). Information on the primers, base pairs, PCR protocols, and models of nucleotide substitution are indicated.

| Locus | Primers used (reference) | PCR protocol: Annealing temp. & cycles | Nucleotide substitution models | Included sites (# of excluded sites) | Phylogenetically informative sites (%) | Uninformative polymorphic sites | Invariable sites |
|-------|---------------------------|----------------------------------------|---------------------------------|-------------------------------------|--------------------------------------|-------------------------------|-----------------|
| Act   | Taclf, Talc2 (Samuels et al. 2006) | 65 °C, 30 s, 15´ | TrN+G | 649 (91) | 47 (7 %) | 40 | 562 |
| ITS   | IT55, IT54 (White et al. 1990) | 53 °C, 1 min, 35´ | TrNe+G | 475 (592) | 38 (8 %) | 19 | 418 |
| LSU   | LR5, LROR (Vilgalys n.d.) | 53 °C, 1 min, 35´ | TIM1+H+G | 814 (260) | 18 (2 %) | 14 | 782 |
| Rpb1  | cebp1a, rpb1c (Castlebury et al. 2004) | 50 °C, 2 min, 40´ | TrN+G | 621 (123) | 111 (18 %) | 120 | 390 |
| Tefl  | tefl-728, tefl-1567 (Carbone & Kohn 1999, Rehner 2001) | 66 °C, 55 s, 9´ | TrN+G | 828 (186) | 158 (19 %) | 36 | 634 |
| Tub   | βtub-T1, βtub-T2 (O’Donnell & Cigelnik 1997) | 55 °C, 30 s, 35´ | TPM3uf+G | 527 (135) | 88 (17 %) | 138 | 301 |
| Total |                          |                               |                                 | 3914 | 460 (12 %) | 367 | 3527 |

DNA extraction, PCR, and sequencing

The forty-five cultures of N. cinnabarina used in the phylogenetic analyses (Table 1) and representatives of other species of Nectria s. str. were grown in Difco™ potato dextrose broth in 6 cm diam Petri plates for about 3 wk. Mycelial mats were harvested in a laminar flow hood and dried with clean, absorbent paper towels. DNA was extracted with Ultra Clean™ Plant DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California, USA). Sequences are deposited in GenBank (Table 1).

Phylogenetic analyses

Sequences of the six genes were aligned with MAFFT v. 6 (Katoh 2008) and the alignment was visually improved with Mesquite v. 2.6 (Maddison & Maddison 2009). Maximum likelihood (ML) and Bayesian (BI) analyses were carried out with all sequences, first each locus separately, then with the combined/concatenated data sets. Representative members of the Nectriaceae, namely Cosmosporea coccinea, Cyanonectria cyanostoma, and Thelonectria westlandica, were used as outgroups for inferring intrageneric relationships (Fig. 1). Nectria balansae, N. pseudotrichia, and N. pseudocinnabarina were used as outgroup taxa for the NCSC tree, including 45 isolates in the NCSC (Fig. 2). JMODELTEST (Posada 2008) was used to calculate the models of nucleotide substitutions of each gene/partition for the ML and BI analyses. The number of substitution schemes was set to 11, base frequencies +F, rate variation +I and +G, and the base tree for likelihood calculations was set to "ML
位於Koshikihara et al. (2001)。“optimized”。88 models were compared. After the likelihood scores were calculated, the models were selected according to the Akaike information criterion (AIC) (Posada & Buckley 2004). Under the AIC settings, the AICc corrected for smaller samples was selected. After jMODELTEST was run, likelihood settings for trees of the *Nectria* tree and NCSC tree were set to each gene (Tables 2, 3). For the ML and bootstrap analyses (BP), GARLI version 0.96 (Zwickl 2006) was computed through the Grid computing (Cummings & Huskamp 2005) and The Lattice Project (Bazinet & Cummings 2008), which includes clusters and desk tops in one integrated network (Myers et al. 2008). In GARLI, the starting tree was made by stepwise-addition and the number of runs or search replicates was set to 50. 2000 ML BP replicates were done in GARLI with the starting tree chosen randomly. Bayesian analysis (BI) was done using MrBayes v. 3.1.2 (Huelsenbeck et al. 2001, 2002). In MrBayes, data were partitioned by locus and the parameters of the nucleotide substitution models for each partition were set as described (Tables 2, 3). For this analysis, two independent analyses of two parallel runs and four chains were carried out for 5 000 000 generations using MrBayes. Analyses were initiated from a random tree and trees sampled every 100th generation. The first 20 % of the resulting trees were eliminated (= “burn in”). A consensus tree (“sumt” option) and posterior probabilities (PP) were calculated in MrBayes, which combines the results from both parallel runs. A reciprocal 70 % BP threshold was used to detect topological incongruence among genes/partitions (Mason-Gamer & Kellogg 1996, Reeb et al. 2004).

**RESULTS**

**Phylogenetic analyses**

Sequencing and alignment of the six loci for 23 taxa in *Nectria* resulted in 3 673 base pairs, 807 (22 %) phylogenetically informative, and 2 592 invariable sites; 325 sites presented unique non-informative polymorphic sites (Table 2). Sequencing and alignment of the six loci for 48 taxa for the NCSC tree included 3 914 base pairs, 460 (12 %) phylogenetically informative, and 3 087 invariable sites; 325 sites presented unique non-informative polymorphic sites (Table 3). Ambiguously aligned and poly-T/A regions were excluded from the analyses. For the species of *Nectria*, the ML and BI analyses of the combined six loci produced one tree with Ln likelihoods of −21393.478926 and −21514.704, respectively (Fig. 1). For the NCSC tree, ML and BI analyses produced one tree with Ln likelihoods of −11339.862470 and −11408.155, respectively (Fig. 2). The topologies of the ML and BI trees were congruent.

The topologies of each gene tree did not contradict each other, although the tef1 tree does not include *N. asiatica* (results not shown). All individual gene trees reveal three clades in *N. dematioida* species complex. Among these trees, the act tree provides the best resolution with best BP support as evidenced in the high BP and PP support in most nodes.

The combined ML and BI analyses of six loci indicated that *Nectria* comprises two major clades: species with *Tubercularia* anamorphs (0.73 BI PP, 52 % ML BP) and species with pycnidial anamorphs (1.00 BI PP, 100 % ML BP) (Fig. 1). All isolates initially identified as *N. cinnabarina* formed a monophyletic *Nectria- Tubercularia* clade supported by high BI PP and ML BP value (1.00 BI PP, 100 % ML BP).
The combined ML and BI analyses of six loci using 45 isolates of the NCSC resolved four distinct species (Fig. 2). One major clade (clade II) included three species with high support (BI PP 0.96, ML BP 75 %). One of the species in clade II represents *N. cinnabarina* s. str. and includes the ex-epitype isolate from a hardwood tree in Europe with isolates on hardwoods in Europe and North America. *Nectria cinnabarina* s. str. is highly supported (BI PP 1.00, ML BP 99 %). A second segregate species occurring only in Asia is here described as a new species, *N. asiatica*. This species was supported by moderate values (BI PP 0.69, ML BP 80 %). A third species is recognised as *N. nigrescens*, previously considered a synonym of *N. cinnabarina*. *Nectria nigrescens* also occurs on hardwoods in Europe and North America. This species is highly supported (BI PP 1.00, ML BP 99 %). A fourth segregate species, recognised as *N. dematiosa* (clade I), a previous synonym of *N. cinnabarina*, constitutes a sister clade to clade II. Within *N. dematiosa*, three subclades are highly supported (BI PP 1.00, ML BP 97 % for subclade A; BI PP 1.00, ML BP 100 % for subclade B; and BI PP 0.96, ML BP 80 % for subclade C). However, clades I was poorly supported (BI PP 0.62, ML BP 54 % for clade I) (Fig. 2). *Nectria dematiosa* subclade A is known from Europe and North America. *N. dematiosa* subclade B is represented by two isolates from Canada, while *N. dematiosa* subclade C is known only from Asia.
Morphological, colony growth, and temperature analyses

Morphological characters of the teleomorph and anamorph in the natural environment and cultural characteristics are useful in distinguishing species in the NCSC. Perithecial characters, such as colour, surface, and cell wall structure, are generally reliable for identifying the species complex, but not the segregate species. The perithecial wall surface of species in the NCSC is roughened, with conspicuous to small warts, 10–20 μm high, rarely smooth. In all species of the NCSC, the perithecial walls are about the same thickness and cell walls form similar textura globulosa or t. angularis; thus, perithecial wall structure is not useful in distinguishing species. Differences in ascospore septation correlate with phylogenetic species recognised in the NCSC. *Nectria asiatica* has up to 1-septate ascospores, *N. cinnabarina* and *N. dematiosa* have up to 2-septate ascospores, and *N. nigrescens* has up to 3-septate ascospores. The size ranges of ascospores in the four species overlap. However, in comparing 95 % confidence intervals of length/width ratios of ascospores on natural substrate, those of *N. asiatica* are greater than the other species while those of *N. cinnabarina* on *Ribes* are less than the other species (Fig. 3).

Anamorph characters on natural substrate, especially presence or absence and length of the stipe of the sporodochia, are useful in distinguishing species. A distinction is made here between sporodochia that are astipitate i.e. lack any kind of stipe and sporodochia that are stipitate having a short stipe, less than 800 μm high, or a long stipe, 700–1600 μm high. The sporodochia of *N. dematiosa* are astipitate. In clade II, which includes *N. cinnabarina*, *N. asiatica*, and *N. nigrescens*, the sporodochia are short to long stipitate. *Nectria asiatica* has short stipitate sporodochia, *N. cinnabarina* has long stipitate sporodochia, and *N. nigrescens* has short to long stipitate sporodochia. The long stipitate sporodochia of *N. cinnabarina* and *N. nigrescens* have marginal cells arranged in a palisade, while the short stipitate sporodochia of *N. asiatica* and *N. nigrescens* lack these cells.

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Fig. 3. Graphs of 95 % confidence intervals of length to width ratios of ascospores and conidia.
Additional morphological characteristics of the anamorph were also evaluated. These characteristics include the number of conidiophore branches and conidial size in the natural environment. No differences were found between species. The sizes of conidia among the four species overlap; however, in comparing 95% confidence intervals of length/width ratios of conidia on natural substrate, those of *N. asiatica* are larger than other members of the NCSC (Fig. 3).

The optimal temperature for growth on PDA for *N. dematiosa* is 20 °C while that for *N. asiatica, N. cinnabarina, and N. nigrescens* is 25 °C (Fig. 4). In macroscopic appearance these colonies look similar.
Conidia produced in culture show differences that correlate with species. The size of conidia varies considerably when grown on different media (CMD, PDA, and SNA). On SNA conidia were classified into two types, namely young and mature conidia. Mature conidia appear after 3 to 4 d and are defined by extreme swelling to twice their original size, becoming 1-septate, often including vacuoles. The 95% confidence interval of length/width ratios of young conidia in culture of N. asiatica was larger than that of other species of the NCSC (Fig. 3). By observing mature conidia on SNA, we could distinguish species in the NCSC. Mature conidia of N. cinnabarina budded abundantly while those of N. asiatica and N. nigrescens rarely budded. Mature conidia of N. dematiosa did not bud at all. In evaluating the 95% confidence intervals of length/width ratios of mature conidia in culture, N. cinnabarina, N. dematiosa subclade B, and N. nigrescens were smaller than other members of the NCSC. Each subclade in N. dematiosa can be distinguished by the morphology of the anamorph in culture. Mature conidia of subclade A produced almost straight germ tubes that do not penetrate the agar immediately, while mature conidia of subclades B and C produced sinuous germ tubes that penetrate the agar after germination. The 95% confidence interval of length/width ratio of mature conidia of subclade B was statistically different from subclades A and C (Fig. 3). On PDA at 25 °C for 7 d, subclade B grew more slowly than subclades A and C (Fig. 4).

In summary, clades I includes N. dematiosa with subclades A, B, and C. This species is characterised by ascospores that are generally 1-septate, rarely 0- or 2-septate, sessile sporodochia or anamorph lacking, mature conidia that do not bud, and an optimum growth temperature of 20 °C on PDA. Clade II includes N. asiatica, N. cinnabarina and N. nigrescens, all of which have short to long stipitate sporodochia, mature conidia that bud, although sometimes only rarely, and an optimum growth temperature of 25 °C on PDA. Nectria cinnabarina has 1-septate, rarely 0- or 2-septate ascospores, long stipitate sporodochia, and mature conidia that bud abundantly. Nectria asiatica has 1-septate, rarely 0-septate ascospores, short stipitate sporodochia, and mature conidia that seldom bud. Nectria nigrescens has 1-, 2-, or occasionally 3-septate ascospores, short to long stipitate sporodochia, and mature conidia that bud infrequently.

Taxonomy

Based on our morphological and molecular analyses, the N. cinnabarina species complex is recognised as four distinct species, each of which is described and illustrated below. A key to these four species is provided.

**Nectria asiatica** Hirooka, Rossmann & P. Chaverri, sp. nov. MycoBank MB516721. Fig. 5. Anamorph: Tuberculina vulgaris-like.

**Etymology:** Asia + -atica - indicates the area from which this species is known.

**Anamorph in culture:** Optimum temperature for growth on PDA 25 °C, maximum temperature 30 °C; after 7 d at 25 °C colonies 40–75 mm diam (average 51 mm). Colony surface on PDA radiating sometimes wavy, slightly cottony with aerial mycelium, white to whitish saffron; aerial mycelium developing in a few isolates (CBS 125151, MAFF 241448); after 3 wk abundant white to whitish yellow sporodochial conidial masses produced; reverse white to slightly whitish yellow. Odour on PDA slightly fruity. Sporulation on SNA from lateral phialidic pegs on submerged or aerial hyphae, 3.0–5.0 μm long, 1.5–2.5 μm wide at base. Aerial conidiophores developing abundantly on aerial hyphae, unbranched, sometimes verticillate, textura angularis, with pigmented walls ca. 1.5 mm thick. 

**Anamorph on natural substrata:** Stromata erumpent through epidermis, orange to red. Sporodochial conidiomata with stroma, cells forming textura angularis to t. angularis, continuous with stroma, usually with wider cells in centre. Hymenium arising directly from textura prismatic, elongating from textura angularis, up to 110 μm long, of cells 2.0–7.0 μm wide, without curved margin. Conidiophores monoverticillate or rarely bi-verticillate, then developing acropleurogenously for 3–6 levels, strongly coiled, hyaline, rarely slightly pale green. Phialides intercalary, occurring below each septum, rarely terminal; intercalary phialides monophialidic, up to 3.5–7.5 μm long, 1.5–2.5 μm wide; terminal cells monophialidic, sometimes sterile, without colarettes. Conidia hyaline, narrowly long ellipsoidal to cylindrical, straight or slightly curved, non-septate, 4.5–5.5–7.5(–9.5) μm × (1.0–)2.0–2.5(–3.0) μm (n = 258), smooth-walled.

**Teleomorph on natural substrata:** Mycelium not visible around perithecia or on host. Stromata up to 1.0 mm high and 3 mm diam, erumpent through epidermis, whitish yellow to bay, sometimes darker red, KOH+ dark red, LA+ yellow, pseudoparenchymatous; cells forming textura angularis to t. prismatic with cells oriented more or less vertically; cells 3–15 μm diam with walls 1–1.5 μm thick, intergrading with ascomatal wall. Perithecia superficial on well-developed stroma, solitary or caespitose, up to 20 on stroma, rarely clustered around base of stipitate sporodochia, subglobose to globose, 285–400 μm high × 250–380 μm diam (n = 39), red to reddish brown, sometimes cupulate upon drying, non-papillate, apical region darker, KOH+ dark red, LA+ yellow, surface with rough or concolourous warts, but sometimes smooth. Perithecial surface cells forming textura globulosa to t. angularis, with pigmented walls ca. 1.5 mm thick. Perithecial wall ca. 40–70 mm thick, of two distinct regions: outer region ca. 30–50 mm thick, intergrading with stroma, cells forming textura globulosa to t. angularis, walls pigmented, about 1.5 mm thick; inner region about 10–18 mm thick, of elongated, thin-walled, hyaline cells, forming textura prismatic. Asci uniloculate, (74–)89–101(–117) × (8.5–)10.0–12.5(–14.0) μm (n = 89), cylindrical to narrowly clavate, with an inconspicuous ring at apex, 8-spored, ascospores biseriate above, uniseriate below. Ascospores ellipsoidal to fusiform, straight, rarely slightly curved, hyaline, (0–)1-septate, (10.5–)14.5–17.5(–19.0) × (3.0–)3.5–5.0(–6.0) μm (n = 251), smooth-walled.

**Holotype:** Japan, Kanagawa Prefecture, Ashigarakami-gun, on dead wood, Oct. 2004, Y. Hirooka, holotype BPI 879972; ex-holotype culture MAFF 241439.

**Results:** 

**Perithecia:** spiny, arising below each septum, rarely terminal; intercalary, occasional, but sometimes sterile, without colarettes. Conidia hyaline, narrowly long ellipsoidal to cylindrical, straight or slightly curved, non-septate, 4.5–5.5–7.5(–9.5) μm × (1.0–)2.0–2.5(–3.0) μm (n = 258), smooth-walled.
1–3 branched, becoming loosely to moderately densely branched, 6.0–25.5 μm long, 2.0–5.0 μm wide at base. Conidiogenous cells monophialidic, cylindrical, slightly tapering toward tip or narrowly flask-shaped with widest point in middle 7.5–22.5 μm long, 2.0–3.0 μm wide at base. Young conidia developing from monophialides on submerged or aerial hyphae, produced abundantly on slimy heads, non-septate, ellipsoidal, oblong to cylindrical, hyaline, smooth, straight or slightly curved, rounded at both ends, (4.0–) 6.0–12.0(–23.0) × (1.5–)2.0–3.0(–5.0) μm (n = 210). Mature conidia swollen, mostly 0-, rarely 1-septate, ellipsoidal, oblong or allantoid,
rarely ellipsoidal with slightly constricted centre, smooth, straight or slightly curved, rounded at both ends, germinating or budding mature conidia (7.0–)11.5–17.5(–25.5) × (3.0–)3.5–4.5(–6.0) μm (n = 168). Chlamydospores and perithecia not produced in culture.

**Distribution:** Asia (China, Japan).

**Habitat:** On dead woody substrata, known in this study from *Acer* sp., *Betula lutea*, *Prunus* sp., *Sorbus commixta*, and *Zelkova serrata*.

Specimens and isolates examined: *China*, on dead wood, W.Y. Zhuang, culture CBS 126568 = A.R. 4639. *Japan*, Kanagawa Prefecture, Ashigarakami-gun, on bark of dead wood, Oct. 2004, Y. Hirooka, BPI 879973, culture MAFF 241435; Kanagawa Prefecture, Ashigarakami-gun, on dead twig, Apr. 2005, Y. Hirooka, BPI 879974, culture MAFF 241448; Kumamoto Prefecture, Kikuchi city, Kikuchi valley on dead wood of *Zelkova serrata*, Dec. 2000, Y. Hirooka, BPI 879975, culture MAFF 241396; Kumamoto Prefecture, Kikuchi city, Kikuchi valley, on twig of *Prunus* sp., Dec. 2000, Y. Hirooka, BPI 879976, culture MAFF 241399; Hokkaido, Kamiga-wan-gun, mie-cho, on dead stem of *Sorbus* commixta, Sep. 1999, Y. Ono, BPI 879977, culture MAFF 241400; Nagano Prefecture, Ina city, on dead wood, Aug. 7, 1999, Y. Ono, BPI 879978, culture MAFF 241401; Saitama Prefecture, Kawaguchi city, Angyo, on dead twig of *Prunus* sp., Sep. 2002, Y. Hirooka, BPI 879979, culture MAFF 241405; Tokyo, Setagaya-ku, Tokyo University of Agriculture, on dead wood, Oct. 2002, Y. Hirooka, BPI 879980, culture MAFF 241408.

**Notes:** *Nectria asiatica* is known only from China and Japan, a range it shares with *N. dematiosa* subclade C. To differentiate these species, it is necessary to consider morphological characters of both the teleomorph and anamorph. *Nectria asiatica* has up to 1-septate ascospores (Fig. 5F) and budding mature conidia on SNA (Fig. 5Q, R) while *N. dematiosa* subclade C has up to 2-septate ascospores (Fig. 7E) and mature conidia that do not bud on SNA (Fig. 7R–W). In addition, *N. asiatica* has an optimal temperature for growth of 25 °C on PDA while *N. dematiosa* including subclade C has an optimal temperature for growth of 20 °C on PDA (Fig. 4). Although *N. cinnabarina* and *N. nigrescens* also produce budding mature conidia, *N. asiatica* forms up to 1-septate ascospores and stipitate sporodochia shorter than the former two species.

Hara (1918) described *Nectria cinnabarina* f. *stromatica* on *Dothichiza* sp. (*Dothioraceae, Dothideales*) in Japan. He did not mention a type specimen and one could not be located. Based on his original description, this species had superficial, red,warted perithecia, asci with eight ascospores, and 1-septate ascospores. No anamorph was mentioned; however, it seems possible that the black stroma of the *Dothichiza* sp. listed as the substrate was actually the dark sporodochia of a *Tubercularia* anamorph. Most specimens of *N. asiatica* collected in Japan have chestnut to black sporodochial conidiomata. Because no type specimen could be located, we do not consider *Nectria cinnabarina* f. *stromatica* to be a synonym of *N. asiatica*.

One isolate (MAFF 241400) is phylogenetically distinct from the other isolates of *N. asiatica*; however, the BI posterior probabilities and ML bootstrap values are not high enough to clearly segregate this strain from *N. asiatica* (0.69 BI PP, 80 % ML BP) (Fig. 2). In addition, the specimen of this isolate forms up to 1-septate ascospores, short stipitate sporodochia, and ellipsoidal, budding mature conidia with slightly constricted centres, morphological characteristics typical of *N. asiatica*. Based on these morphological and molecular phylogenetic analyses, we include MAFF 241400 in *N. asiatica*.

**Nectria cinnabarina** (Tode : Fr.) Fr., Summa Veg. Scand. 2: 388. 1849. Fig. 6.

**Basionym:** *Sphaeria cinnabarina* Tode : Fr., Tode, Fungi Mecklenb. sel. 2: 9, 1791; Fries, Syst. Mycol. 2: 412. 1823.

≡ *Cucurbitaria cinnabarina* (Tode : Fr.) Pers., Persoon, Neues Magazin für Botanik, Römer 1: 83. 1794; Fries, Syst. Mycol. 2: 412. 1823.

≡ *Sphaeria decolorans* Pers., Persoon, Elenchus Fungorum 2: 81. 1827.

≡ *Nectria russelli* Berk. & M.A. Curtis in Berkeley, Grevillea 4: 45. 1875.

≡ *Nectria diffusa* Berk. & M.A. Curtis in Berkeley, Grevillea 4: 45. 1875.

**Anamorph:** *Tubercularia vulgaris* Tode : Fr., Tode, Fungi Mecklenb. sel. 1: 18, 1790; Fries, Syst. Mycol. 3: 464. 1832.

**Teleomorph from natural substrata:** *Myelium* rarely visible around perithecia and on host. *Stromata* up to 2.0 mm high and 5 mm diam, erumpent through epidermis, whitish yellow to bay, KOH+ dark red, LA+ yellow, pseudoparenchymatous, cells forming *textura angularis* to *t. prismatica* with cells oriented more or less vertically; cells 5–20 μm diam, with 1–2 μm thick walls, intergrading with ascomatal wall. *Perithecia* superficial on well-developed stroma, solitary or caespitose, up to 25 on stroma, sometimes clustered around base of stipe-like sporodochia, subglobose to globose, 275–450 μm high × 250–370 μm thick (n = 55), red to reddish brown, sometimes cupulate upon drying, non-papillate, apical region darker, KOH+ dark red, LA+ yellow, surface roughened with concolourous warts, but sometimes smooth. *Perithecial surface cells* forming *textura globulosa* or *t. angularis*, with walls pigmented ca. 1.5 mm thick. *Perithecial wall* ca. 40–60 mm thick, of two distinct regions: outer region ca. 35–55 mm thick, intergrading with stroma, cells forming *textura globulosa* or *t. angularis*, walls pigmented ca. 1.5 mm thick; inner region ca. 15–20 mm thick, of elongated, thin-walled, hyaline cells, forming *textura prismatica*. Asci unitunicate, (81–)85–96(–105) × (7.5–)8.0–9.5(–11.0) μm (n = 129), cylindrical to narrowly clavate, with inconspicuous ring at apex, 8-spored, ascospores biseriate above, uniseriate below. Ascospores ellipsoidal to fusiform, straight, sometimes slightly curved, hyaline, (0–)1(–2) septate, (11.5–)14.0–17.5(–21.5) × (3.0–)4.0–5.5(–7.0) μm (n = 558), smooth-walled.

**Anamorph on natural substrata:** *Stromata* erumpent through epidermis, pale yellow to orange, rarely reddish brown. *Sporodochial condidiomata* with stipe, superficial on well-developed stroma, smooth, cerebriform, or tubercularoid, scattered, solitary or 2–4 gregarious, stipitate, pustulate, discoid, or cylindrical-capitate, up to 700–1600 mm high including stipe, 300–2500 mm wide, white, whitish yellow to orange, sometimes darker red. *Stipe* white to whitish red, rarely darker red, up to 250–600 mm wide, solitary or 2–6 gregarious; *stipe cells* almost *textura angularis*, continuous with stroma, usually with wider cells in centre. *Hymenium* arising directly from *textura prismatica*, elongating from *textura angularis*, up to 150 μm long, of cells 2.5–5 μm wide; in stipitate forms marginal cells arranged in a palisade as described above for surface of stroma; curved margin, up to 100 μm long, of parallel hyphae 1.5–2.5 μm wide. *Conidiophores* monoverticillate or rarely bi-verticillate, then developing acropleurogenously for 3–10 levels, straight, curved. *Phialides* intercalary, occurring below each septum, or rarely terminal; *intercalary phialides* monophialidic, up to 3–9 μm long, *terminal cells* monophialidic, sometimes sterile, no collarettes. *Conidia* hyaline, narrowly long ellipsoidal to cylindrical, straight or slightly curved, non-septate, (4.0–)5.2–7.0(–8.5) × (1.3–)1.9–2.7(–3.4) μm (n = 355), smooth-walled.
Fig. 6A–R. Nectria cinnabarina. A. Perithecia and long stipitate sporodochia in the natural environment. B. Perithecia in the natural environment. C. Median section of perithecium. D. Median section of perithecial wall. E. Ascus. F. 0–2 septate ascospores. G. Long stipitate sporodochia in the natural environment. H. Median section of long stipitate sporodochia. I. Conidia in the natural environment. J. Acropleurogenous conidiophore in the natural environment. K. Aerial conidiophores and conidial mass on SNA. L. Lateral phialidic pegs on SNA. M. Aerial conidiophores and young conidia. N. Densely blanched aerial conidiophores and young conidia. O. Mature conidia on SNA. P. Budding mature conidia and secondly conidia on SNA. Q. Slimy head of young and mature conidia on lateral phialidic peg on SNA. R. Budding and germinating mature conidia (arrow) that were streaked onto SNA. Scale bars: A = 500 μm; C = 300 μm; D, = 100 μm; E, J, L, M, N, P, R = 30 μm; F, I, O, Q = 15 μm; B, G, H, K = 1 mm.
Anamorph in culture: Optimum temperature for growth on PDA 25 °C, maximum temperature 30 °C. After 7 d at 25 °C, colonies 60–85 mm (average 73 mm) diam. Colony surface radial, sometimes wavy, slightly cotonny with aerial mycelium, white to whitish saffron; aerial mycelium developed, in some isolates (A.R. 4338, CBS 127668, CBS 125154, CBS 125157, CBS 125165) abundant, white to whitish yellow sporodochial conidial masses produced after 2 wk; reverse white to slightly whitish yellow. Odour on PDA slightly fruity. Sporulation on SNA from lateral phialide pegs common, 1.5–4.5 µm long, 1.0–1.5 µm wide near aperture. Aerial conidiophores abundantly formed, unbranched, sometimes verticillate, 1–3 branched, becoming loosely to moderately densely branched, 5.5–38.0 µm long, 2.0–3.5 µm wide at base. Conidigenous cells monomorphic, cylindrical and slightly tapering toward tip or narrowly flask-shaped with widest point in middle, 5–22 µm long, 2.0–3.2 µm wide at base. Young conidia formed from monophialides on submerged or aerial hyphae, formed abundantly on slimy heads or sporodochia, ellipsoidal, oblong to cylindrical, hyaline, smooth, straight or slightly curved with rounded at both end, non-septate, (3.0–) 5.5–9.0–(15.0) × (1.5–)2.0–3.0–(3.5) µm (n = 764), smooth-walled. Mature conidia swollen, mostly 0–1 rarely 1-septate, allantoid, oblong, ellipsoidal, or ellipsoidal with strongly constricted centre, hyaline, smooth, straight or slightly curved, rounded at both ends, germinating and budding on media, (5.5–)10.5–17.0–(27.0) × (3.0–)4.0–5.0–(7.0) µm (n = 668). Chlamydomspores rarely present, globose, subglobose, broadly ellipsoidal, 0–(1)-septate, solitary or chains, 8.5–12 µm diam. Perithecia not produced in culture.

Distribution: Europe (Austria, Denmark, France, Germany, Ireland, Netherlands, Poland, Sweden, Ukraine, UK) and North America (Canada, USA).

Habitat: On dead woody substrate including Acer campestre, A. platanoides, A. pseudoplatanus, A. saccharum, Acer sp., Aesculus sp., Celastris scandens, Fagus sp., Gleditsia sp., Populus tremula, Sorbus aria, Spiraea trilobata, Tilia sp., and Ulmus x hollandica.

Lectotype of Sphaeria cinnabarina designated here: figs 68a–e in the copy of Tode HJ (1791). Fungi Mecklenburgenses select. 2: 9 associated with BPI.

Epitype of Sphaeria cinnabarina designated here. France: Villiers en Bois, on dead twigs of Aesculus sp., Feb. 13, 2008, C. Lechat, epitype BPI 879981 = C.L.L. 7152, ex-epitype culture CBS 125165 = A.R. 4477.

Additional type specimens examined: The type specimen of Sphaeria tremelloides exists at K but these specimens are no longer sent for examination. This name is retained as a synonym of N. cinnabarina. A lectotype for Sphaeria decolorans was designated here: Country unknown: on branch of Acer platanoides, ex Herb. Persoon, BPI 799523). Additional Persoon material examined: Country unknown: on bark of Ribes rubrum, Mouget, ex Herb. Persoon, BPI 799524). The lectotype and additional specimens of Sphaeria decolorans were examined, but these lacked the anamorphic structures needed to identify species within the NCSC. This name is retained as a synonym of N. cinnabarina. Type specimen of Sphaeria celastri: USA, Philadelphia, on dead branch of Celastrus scandens L., coll. possibly L.D. Schneenitz, holotype Schweinitz Syn. FH 1421. Type of Nectria russelli: USA Massachusetts, Jan. 1856, L.L. Russell, holotype FH 284394. Lectotype of Nectria officinata designated here: USA, South Carolina, on Hibiscus synicus L., lectotype BPI, Michener Collection 32, Sheet 12.

Additional specimens and isolates examined: Austria, Vienna, 19th district, base of the mountain Kahlenberg, MTB 77632, on Acer campestre L., 25 May 2006, W. Jaklitsch, BPI 878316, culture CBS 125151 = A.R. 4303; Vienna, on Acer pseudoplatanus L., 25 May 2006, coll W. Jaklitsch, BPI 878317, culture CBS 125165 = A.R. 4302. Canada, Ontario, Ottawa, on Acer sp., 26 Sep. 2006, K.A. Seifert 961, culture CBS 125154 = A.R. 4327; Quebec, Gatineau Park, Lac Philippe sector, ca. 45°35’24”N 75°59’25”W, on Acer saccharum Marsh., 15 Sep. 2006, K.A. Seifert, W. Garms, T. Graflener, BPI 878311, culture CBS 125157 = A.R. 4341; Quebec, Quebec City. Lake St. Charles, on Spiraea × arguta L., Aug. 2006, G. Lafamme, BPI 878335, culture CBS 125165 = A.R. 4340. Denmark, on bark of Tilia sp., 21 May 2006, T. Laesoe, BPI 879982, culture CBS 125152 = A.R. 4304; Sjælland, Gadevang, on Acer pseudoplatanus L., 25 Aug. 2006, W. Jaklitsch, BPI 878312, culture CBS 127668 = A.R. 4337. France, Chize, on Acer sp., Jan. 18, 2007, C. Lechat 7027, BPI 679963, culture CBS 125163 = A.R. 4397.

Germany, on Sorbus alnifolia (L.) Crantz, Oct. 166, H. Reinitz, anamorph only, culture CBS 189.87. Ireland, Dublin, Phoenix Park 53°20’59.9”N 6°17’56.8”W, on twigs, 21 Sep. 2006, K. Seifert, BPI 878313, culture CBS 125156 = A.R. 4379. Netherlands, on stem of Ulmus sp. (culture CBS 255.47, ATCC 11432; on twig of Ulmus sp., culture CBS 256.47. Poland, Sudeles, Zloty Mts., Zloty Stok, on twigs of Acer pseudoplatanus L., 6 Jun. 2008, A. Chlebicki, BPI 877822, culture A.R. 4388. Sweden, Fries, Scleromyceti Sueciae no. 184 as Sphaeria cinnabarina, BPI 779329, BPI 779330, BPI 779331, UPS,

Notes: Nectria cinnabarina is the type species of the genus Nectria. Tode (1791) described and illustrated the superficial, red, warted perithecia and 1-septate ascospores, but did not mention any detailed morphology of perithecial wall structure or stroma. Because the type specimen was lost, the name Sphaeria cinnabarina is lectotypified by the original illustration in the copy of Tode (1791) associated with BPI. A stipitate sporodochium with perithecia at the base is clearly illustrated by Tode (1791), thus assuring the identity of N. cinnabarina. Based on Article 7.8 of the ICBN (McNeill et al. 2006), an illustration from the protologue may serve as a lectotype, thus this lectotypification supersedes the neotypification by Rossman et al. (1999). We here epitypify N. cinnabarina with BPI 879981, a specimen collected in France with abundant mature perithecial and anamorphic structures as well as a living culture.

Nectria cinnabarina can be identified by morphological characteristics of the teleomorph and anamorph in the natural environment and in culture. On natural substrate, N. cinnabarina has up to 2-septate ascospores and long stipitate sporodochia (Fig. 6A, F–H). Among species in the NCSC, N. cinnabarina is similar to N. nigrescens in having long stipitate sporodochia; however, N. nigrescens is distinct in having up to 3-septate ascospores. Unlike N. asiatica, N. dematiosa, and N. cinnabarina, N. cinnabarina is distinguished in culture by abundant budding mature conidia that are ellipsoidal and strongly constricted in the centre (Fig. 6B, P).

Nectria dematiosa (Schwein.) Berk., Grevillea, 4: 16. 1875. Fig. 7.

Basionym: Sphaeria dematiosa Schwein., Trans. Amer. Philos. Soc. II, 4: 205. 1832.

≡ Cucurbitaria dematiosa (Schwein.) Kunze, Revisio Generum Plantarum 3: 461. 1898.

≡ Nectria sambuci Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 1890: 246. 1891.

≡ Nectria cinnabarina subsp. amygdalina P. Karst., Rev. Mycol. 37: 205. 1889.

≡ Nectria amygdalina (P. Karst.) Musser in Saccardo, Syll. Fung. 15: 225. 1901.

Anamorph: Tubercularia vulgaris-like.

Teleomorph on natural substrata: Mycelium not visible around perithecia and on host. Stromata up to 0.3 mm high and 2 mm

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Fig. 7. A–W. *Nectria dematiosa* species complex. A. Perithecia in the natural environment. B. Median section of perithecium. C. Median section of perithecial wall. D. Ascus. E. 1–2 septates ascospores. F. Astipitate sporodochium in the natural environment. G. Median section of stipitate sporodochium. H. Acropleurogenous conidiophore in the natural environment. I. Conidia in the natural environment. J. Aerial conidiophores and conidial mass on SNA. K. Young conidia on SNA. L. Lateral phialidic pegs and young conidia on SNA. M. Short aerial conidiophores and conidia on SNA. N. Densely blanched aerial conidiophores on SNA. O. Mature conidia and young conidia of *N. dematiosa* subclade A. P. Mature conidia and young conidia of *N. dematiosa* subclade B. Q. Mature conidia and young conidia of *N. dematiosa* subclade C. R. Germinating mature conidia (arrows) of *N. dematiosa* subclade A on SNA. S. Germinating mature conidia (arrows) of *N. dematiosa* subclade B on SNA. T. Germinating mature conidia (arrow) of *N. dematiosa* subclade C on SNA. U. Germinating mature conidia of *N. dematiosa* subclade A that were streaked onto SNA. V. Germinating mature conidia of *N. dematiosa* subclade B that were streaked onto SNA. W. Germinating mature conidia of *N. dematiosa* subclade C that were streaked onto SNA. Scale bars: A, J = 1 mm; B = 300 μm; C, G = 100 μm; D, H, I, R = 30 μm; E, K, L–W = 15 μm.
Hirooka surface with rough or concolourous warts, but sometimes smooth. In

= 40), red to reddish brown, sometimes cupulate upon drying, (subgloi-se to globose, 260–380 μm high × 220–380 μm diam

= 213); subclade C: (8.2–)10.7–17.9(–27.8) × (2.9–)3.6–5.0(–6.1) μm (n = 136); subclade B: (7.1–)9.7–16.7–29.3) × (3.5–)4.3–6.1–7.9 μm (n = 211); subclade C: (8.0–)10.7–15.9(–23.2) × (2.8–)3.3–4.7(–5.6) μm (n = 82). Chlamydospores and perithecia not produced in culture.

Anamorph on natural substrata: Stromata erumpent through epidermis, orange to red. Sporodochia conidiforma without stipe, superficial on well-developed, erumpent stroma, solitary or caespitose, up to 20 on a stroma, rarely clustered around sessile sporodochia, subglobose to globose, 260–380 μm high × 220–380 μm diam (n = 40), red to reddish brown, sometimes cupulate upon drying, non-papillate, apical region darker, KOH+ dark red, LA+ yellow, pseudoparenchymatous, surface with rough or concoulourous warts, but sometimes smooth. Perithecial surface cells forming textura globulosa or t. angularis, with walls pigmented, ca. 1.5 mm thick. Perithecial wall ca. 35–60 mm thick, of two distinct regions: outer region ca. 25–40 mm thick, intergrading with stroma, cells forming textura globulosa or t. angularis, walls pigmented, ca. 1.5 mm thick; inner region ca. 10–20 mm thick, of elongated, thin-walled, hyaline cells, forming textura prismatica. Ascii unistuniculate, (64–)77–91(–108) × (6.3–)9.4–11.0(–12.0) μm (n = 68), cylindrical to narrowly clavate, with an inconspicuous ring at apex, 8-spored, ascospores biseriate above, uniseriate below. Ascospores ellipsoid to fusiform, sometimes long fusiform, straight or slightly curved, hyaline, smooth-walled, (0–)1(–2)-septate, (12.6–)15.2–17.2(–22.2) × (3.2–)4.3–5.7(–6.4) μm (n = 150); subclade A: (12.6–)13.9–16.9(–18.5) × (3.4–)3.9–4.9(–5.3) μm (n = 30); subclade B: (13.6–)14.7–17.9(–20.5) × (3.8–)4.7–5.7(–6.4) μm (n = 60); subclade C: (12.6–)14.3–18.9(–22.2) × (3.2–)4.3–5.7(–6.2) μm (n = 60).

Anamorph on natural substrata: Stromata erumpent through epidermis, orange to red. Sporodochia conidiforma without stipe, superficial on well-developed stroma, smooth, cerebriform or tubercularoid, scattered, solitary, rarely caespitose, astipitate, elongating from (0–)1(–2)-septate, (12.6–)15.2–17.2(–22.2) × (3.2–)4.3–5.7(–6.4) μm (n = 150); subclade A: (12.6–)13.9–16.9(–18.5) × (3.4–)3.9–4.9(–5.3) μm (n = 30); subclade B: (13.6–)14.7–17.9(–20.5) × (3.8–)4.7–5.7(–6.4) μm (n = 60); subclade C: (12.6–)14.3–18.9(–22.2) × (3.2–)4.3–5.7(–6.2) μm (n = 60).

Anamorph in culture: Optimum temperature for growth on PDA 20 °C, colonies 65–85 mm (average 70 mm) diam at 20 °C after 7 d, maximum temperature 30 °C. Colony surface on PDA, radial, sometimes wavy, slightly cotty with aerial mycelium, white to whitish saffron; aerial mycelium developing in a few isolates (CBS 125127, CBS 126570), white to whitish yellow to orange, 35–70 mm high, 250–1000 mm wide, white, whitish yellow to orange, sometimes brown.

Nectria dematiosa described here: USA, Pennsylvania, on Morus sp., Bethlehem, Schweinitz, lectotype BPI 799536, isotypelectotype BPI 799535 anamorph only. The two isotype specimens of S. dematiosa have sessile sporodochia; on BPI 799536 ascospores up to 2-septate were observed. This specimen has only 4 or 5 perithecia and a few sessile sporodochia.

Epitype of Nectria dematiosa described here: USA, North Carolina, Highlands, Macon Co. Highlands Biological Station, Lake Ravenel, on bark, 31 Aug. 1994, G.J. Samuels & H.-J. Schroers, epitype BPI 749337, ex-epitype culture CBS 126570 = G.J.S. 94-37.

Additional type specimens examined. Holotype of Nectria sambuci: USA, Nebraska, Lincoln, on Sambucus nigra ssp. canadensis, Aug. 1888, H.J. Webber, holotype NY 000927949. Holotype of Nectria cinnabária subsp. amygdalina: Finland, Mustiala, on dead branch of Amygdalus nana, now to be considered to be Prunus tenella, 28 May 1989, P.A. Karsten. Holotype H 8000734.

Specimens and isolate examined. Canada, British Columbia, Sidney, Dogwood, on dead twig of Acer macrophyllum, 2 May 1992, M.E. Barr, BPI 802212, culture CBS 125125 = A.R. 2699; British Columbia, Sidney, on dead twig of Rosa sp., 5 Feb. 1992, M.E. Barr, BPI 802215, culture CBS 125127 = A.R. 2702; Ontario, Ottawa, on Acer sp., K. Seifert 1450, culture CBS 125155 = A.R. 4328. China, Jun. 2009, W.Y. Zhaung, culture CBS 127667 = A.R. 4638. Japan, Gunma Prefecture, Seta-gun, Fujimi-son, on twig of Weigela coraeensis Thunb., May 2003, Y. Hirooka, BPI 879984, culture MAFF 244116: Tokyo, Okutama-gun, on twig, Nov. 2003, Y. Hirooka, BPI 879985, culture MAFF 244130. New Zealand, Otago, on dead twig of Ribes sativum, 1 Feb. 1948, BPI 880708. Poland, Bialowieza forest, NW part of the forest near Lipiny reserve, section 271c, alt. 170 m. 52°45'13''N 23°37'59''E, on Acer pseudoplatanus, culture CBS 279.48; on Ribes sp., culture CBS 278.48.

Notes: Nectria dematiosa is distinguished from other species of the NOSC by sessile sporodochia and ascospores that are up to 2-septate. Care must be taken in observing these characters, because the short stipele sporodochia of N. asiatica and N.
Nigrescens are often covered by a mass of conidia, thus appearing sessile. In addition, the 2-septate ascospores of *N. dematiosa* occur relatively infrequently (Fig. 7E). Additional differences include mature conidia of *N. dematiosa* that never bud on SNA (Fig. TR-W). Finally, the optimum temperature for growth of *N. dematiosa* on PDA is 20 °C, while the optimum temperature for growth of *N. asiatica*, *N. cinnabarina*, and *N. nigrescens* is 25 °C (Fig. 4).

Our molecular phylogenetic analyses suggest that three subclades can be distinguished within *N. dematiosa* (Fig. 2). Some subtle differences among subclades were observed specifically in the shape and behavior of germ tubes, mycelial growth at 25 °C on PDA, and geographic range. Mature conidia of subclade A produce almost straight germ tubes that did not grow into the agar immediately, while mature conidia of subclades B and C produced sinuate germ tubes that grew into the agar after germination (Fig. 7U–W). The 95% confidence intervals of mature conidial length/width ratio of subclade B were statistically different from subclades A and C (Fig. 3). According to mycelial growth at 25 °C for 7 d on PDA, subclade B showed slower growth than subclades A and C (20–30 mm vs. 40–70 mm) (Fig. 4).

For several reasons, we do not recognize these *N. dematiosa* subclades as distinct species. First of all, in subclade A the five collections from Canada, Poland and the USA contain only one specimen with the teleomorph (BPI 749337), while anamorphs on natural substrate were observed on only two specimens (BPI 749337, BPI 878308). In subclade B, there are only two specimens both collected in Canada (BPI 802212, BPI 802215). In addition, the anamorph of BPI 802215 was not found on natural substrate. Subclade C is known only from Asia and no anamorph was observed on natural substrate (Fig. 2). The number of samples available is relatively small and the few specimens were insufficient to determine if morphological differences exist and are constant on natural substrate.

Jagensen (1952) found morphological differences between typical *N. cinnabarina* and *N. cinnabarina* on Ribes. Jagensen (1952) also mentioned that the fungus grew faster than *N. cinnabarina* from other hosts. One isolate was obtained of *N. cinnabarina* on Ribes (CBS 278.48). In growth trials this isolate showed growth similar to that of *N. dematiosa* subclade A (Fig. 4). Based on our phylogenetic analysis, this isolate falls in *N. dematiosa* subclade A with isolates collected on *Acer pseudoplatanus* and *Acer* sp. **Nectria nigrescens** Cooke, Grevillea 7: 50. 1878. Fig. 8.

- *Nectria cinnabarina* f. *dendroidea* Fuckel, Fungi hennai 2657. 1874.
- *Nectria cinnabarina* var. *dendroidea* (Fuckel) Wollenw., Angew. Bot. 8: 186. 1926.
- *Nectria cinnabarina* var. *minor* Wollenw., Angew. Bot. 8: 185. 1926.
- *Nectria melanis* Earle, Bull. Torrey Bot. Club 25: 364. 1898.
- *Nectria fuscoscapulare* Wakef., Kew Bull., p. 232. 1918.

**Anamorph:** Tubercularia vulgaris-like.

**Teleomorph on natural substrata:** Mycelium rarely visible around perithecia and on host. Stromata up to 2.0 mm high and 4.0 mm diam, erumpent through epidermis, whitish yellow to brown, sometimes darker red, KOH+ dark red, LA+ yellow, surface with rough or concolourous warts, but sometimes smooth. Perithecial surface cells forming textura globulosa or *t.* angularis, with walls pigmented ca. 1.5 mm thick. Perithecial wall ca. 40–65 mm thick, of two distinct regions: outer region about 25–45 mm thick, intergrading with stroma, cells forming textura globulosa or *t.* angularis, walls pigmented, ca. 1.5 mm thick; inner region ca. 7–18 mm thick, of elongated, thin-walled, hyaline cells, forming textura prismatica. Asci uniloculate, (62–)70–98–(113) × (6.5–)7.5–10.0–(11.5) μm (n = 63), cylindrical narrowly clavate, with an inconspicuous ring at apex, 8-spored, ascospores biseriate above, uniseriate below. Ascospores ellipsoidal to fusiform, straight, sometimes slightly curved, hyaline, (0–1)–(3)–septate, (10.5–)13.5–18.0(–22.0) × (2.5–)3.5–5.5(–8.0) μm (n = 320), smooth-walled.

**Anamorph on natural substrata:** Stromata erumpent through epidermis, pale yellow to orange, rarely reddish brown. Sporodochial conidiomata with stipe, superficial on well-developed stroma, smooth, cerebriform or tuberculoid, scattered, solitary, or 2–4 gregarious, stipitate, pustular, discoid or cylindrical-capitate, up to 250–1700 mm high, 300–1700 mm wide, white, whitish yellow to orange, sometimes brown, red or dark red; stipe white to whitish red, rarely dark red, up to 340–640 mm wide; stipe cells almost textura angularis, continuous with stroma, usually with wider cells in centre. Hymenium arising directly from textura prismatica elongating from textura angularis, up to 120 μm long, of cells 2.5–6.0 μm wide, curved margin, up to 150 μm long, of parallel hyphae 1.5–2.5 μm wide. Conidiophores monoverticillate or rarely bi-verticillate, then developing acropleurogenously for 3–7 levels, straight to curved, sometimes coiled. Phialides intercalary, occurring below each septum, or rarely terminal; intercalary phialides mononphialidic, up to 3.0–5.0 μm long, 1.0–2.0 μm wide; terminal cells monophrilidic, sometimes sterile, no collarettes. Conidia hyaline, narrowly long ellipsoidal to cylindrical, straight or slightly curved. (4.7–)5.5–6.9–(8.4) × (1.6–)2.1–2.7(–3.0) μm (n = 343), non-septate.

**Anamorph in culture:** Optimum temperature for growth on PDA 25 °C, maximum temperature 35 °C, after 7 d colonies 70–85 mm (av. 80 mm) diam. Colony surface on PDA, radial, sometimes wavy, slightly cottony with aerial mycelium, white to whitish saffron; aerial mycelium developing only in CBS 125148, white to whitish yellow, sporodochial conidial masses produced after 2 wk; reverse white to slightly whitish yellow. Odour on PDA slightly fruity. Sporulation on SNA from lateral phialidic pegs on submerged or aerial hyphae common, 2.4–5.3 μm long, 1.9–1.9 μm wide near apex. Aerial conidiophores abundantly developed on aerial hyphae, unbranched, sometimes verticillate, 1–2-branched, becoming loosely to moderately densely branched, 5.5–21.5 μm long, 2.0–3.0 μm wide at base. Conidigenous cells mononphialidic, cylindrical, slightly tapering toward tip or narrowly flask-shaped with widest point in middle, 9.5–17.0 μm long, 1.5–2.0 μm wide at base. Young conidia formed by monophialides on submerged or aerial hyphae, formed abundantly on slimy heads, non-septate, ellipsoidal, oblong to cylindrical, hyaline, smooth, straight or slightly curved with rounded ends, (3.0–)4.0–7.0(–14.5) × (1.5–)2.0–2.5(–3.5) μm (n = 250). Mature conidia swollen, mostly 0-, rarely 1-septate, ellipsoidal, oblong, or allantoid, rarely ellipsoidal with slightly constricted centre, hyaline, smooth, straight or slightly curved, rounded at both ends, germinating or budding secondary conidia on media, (5.0–)7.5–14.6(–24.3) × (2.3–)3.5–4.9(–6.8) μm (n = 180). Chlamydospores rare, globose, subglobulose, broadly ellipsoidal, 0–(1)–septate, solitary or chains, 8.0–13.0 μm wide. Perithecia not produced in culture.
Fig. 8. A–S. *Nectria nigrescens*. A. Perithecia and short stipitate sporodochia in the natural environment. B. Perithecia in the natural environment. C. Median section of perithecia. D. Median section of perithecial wall. E. Ascus. F. One and three septata ascospores. G. Long stipitate sporodochia in the natural environment. H. Median section of long stipitate sporodochium. I. Edge of long stipitate sporodochium. J. Acropleurogenous conidiophore in the natural environment. K. Conidia in the natural environment. L. Aerial conidiophores and conidial mass on SNA. M. Young conidia on SNA. N. Lateral phialidic pegs on SNA. O. Short and densely blanched aerial conidiophores, and conidia on SNA. P. Mature conidia and young conidia on SNA. Q, R. Budding mature conidia on SNA. S. Germinating mature conidia that were streaked onto SNA. Scale bars: A, G, H, L = 1 mm; B, C = 300 μm; D, I = 100 μm; E, J, K, O, R = 30 μm; F, M, N, P, Q, S = 15 μm.
Distribution: Europe (France, Germany, UK), North America (Canada, USA).

Habitat: On dead woody substrata including Acer sp., Betula lutea, Celtis occidentalis, and Fagus sylvatica.

Holotype of Nectria nigrescens: USA, South Carolina, on Gleditsia sp.; S.C. Aiken, K 165219, Ravenel, American Fungi 2380a.

Epitope of Nectria nigrescens designated here. USA, North Carolina, Haywood Co., Great Smoky Mountains National Park, Purchase Knob. Cataloochees Divide Trail, alt. 5000 ft. 35°35'9.9"N 83°4'25.5"W, on dead twig of Acer sp., 7 Sep. 2005, A.Y. Rossman, epitope BPI 871083, ex-epitope culture CBS 125148 = A.R. 4211.

Additional type specimens examined. Holotype of Nectria cinnabarina f. dendroidea: Germany, Fungi Rehnani 2657, FH. Holotype of N. nigrescens: USA, South Carolina, on Gleditsia sp.; S.C. Aiken, K 98615. Neotype of Nectria meliae designated here: USA, Alabama, on Melia sp., 1 Dec. 1996, C.F. Baker, BPI 552568.

Specimens and isolates examined: Canada, Ontario, Carleton Place, near the Mississippi River, on twigs of Celtis occidentalis, 31 Jun. 2007, T. Gräfenhan, BPI 878449, culture CBS 125162 = A.R. 4394. France, Foret le Chize, Les Essarts, on twig of Fagus sylvatica, 27 Nov. 2007, C. Lechat, BPI 878457, culture CBS 125164 = A.R. 4475. Foret le Chize, Puymartier, on dead twig of Acer sp., 18 May 2006, C. Lechat, BPI 878455A = C.O.L. 684, culture A.R. 4282. USA, Tennessee, Sevier Co., Great Smoky Mountains National Park. Alum Cave Bluff Trail, alt. 3900 ft. 35°37'43.3"N 83°27'32"W, on dead twig of Betula lutea, 9 Sep. 2005, A.Y. Rossman, BPI 871084, culture CBS 125149 = A.R. 4213; Vermont, Windham County, Putney, Fort Hill Road, along a stream in a wet site, on dead twig, 17 Oct. 2008, G.J. Samuels, BPI 879986, culture CBS 127668 = A.R. 4211.

Notes: Nectria nigrescens resembles N. asiatica and N. cinnabarina in producing short to long stipitate sporodochia and mature conidia that bud (Fig. 8A, G, H, Q, R). Nectria nigrescens has up to 3-septate ascospores, short or long stipitate sporodochia, and length/width ratios of young and mature conidia that are somewhat smaller than the other species of the NCSC (Figs 3, 8A, F, G, H). Budding mature conidia of N. nigrescens on SNA (Fig. 8Q, R) are less commonly observed than in N. asiatica and N. cinnabarina.

The name N. cinnabarina f. dendroidea was published on the label of Fuckel's Fungi Rhenani 2657, issued in 1874 (Pfister 1895). Fuckel (1874) provided a name on this label that referred to a previously published description of the specimen (Fuckel 1873). We examined photographs and a microscope slide of the exsiccati (Fuckel, Fungi Rhen. 2657 from FH) and determined this name to be a synonym of N. nigrescens. Wollenweber (1926) attributed his name Nectria cinnabarina var. dendroidea (Fuckel) Wollenw. to Fuckel (1873). Wollenweber (1926) noted the presence of long, stipitate sporodochia on the type specimen and was the first to regard this as an important characteristic. He described and illustrated both N. cinnabarina var. dendroidea and N. cinnabarina var. minor as having 1-septate ascospores. His later illustration of N. cinnabarina var. minor showed this variety with up to 3-septate ascospores (Wollenweber 1930, no. 778). Although Wollenweber (1926) did not document stipitate sporodochia of N. cinnabarina var. minor, his illustration showed well developed stroma (Wollenweber 1926, table 3, 21f). From these reasons, we include N. cinnabarina f. dendroidea and N. cinnabarina var. minor as synonyms of N. nigrescens.

The holotype specimen of N. meliae is lost; therefore, a specimen collected in the same year, on the same genus of host, and at the same place i.e. a tootype, specifically BPI 552588, is designated the neotype of N. meliae.

Our phylogenetic analyses suggest a sister-group relationship between N. nigrescens and CBS 125162, supported by high BI posterior probabilities and ML bootstrap (1.00 BI PP, 99 % ML BP) (Fig. 2). However, based on morphological characters in the natural environment and culture, CBS 125162 completely matches N. nigrescens and is regarded as N. nigrescens.

**SPECIES EXCLUDED OR OF UNCERTAIN STATUS**

**Nectria cinnabarina var. ribis** (Tode) Wollenw., Fusaria autographica delineata, Edn 1: no. 787. 1930.

Basionym: Sphaeria ribis Tode, Fungi Mecklenb. sel. 2: 31. 1791.

≡ Hypoxylon ribis (Tode) J. Kickx, Fl. Crypt. Louvain p. 113. 1835.

≡ Nectria ribis (Tode) Nießl, Verh. Naturf. Vereins Brünn 3: 171. 1865.

≡ Nectria ribis (Tode) Rabenh. in Sacc., Syll. Fung. 2: 480. 1883.

Notes: Nectria cinnabarina var. ribis was originally described as Sphaeria ribis by Tode (1791). Because Tode’s specimens were destroyed (Kirk et al. 2008), his illustrations are regarded as lectotype (tabula XII, fig. 103a–f). Tode (1791) described and illustrated smooth, pyriform perithecia immersed at the base of a well-developed stroma, possibly as a parasite, and thus do not belong in the N. cinnabarina species complex. Rather it appears to be related to Cosmospora.

**Tremella purpurea** L., Spec. Plant. 2: 1158. 1753.

Basionym: Nectria purpurea (L.) G.W. Wilson & Seaver, J. Mycol. 13: 51. 1907.

≡ Cucurbitaria purpurea (L.) Seaver, Mycologia 1: 184. 1909.

Notes: The name Tremella purpurea was listed as a synonym of N. cinnabarina (Rossman et al. 1999). However, according to Spencer et al. (2009), this name is invalid because the genus was not validly published by Linnaeus (1753). Names based on this invalidly published name are either invalid or illegitimate.

**KEY TO THE SPECIES IN THE NECTRIA CINNABARINA SPECIES COMPLEX**

On natural substrate

1. Ascospores up to 3-septate, 1-septate (91 %), 2-septate (5 %), 3-septate (4 %); sporodochia short (65 %) to long stipitate (35 %), 250–1700 μm high; Europe or North America ........................................................................................................... N. nigrescens

2. Ascospores up to 1- or rarely 2-septate; sporodochia sessile or stipitate; Asia, Europe or North America .............................................. N. asiatica

3. Ascospores up to 1- or rarely 2-septate (3 %); sporodochia sessile or long stipitate; Asia, Europe or North America .................................
3. Sporodochia 700–1600 μm high, long stipitate (70%); Europe or North America ................................................. N. cinnabarina
3. Sporodochia sessile or anamorph lacking; Asia, Europe or North America ....................................................... N. dematiosa

In pure culture

1. Mature conidia not budding on SNA after 7 d; optimum temperature for growth 20 °C on PDA ........................................ N. dematiosa, subclades A–C, go to 4
1. Mature conidia budding on SNA after 7 d; optimum temperature for growth 25 °C on PDA ........................................ 2

2. Mature conidia ellipsoidal, strongly constricted, budding; Europe or North America ............................................. N. cinnabarina
2. Mature conidia ellipsoidal, straight, or slightly curved, rarely slightly constricted, rarely budding; Asia, Europe or North America .......... 3

3. Young conidia averaging 10 μm long; mature conidia averaging 15 μm long; Asia ....................................................... N. asiatica
3. Young conidia averaging 5 μm long; mature conidia averaging 10 μm long; Europe or North America ......................... N. nigrescens

4. Germ tubes more or less straight, not penetrating agar immediately; Canada, Poland, USA ............................. N. dematiosa subclade A
4. Germ tube sinuate, penetrating agar immediately after germination; Canada, China, Japan .............................................. 5

5. Mean of 95 % confidence intervals of mature conidial length/width ratio 2.5; mycelial growth 20–30 mm after 7 d at 25 °C; Canada .................................................. N. dematiosa subclade B
5. Mean of 95 % confidence intervals of mature conidial length/width ratio 3.5; mycelial growth 40–50 mm after 7 d at 25 °C; China, Japan .................................................. N. dematiosa subclade C

DISCUSSION

_Nectria cinnabarina_ and other species in the NCSC form a monophyletic group within _Nectria_, all having _Tuberularia_ anamorphs (Fig. 1). The molecular analyses of the NCSC resolve four phylogenetically distinct species (Fig. 2), each of which is described and illustrated above.

The anamorph of _N. cinnabarina sensu lato_ has been referred to as _Tuberularia vulgaris_. Many synonyms are known for _T. vulgaris_ (Jørgensen 1952, Booth 1959, Seifert 1985) for which authentic and type specimens were examined by Seifert (1985). Although differences exist in stipe length in the natural environment and in size and shape of conidia in culture, it is not possible to determine which synonym of _T. vulgaris_ represents each species in the NCSC. Thus, the anamorph of _N. cinnabarina_ is referred to as _T. vulgaris_, while the anamorph of other species in the NCSC is referred to as _Tuberularia vulgaris_-like.

Seifert (1985) recognised that _T. vulgaris_ in the natural environment had two types of sporodochia, i.e. sessile and stipitate sporodochia with marginal cells arranged in a palisade. These differences correlate with the species recognised here. Specifically, _N. dematiosa_ has sessile sporodochia while _N. asiatica_, _N. cinnabarina_, and _N. nigrescens_ have short to long stipitate sporodochia. Except for conidia in culture, no differences were found in other morphological characteristics of the anamorph including the number of conidiophore branches and conidial size in the natural environment. Morphological heterogeneity of conidia in culture was noted for many years (Mayr 1883, Brefeld 1891, Beck 1902, Jørgensen 1952). Beck (1902) observed conidia that were much larger than normal conidia and suggested that their size depended on the nutritional content of the media. To standardise cultural conditions, Jørgensen (1952) used a detached branch instead of artificial media. He determined that the range of conidial size was variable but not useful in distinguishing taxa within specimens identified as _N. cinnabarina_. By observing mature conidia on SNA, we could distinguish species in the NCSC including the subclades within _N. dematiosa_. Budding of mature conidia in culture was observed for _N. asiatica_, _N. cinnabarina_, and _N. nigrescens_, a characteristic not noted for other _Nectria_-like fungi.

Differences in the size of mature conidia and the shape of its germ tube can be used to distinguish the subclades in the _N. dematiosa_ clade.

_Nectria cinnabarina sensu lato_ has been considered a cosmopolitan species (Farr & Rossman 2010). This study shows that _N. cinnabarina_, _N. nigrescens_, and _N. dematiosa_ subclades A and B are widespread on hardwood trees and woody shrubs in Europe and North America, while _Nectria asiatica_ and _N. dematiosa_ subclade C are known only in Asia. _Nectria cinnabarina_ has been reported in tropical regions and the Southern Hemisphere (Cunningham 1922, Tunstall 1923, Booth 1977, Debons et al. 1993), however, none of these reports could be confirmed because of the lack of specimens and cultures.

Species of the NCSC occur on a wide range of woody shrubs and trees in many families including the Arecaceae and Pinaceae; it is occasionally reported on herbaceous hosts (Farr & Rossman 2010). Most specimens used in this study were collected on newly killed branches suggesting that these fungi may exist as endophytes that then sporulate when the substrate dies (Wang et al. 2000). _Nectria cinnabarina sensu lato_ causes a disease referred to as “oral spot _Nectria_ canker” because of the conspicuous erumpent pink sporodochia of the anamorph (Sinclair & Lyon 2005). Trees and woody plants growing in plantations and nurseries or those damaged by frost or other causes appear to be especially susceptible. The pathogenicity of this fungus has been proven by host inoculation studies (Bedker & Blanchette 1984, Yasuda & Izawa 2007). Jørgensen (1952) demonstrated that _N. cinnabarina_ was a facultative parasite and saprobe of mainly deciduous trees but was unable to correlate his results with specific hosts. Although _N. cinnabarina_ and _N. nigrescens_ produce chlamydospores, they are rarely found in soil.

Chaverri and Samuels (2003) used a morphological species concept (John & Maggs 1997, Kirk et al. 2008) and genealogical concordance phylogenetic species recognition (Taylor et al. 2000) to delimit Hypocreai-Trichoderma species with green ascospores. According to their species concept, each of the three subclades (A, B, and C) in _N. dematiosa_ would be a distinct species. Even though we found that the subclades of _N. dematiosa_ could be distinguished...
by subtle anamorph characters in culture and by biogeography, we prefer not to give them names because of the small number of available specimens.

Our study clearly indicates that to define and characterise species in the N. cinnabarina species complex, an integrated approach should be used. The use of phylogenetic analyses of DNA sequences from six loci, observations and analyses of morphological characters of teleomorph and anamorph, mycelial growth, and geographical data indicates the existence of four species within the NCSC. This study will pave the way for understanding the evolutionary diversification and taxonomic implications of morphology using robust phylogenetic analyses and comprehensive character sampling.

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