available online at www.studiesinmycology.org
doi:10.3114/sim.2011.68.02

Studies in Mycology 68: 35–56. 2011.

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. *Nectria cinnabarina s. str.* is recircumscribed as having 2-septate ascospores and long stipitate sporodochia. *Nectria dematiosa*, previously considered a synonym of *N. cinnabarina*, has up to 2-septate ascospores and sessile sporodochia or no anamorph on the natural substrate. A third species, *Nectria nigrescens,* has up to 3-septate ascospores and short to long stipitate sporodochia. One newly described species, *Nectria asiatica* with a distribution restricted to Asia, has (0–)1-septate ascospores and short stipitate sporodochia. Young and mature conidia developing on SNA were observed for each species. 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 *Hydropisphaera* (Cannon & Hawksworth 1983).

In studying the species of *Nectria* in the UK, Booth (1959) emphasised perithecial wall structure when he divided 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 congeneric 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. Jøgensen (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 Paoletti (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). Jøgensen (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 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.

MATERIALS AND METHODS

Source and deposition of specimens and isolates

Fresh specimens of the teleomorph and anamorph were collected from which single ascospores or conidia were isolated. Specimens are deposited in the US National Fungus Collections (BPI), Beltsville, Maryland, USA, or elsewhere as indicated in Table 1. Specimens were also obtained from other herbaria as listed in the specimens examined; herbaria are indicated using abbreviations according to Holmgren & Holmgren (1998). To obtain cultures from fresh material, a suspension in sterilised water was made from ascospores or conidia from a crushed fruiting body, streaked onto 2 % (w/v) water agar (WA) with streptomycin (streptomycin sulfate; Sigma Chemicals, St. Louis, Missouri, USA) or Difco™ cornmeal dextrose agar (CMD; Difco, Detroit, Michigan, USA, cornmeal agar + 2 % w/v dextrose) supplemented with antibiotics 0.2 % each neomycin (neomycin trisulfate salt hydrate; Sigma Chemicals, St. Louis, Missouri, USA), and incubated at 25 °C. After 24 h, a single germinating ascospore or conidium was transferred directly to slants or plates of Difco™ potato dextrose agar (PDA) with a tungsten needle (Nissin EM Co., Tokyo, Japan). Representative isolates are preserved at the CBS Fungal Biodiversity Centre (CBS, Utrecht, Netherlands), and/or Genebank, National Institute of Agrobiological Sciences (NIAS, Tsukuba, Ibaraki, Japan). Isolates were also obtained from other culture collections, including the CBS Fungal Biodiversity Center and the Global Bioresource Center (ATCC, Manassas, Virginia, USA).

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

Species	Isolate No.	Herbarium No.	Substrate/ Host	Country	GenBank Accession No.					
					act	ITS	LSU	*rpb1*	*tef1*	*tub*
Cosmospora coccinea	A.R. 2741, CBS 114050	BPI 802729	*Inonotus nodulosus*	Germany	GQ505967 [a]	HM484537	GQ505990 [a]	GQ506020 [a]	HM484515	HM484589
Cyanonectria cyanostoma	G.J.S. 98-127, CBS 101734	BPI 748307	*Buxaceae*	France	GQ505961 [a]	HM484558	FJ474081 [a]	GQ506017 [a]	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 aquifolii	A.R. 4108, CBS 125147	BPI 880698	*Ilex aquifolium*	UK	HM484506	HM484538	HM484565	HM484579	HM484522	HM484590
Nectria asiatica	MAFF 241408	BPI 879980	Dead wood	Japan	–	HM484703	HM484744	HM484790	–	HM484815
	A.R. 4639, CBS 126568		Dead wood	China	–	HM484713	HM484727	HM484787	–	HM484811
	MAFF 241401	BPI 879978	Dead wood	Japan	HM484624	HM484716	HM484747	HM484788	–	HM484817
	MAFF 241435	BPI 879973	Bark of dead wood	Japan	HM484625	HM484709	HM484749	HM484794	–	HM484816
	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	HM484643	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	HM484786	–	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 triacanthos*	USA	GQ505960 [a]	HM484555	GQ505988 [a]	GQ506016 [a]	HM484520	HM484597

Table 1. (Continued).

Species	Isolate No.	Herbarium No.	Substrate/ Host	Country	GenBank Accession No.					
					act	ITS	LSU	*rpb1*	*tef1*	*tub*
Nectria balansae	A.R. 4446, CBS 123351	BPI 878477	*Coronilla* sp.	France	GQ505977 [a]	HM484552	GQ505996 [a]	GQ506026 [a]	HM484525	HM484607
Nectria balsamea	A.R. 4478, CBS 125166		*Pinus sylvestris*	Germany	HM484508	HM484540	HM484567	HM484580	HM484528	HM484591
Nectria berolinensis	A.R. 2776, CBS 126112	BPI 746346	Branches standing, *Ribes rubrum*	Austria	HM484510	HM484543	HM484568	HM484583	HM484517	HM484594
Nectria cinnabarina	A.R. 4327, CBS 125154		*Acer* sp.	Canada	HM484642	HM484688	HM484733	HM484778	HM484666	HM484824
	G.J.S. 91-111, CBS 713.97	BPI 1112880	*Acer* sp.	USA	HM484629	HM484693	HM484724	HM484777	HM484665	HM484825
	A.R. 4340, CBS 125156	BPI 878335	*Spiraea trilobata*	Canada	HM484635	HM484695	HM484756	HM484779	HM484664	HM484836
	A.R. 4341, CBS 125157	BPI 878311	*Acer saccharum*	Canada	HM484636	HM484687	HM484741	HM484780	HM484667	HM484822
	G.J.S. 91-109	BPI 1112878	*Fagus* sp.	USA	HM484633	HM484694	HM484723	HM484766	HM484670	HM484833
	A.R. 4379, CBS 125158	BPI 878313	Twigs	Ireland	HM484640	HM484696	HM484739	HM484772	HM484668	HM484830
	A.R. 4337, CBS 127668	BPI 878312	*Acer pseudoplatanus*	Denmark	HM484631	HM484690	HM484726	HM484775	HM484659	HM484826
	A.R. 4477, CBS 125165	BPI 879981	Dead twigs of *Aesculus* sp.	France	HM484503	HM484548	HM484562	HM484577	HM484527	HM484606
	A.R. 4496	BPI 878878	*Populus tremula*	Ukraine	HM484641	HM484712	HM484731	HM484768	HM484658	HM484831
	A.R. 4302, CBS 125150	BPI 878317	*Acer pseudoplatanus*	Austria	HM484627	HM484684	HM484736	HM484765	HM484654	HM484820
	ATCC 11432, CBS 255.47		Stem of *Ulmus* sp.	Netherlands	GQ505975 [a]	HM484710	GQ505997 [a]	GQ506027 [a]	HM484663	HM484832
	CBS 256.47		Twig of *Ulmus* sp.	Netherlands	HM484628	HM484692	HM484755	HM484769	HM484656	HM484828
	A.R. 4303, CBS 125151	BPI 878316	*Acer campestre*	Austria	HM484630	HM484686	HM484740	HM484776	HM484669	HM484821
	CBS 189.87		*Sorbus aria*	Germany	HM484644	HM484699	HM484746	HM484796	HM484671	HM484835
	A.R. 4397, CBS 125163	BPI 879983, C.L.L. 7027	*Acer* sp.	France	HM484638	HM484691	HM484742	HM484773	HM484661	HM484827
	A.R. 4381, CBS 125160	BPI 878310	Root	UK	HM484632	HM484685	HM484752	HM484774	HM484657	HM484837
	A.R. 4304, CBS 125152	BPI 879982	*Tilia* sp.	Denmark	HM484637	HM484698	HM484734	HM484767	HM484655	HM484829
	A.R. 4388, CBS 125161	BPI 878322	Twigs of *Acer pseudoplatanus*	Poland	HM484639	HM484689	HM484735	HM484771	HM484662	HM484823
	CBS 125115, G.J.S. 91-121	BPI 1112890	*Acer* sp.	USA	HM484634	HM484697	HM484753	HM484770	HM484660	HM484834
Nectria coryli	A.R. 4561, Y.H. 0815	BPI 880697	Twigs of *Rhus copallinum*	USA	HM484509	HM484539	HM484566	HM484581	HM484536	HM484596
Nectria cucurbitula	CBS 259.58		*Pinus sylvestris*	Netherlands	GQ505974 [a]	HM484541	GQ505998 [a]	GQ506028 [a]	HM484530	HM484592
Nectria dematiosa	CBS 126570, G.J.S. 94-37	BPI 749337	Bark	USA	HM484502	HM484557	HM484561	HM484576	HM484534	HM484603
	A.R. 4328, CBS 125155		*Acer* sp.	Canada	HM484616	HM484680	HM484725	HM484761	HM484648	HM484799
	CBS 279.48		*Acer pseudoplatanus*		–	HM484700	HM484754	HM484762	HM484649	HM484802
	CBS 278.48		*Ribes* sp.		HM484615	HM484682	HM484729	HM484760	HM484647	HM484800
	A.R. 4380, CBS 125159	BPI 878308	Twig	Poland	HM484614	HM484681	HM484722	HM484759	HM484650	HM484801
	A.R. 2699, CBS 125125	BPI 802212	Dead twig of *Acer macrophyllum*	Canada	HM484612	HM484676	HM484717	HM484757	HM484645	HM484797
	A.R. 2702, CBS 125127	BPI 802215	Dead twig of *Rosa* sp.	Canada	HM484613	HM484677	HM484719	HM484758	HM484646	HM484798
	MAFF 241430	BPI 879985	Branches standing	Japan	HM484617	HM484704	HM484750	HM484795	HM484653	HM484803
	A.R. 4638, CBS 127667		Unknown	China	–	HM484706	HM484718	HM484763	HM484651	HM484805
	MAFF 241416	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.					
					act	ITS	LSU	*rpb1*	*tef1*	*tub*
Nectria lamyi	A.R. 2779, CBS 115034	BPI 746349	*Berberis vulgaris*	Austria	HM484507	HM484544	HM484569	HM484582	HM484518	HM484593
Nectria miltina	A.R. 4391, CBS 121121	BPI 878442	Decaying leaves of *Agave americana*	Italy	HM484514	HM484547	HM484572	HM484587	HM484524	HM484609
Nectria nigrescens	A.R. 4282	BPI 878455A	Dead twig of *Acer* sp.	France	HM484619	HM484711	HM484745	HM484785	HM484673	HM484808
	A.R. 4211, CBS 125148	BPI 871083	Dead twig of dictyledonous tree	USA	HM484618	HM484707	HM484720	HM484781	HM484672	HM484806
	A.R. 4475, CBS 125164	BPI 878457	Twig of *Fagus sylvatica*	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	French Guiana	–	HM484553	HM484574	HM484588	HM484529	HM484608
Nectria pseudotrichia	CBS 551.84		Unknown	Japan	GQ505976 [a]	HM484554	GQ506000 [a]	GQ506030 [a]	HM484532	HM484602
Nectria pyrrhochlora	A.R. 2786, CBS 125131	BPI 746398	*Acer campestre*	Austria	HM484512	HM484545	HM484570	HM484584	HM484519	HM484598
Nectria sinopica	CBS 462.83	CBS H-19479, CBS H-19485	*Hedera helix*	Netherlands	GQ505973 [a]	HM484542	GQ506001 [a]	GQ506031 [a]	HM484531	HM484595
Nectria zanthoxyli	A.R. 4280, CBS 126113	BPI 878445	*Crataegus* sp.	France	HM484513	HM484546	HM484571	HM484585	HM484523	HM484599
Thelonectria westlandica	G.J.S. 83-156, CBS 112464		*Dacrydium cupressinum*	New Zealand	GQ505959	HM484559	GQ505987 [a]	GQ506015 [a]	HM484533	HM484610

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.
[a]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; perithecium 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 Kornerup & 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	T*act*1, T*act*2 (Samuels *et al.* 2006)	65 °C, 30 s, 15´ 48 °C, 30 s, 30´	GTR+G	613 (127)	111 (18 %)	43	459
ITS	ITS5, ITS4 (White *et al.* 1990)	53 °C, 1 min, 35´	TIM3+I+G	539 (279)	62 (12 %)	52	425
LSU	LR5, LROR (Vilgalys n.d.)	53 °C, 1 min, 35´	TIM3+I+G	807 (150)	67 (8.3 %)	39	701
Rpb1	c*rpb1*a, *rpb1*c (Castlebury *et al.* 2004)	50 °C, 2 min, 40´	TIM2+I+G	590 (540)	233 (40 %)	65	292
Tef1	*tef1*-728, *tef1*-1567 (Carbone & Kohn 1999, Rehner 2001)	66 °C, 55 s, 9´ 56 °C, 55 s, 35´	GTR+I+G	645 (261)	142 (22 %)	43	460
Tub	β*tub*-T1, β*tub*-T2 (O'Donnell & Cigelnik 1997)	55 °C, 30 s, 35´	TPM3uf+I+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	T*act*1, T*act*2 (Samuels *et al.* 2006)	65 °C, 30 s, 15´ 48 °C, 30 s, 30´	TrN+G	649 (91)	47 (7 %)	40	562
ITS	ITS5, ITS4 (White *et al.* 1990)	53 °C, 1 min, 35´	TrNef+G	475 (592)	38 (8 %)	19	418
LSU	LR5, LROR (Vilgalys n.d.)	53 °C, 1 min, 35´	TIM1+I+G	814 (260)	18 (2 %)	14	782
Rpb1	c*rpb1*a, *rpb1*c (Castlebury *et al.* 2004)	50 °C, 2 min, 40´	TrN+G	621 (123)	111 (18 %)	120	390
Tef1	*tef1*-728, *tef1*-1567 (Carbone & Kohn 1999, Rehner 2001)	66 °C, 55 s, 9´ 56 °C, 55 s, 35´	TrN+G	828 (186)	158 (19 %)	36	634
Tub	β*tub*-T1, β*tub*-T2 (O'Donnell & Cigelnik 1997)	55 °C, 30 s, 35´	TPM3uf+G	527 (135)	88 (17 %)	138	301
Total				3914	460 (12 %)	367	3087

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).

Six loci were sequenced, namely a-actin (*act*) (Carbone & Kohn 1999), β-tubulin (*tub*) (O'Donnell & Cigelnik 1997), RNA polymerase II subunit one (*rpb1*) (Castlebury *et al.* 2004), the internal transcribed spacer (ITS) (White *et al*. 1990), large subunit nuclear ribosomal DNA (LSU) (Vilgalys n.d.), and translation elongation factor 1-a (*tef1*) (Carbone & Kohn 1999, Rehner 2001). The primers and PCR protocol information are listed in Tables 2 and 3. PCR products were cleaned with ExoSAP-IT® (USB Corporation, Cleveland, Ohio, USA) following the manufacturer's instructions. Clean PCR products were sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, USA) and at MCLAB (Molecular Cloning Laboratories, San Francisco, California, USA). Sequences were assembled and edited with Sequencher v. 4.9 (Gene Codes, Madison, Wisconsin, 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 *Cosmospora coccinea*, *Cyanonectria cyanostoma*, and *Thelonectria westlandica,* were used as outgroups for inferring intrageneric relationships (Fig. 1). *Nectria balansae, N. pseudocinnabarina*, and *N. pseudotrichia* 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

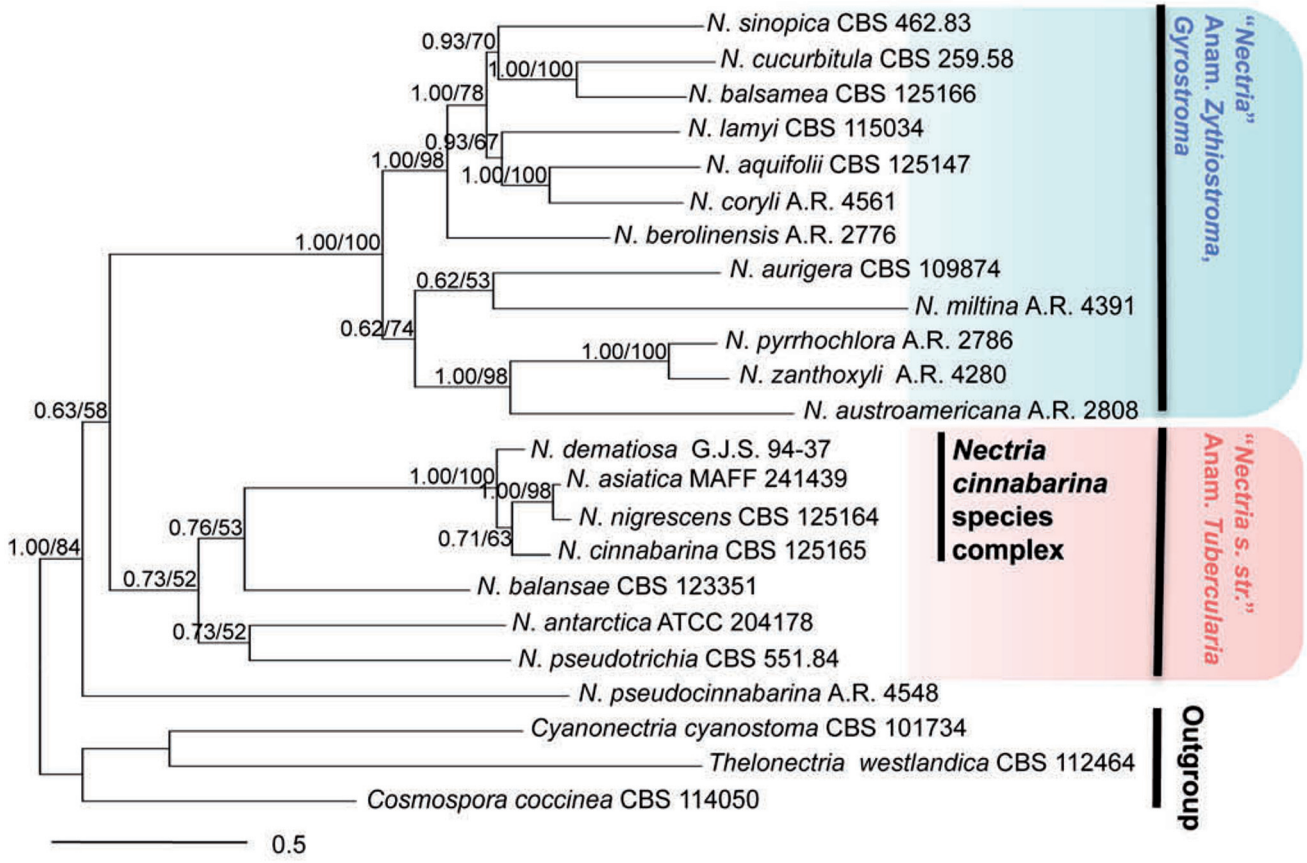

Fig. 1. Members of the genus *Nectria*. Combined *act*, *tub*, *rpb1*, ITS, LSU, *tef1* Bayesian cladogram (Ln −21514.704). BI posterior probabilities/ML bootstrap values indicated at branches.

optimised". 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. dematiosa* 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).

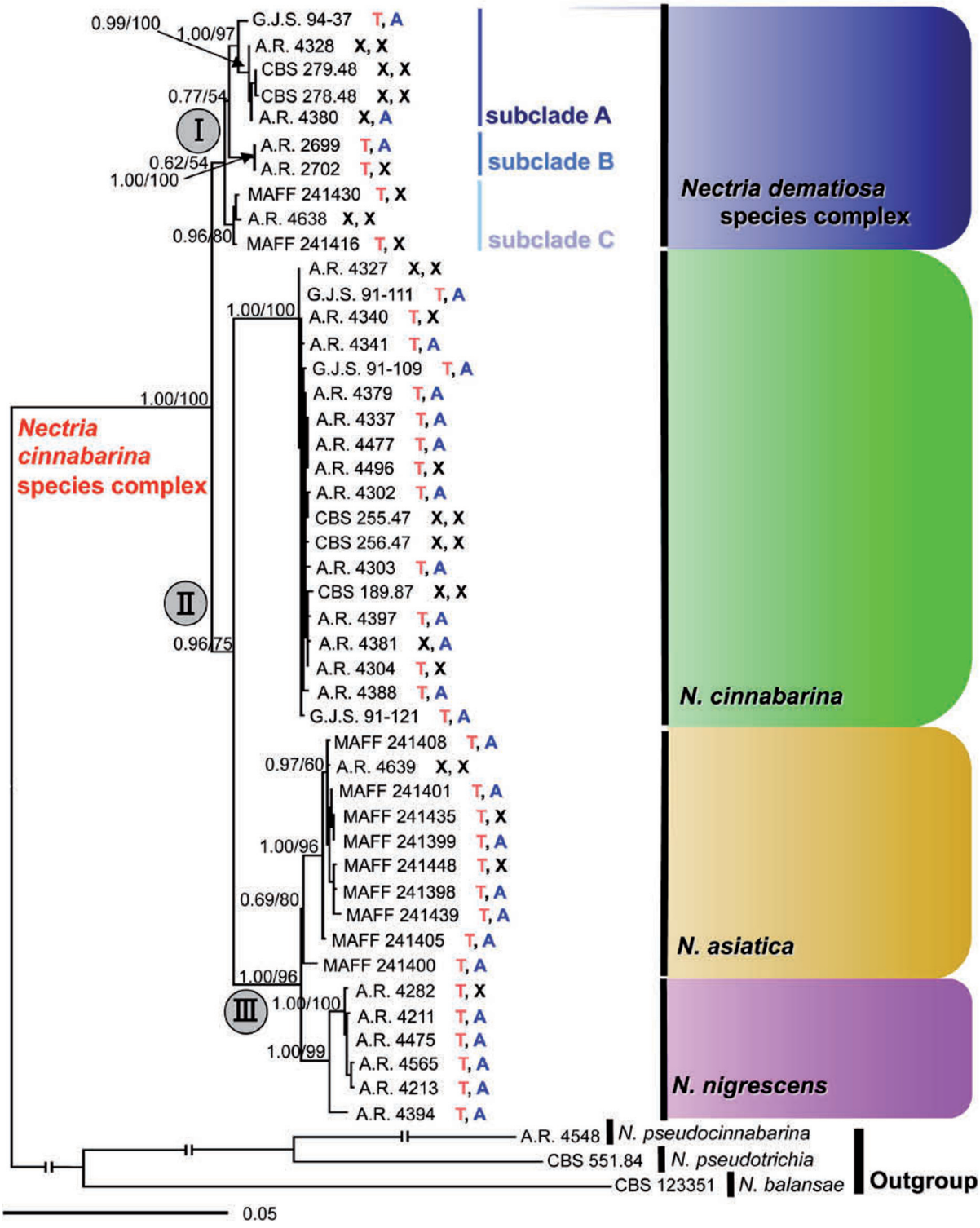

Fig. 2. Members of the *Nectria cinnabarina* species complex (NCSC). Combined *act*, *tub*, *rpb1*, ITS, LSU, *tef1* Bayesian cladogram (Ln −11408.155). BI posterior probabilities/ML bootstrap values indicated at branches. T: Teleomorph observed in the natural environment; A: Anamorph observed in the natural environment; X: no holomorph observed in the natural environment.

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 100 %). A second segregate species occuring 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 representated by two isolates from Canada, while *N. dematiosa* subclade C is known only from Asia.

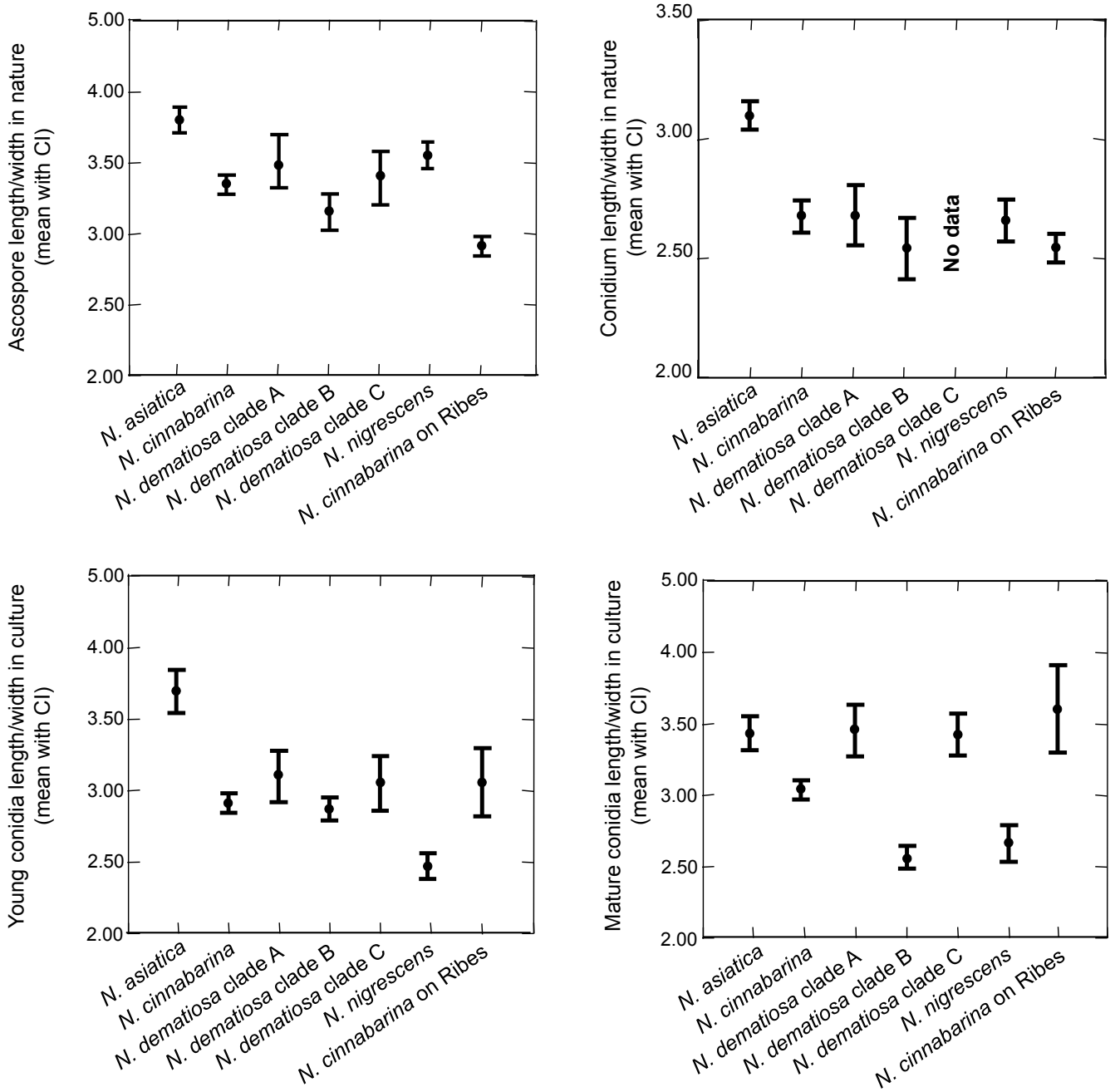

Fig. 3. Graphs of 95 % confidence intervals of length to width ratios of ascospores and conidia.

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 wall cell 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.

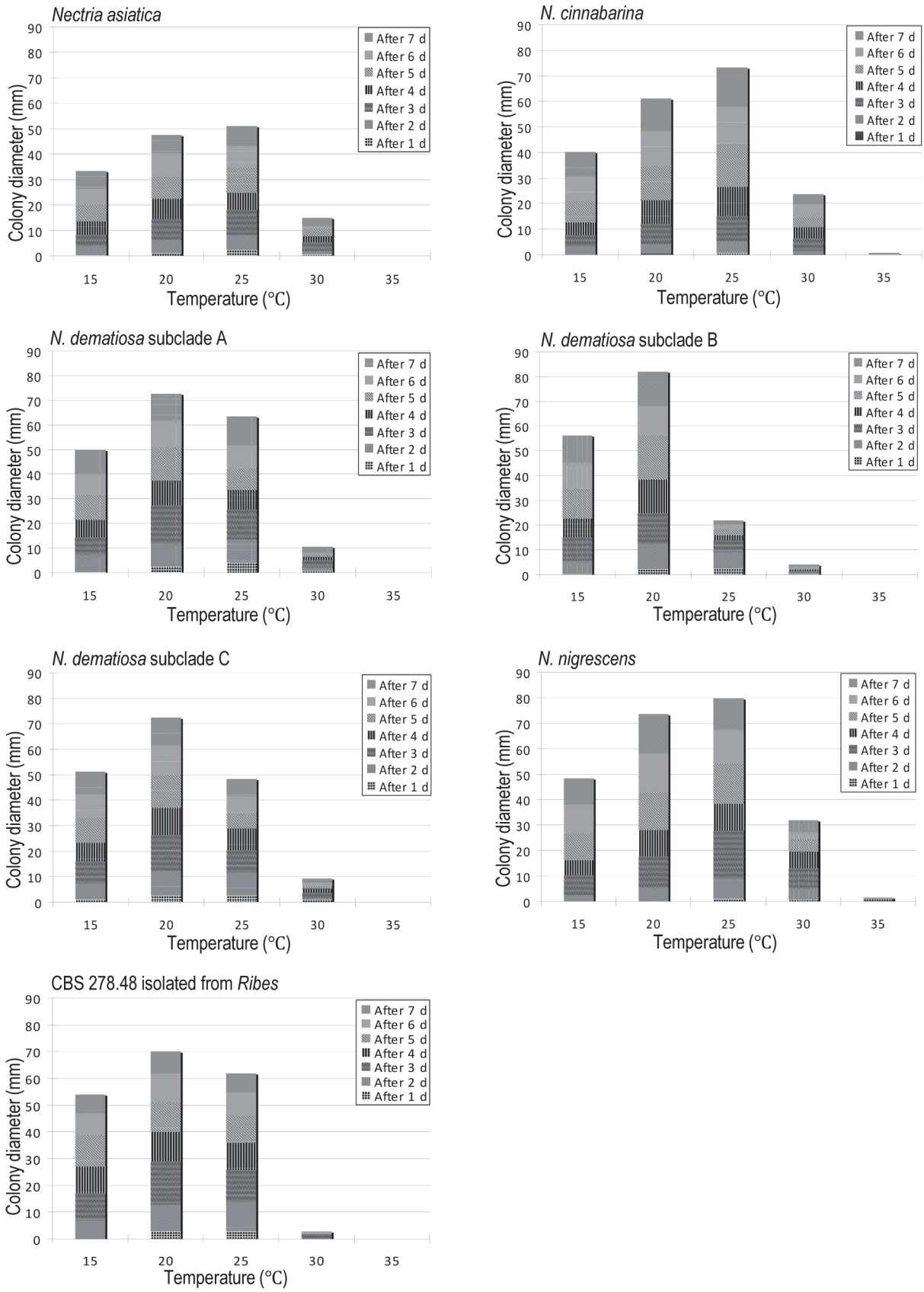

Fig. 4. Mycelial growth of NCSC at different temperatures on PDA.

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. asiastica* 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, Rossman & P. Chaverri, **sp. nov.** MycoBank MB516721. Fig. 5.
Anamorph: *Tubercularia vulgaris*-like.

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

Perithecia in cortice emortuo, solitaria vel gregaria, superficialia, subglobosa, 285–400 µm alta, 250–380 µm diam, rubella, KOH+, LA+. Asci unitunicati, clavate, apice simplici, 74–117 × 8.5–14.0 µm, octospori. Ascosporae ellipsoideae vel fusiformes, 10.5–19.0 × 3.0–6.0 µm, 0–1-septatae, hyalinae, laeves. Anamorphosis sporodochia discoida vel cylindrico-capitata, brevi-stipites, 250–800 mm alti, 300–2000 mm lati, atro-rubella vel raro niger, KOH+. Conidia oblonge ellipsoidea ad Cylindrica, 4.5–9.5 × 1.0–3.0 µm, hyalinae, leaves.

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

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. prismatica* 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 prismatica*. *Asci* unitunicate, (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.

Anamorph on natural substrata: *Stromata* erumpent through epidermis, orange to red. *Sporodochial conidiomata* with stipe, superficial on well-developed stroma, smooth or cerebriform, scattered, solitary, or 2–4 gregarious, stipitate,, pustular, discoid or cylindrical-capitate, up to 250–800 mm high including stipe, 300–2000 mm diam, chestnut to black, sometimes whitish yellow to orange; *stipe* chestnut to black, sometimes dark green, up to 440–610 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 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 collarettes. *Conidia* hyaline, narrowly long ellipsoidal to cylindrical, straight or slightly curved, non-septate, (4.5–)5.5–7.5(–9.5) × (1.0–)2.0–2.5(–3.0) µm (n = 258), smooth-walled.

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,

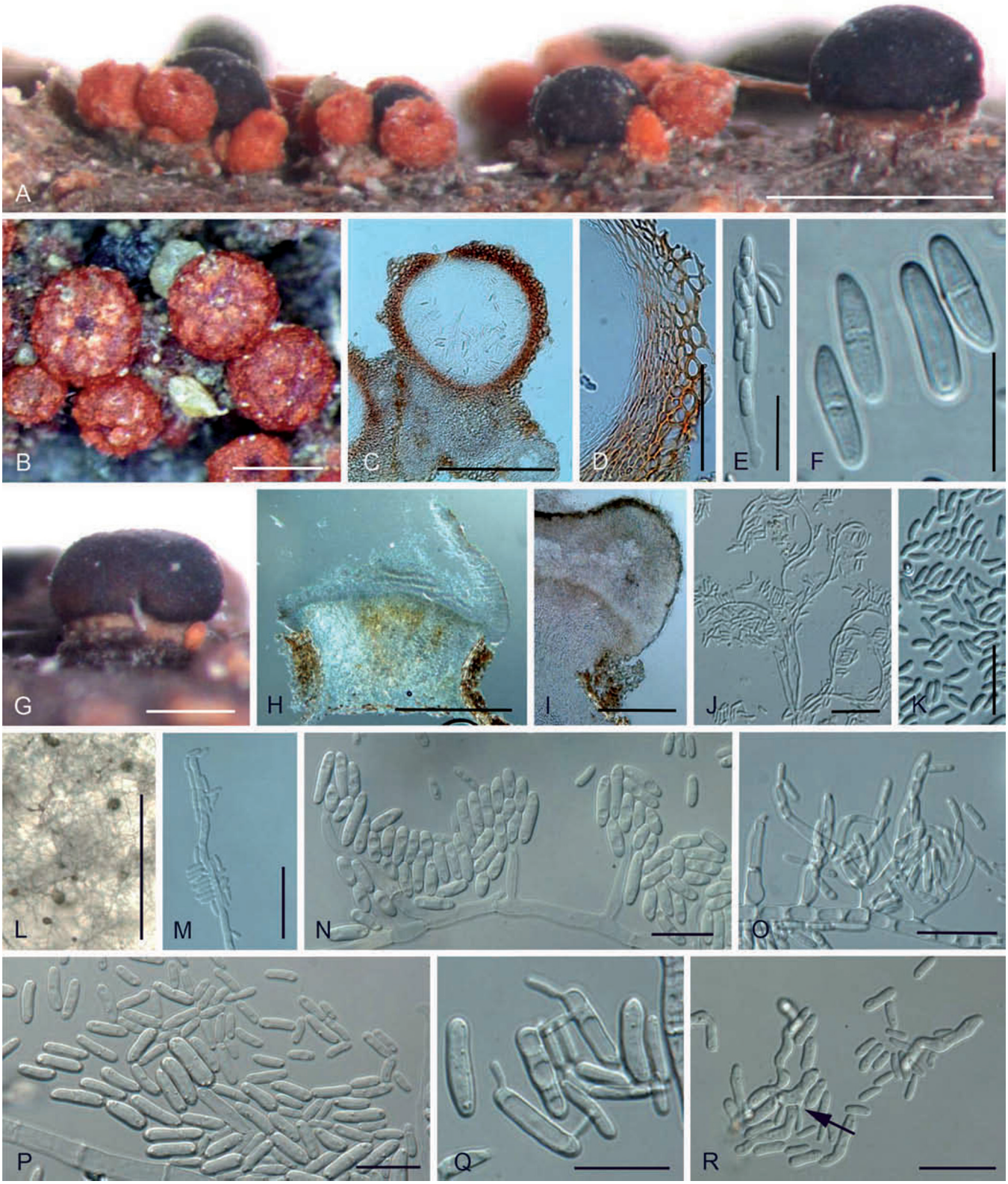

Fig. 5.A–R. *Nectria asiatica*. A. Perithecia and short stipitate sporodochia in the natural environment. B. Perithecia on nature. C. Median section of perithecium. D. Median section of perithecial wall. E. Ascus. F. 0–1 septates ascospores. G. Short stipitate sporodochium in the natural environment. H. Median section of short stipitate sporodochium. I. Edge of short stipitate sprodochium. J. Acropleurogenous conidiophores in the natural environment. K. Conidia in the natural environment. L. Aerial conidiophores and conidial mass on SNA. M. Lateral phialidic pegs and conidia on SNA. N. Short aerial conidiophores and conidia on SNA. O. Densely blanched aerial conidiophores and conidia on SNA. P. Mature conidia and young conidia on SNA. Q. Budding mature conidia on SNA. R. Budding and germinating mature conidia (arrow) that were streaked onto SNA. Scale bars: A, L = 1 mm; B, C, G, H = 300 µm; D, I = 100 µm; E, J, K, M, R = 30 µm; F, N, O, P, Q = 15 µm.

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 241398; Kumamoto Prefecture, Kikuchi city, Kikuchi valley, on twig of *Prunus* sp., Dec. 2000, Y. Hirooka, BPI 879976, culture MAFF 241399; Hokkaido, kamigawa-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. *stromaticola* 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. *stromaticola* 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.) Grev., Scot. Crypt. Fl. 3: 135. 1825.

= *Sphaeria tremelloides* Weigel, Obs. Bot. p. 46, 1772.

= *Sphaeria decolorans* Pers. : Fr., Persoon, Neues Magazin für Botanik, Rőmer 1: 83. 1794 : Fries, Syst. Mycol. 2: 412, 1823.

= *Sphaeria celastri* Fr., Elenchus Fungorum 2: 81. 1827.

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

= *Nectria offuscata* 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: *Mycelium* 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 stipitate sporodochia, subglobose to globose, 275–400 µm high × 250–370 µm diam (*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 conidiomata* 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, 1.5–2 µm wide; *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.

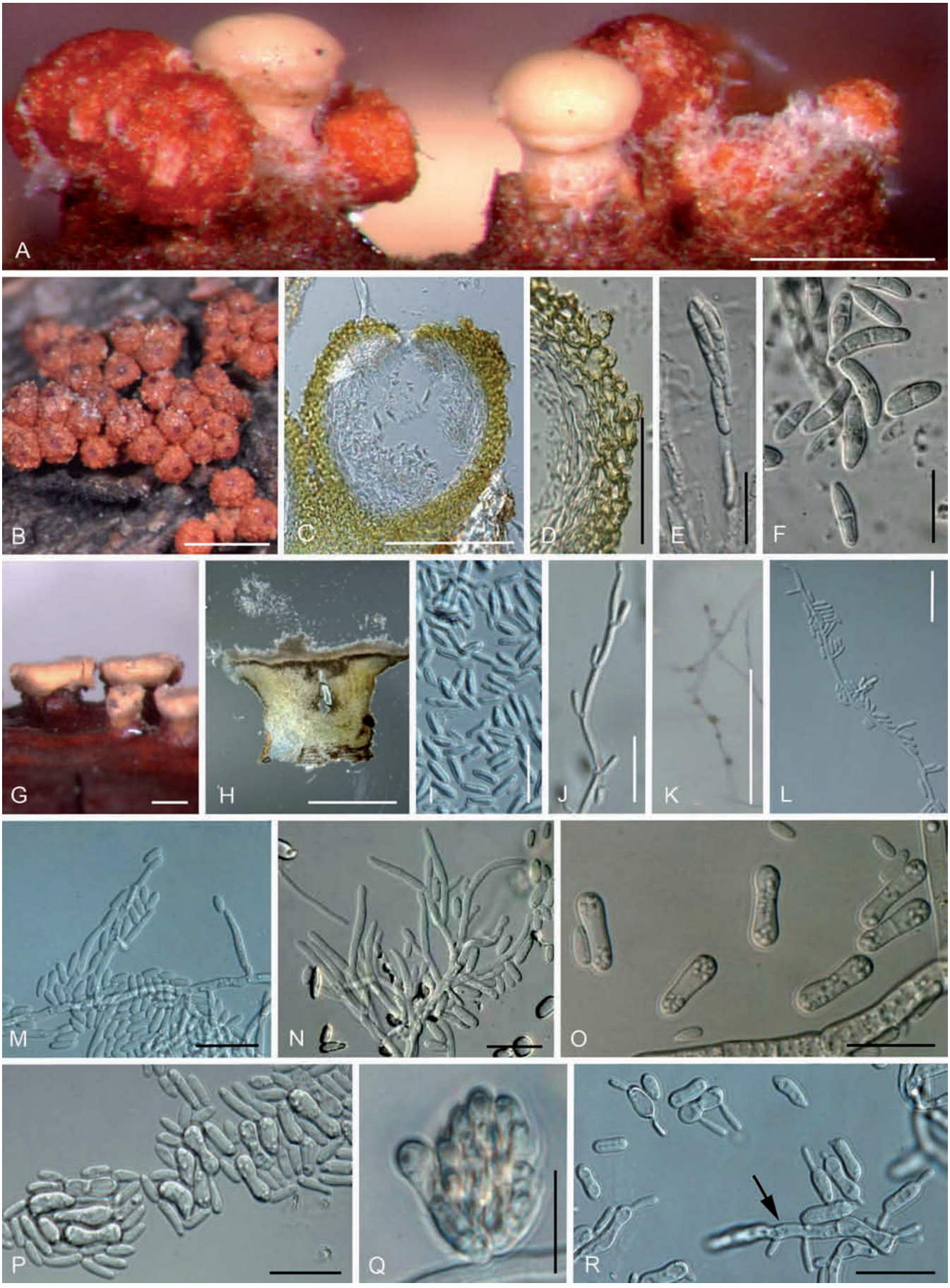

Fig. 6.A–R. *Nectria cinnabarina*. A. Perithecia and long stipitate sporodochia in the natural environment. B. Peritheica in the natural environment. C. Median section of perithecium. D. Median section of perithecial wall. E. Ascus. F. 0–2 septates ascospores. G. Long stipitate sporodochia in the natural 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 cottony 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 phialidic 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. *Conidiogenous cells* monophialidic, 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 round 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-, 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). *Chlamydospores* 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 substrata 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* × *hollandica*.

Lectotype of Sphaeria cinnabarina *designated here*: figs 68a–e in the copy of Tode HJ (1791). Fungi Mecklenburgenses selecti. **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* is 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*, Mougeot, 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. Schweinitz, holotype Schweinitz Syn. PH 1421. Type of *Nectria russellii*: **USA** Massachusetts, Jan. 1856, J.L. Russell, holotype FH 284394. Lectotype of *Nectria offuscata* designated here: **USA**, South Carolina, on *Hibiscus syriacus* L., lectotype BPI, Michener Collection 32, Sheet 12.

Additional specimens and isolates examined: **Austria**, Vienna, 19th district, base of the mountain Kahlenberg, MTB 7763/2, 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 125150 = 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. Gams, T. Gräfenhan, BPI 878311, culture CBS 125157 = A.R. 4341; Quebec, Quebec City. Lake St. Charles, on *Spiraea trilobata* L., 18 Aug. 2006, G. Laflamme, BPI 878335, culture CBS 125156 = A.R. 4340. **Denmark**, on bark of *Tilia* sp., 21 May 2006, T. Laessoe, BPI 879982, culture CBS 125152 = A.R. 4304; Sjaelland, 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 879983, culture CBS 125163 = A.R. 4397. **Germany**, on *Sorbus aria* (L.) Crantz, Oct. 1986, H. Reinartz, anamorph only, culture CBS 189.87. **Ireland**, Dublin, Phoenix Park 53°20'59.91"N 6°17'56.87"W, on twigs, 21 Sep. 2006, K. Seifert, BPI 878313, culture CBS 125158 = 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**, Sudetes, Zlote Mts., Zloty Stok, on twigs of *Acer pseudoplatanus* L., 6 Jun. 2006, A. Chlebicki, BPI 878322, culture A.R. 4388. **Sweden**, Fries, Scleromyceti Sueciae no. 184 as *Sphaeria cinnabarina*, BPI 799329, BPI 799330, BPI 799331, UPS. **Ukraine**, Kharkov-city, University botanic garden, on fallen twigs of *Populus tremula* L., 3 Mar. 2007, A. Akulov, BPI 878878, culture A.R. 4496. **U.K**, Wales, Hafod, logged area, *ca.* 52°22'N 3°51'W, on root, 1 Oct. 2006, K. Seifert, BPI 878310, culture CBS 125160 = A.R. 4381. **USA**, Virginia, Giles Co., Cascades Recreation Site, 4 Mi N of Pembroke, Little Stony Creek, 37d2d'n, 80d35'w. alt. 840 meters, on *Acer* sp., 18 Sep. 1991, G.J. Samuels,C.T. Rogerson, S. Huhndorf, S. Rehner, BPI 1112890, culture CBS 125115 = G.J.S. 91-121; Virginia, Giles Co., Mountain Lake, alt. 1160 meters, 37d22'n,80d31'w, near hotel pond drain, on *Fagus* sp., 17 Sep. 1991, G.J. Samuels, BPI 1112878, culture G.J.S. 91-109; Virginia, Giles Co., Mountain Lake, alt. 1160 meters, 37d22'n, 80d31'w, near hotel, Pond Drain, on *Acer* sp., 17 Sep. 1991, G.J. Samuels, BPI 1112880, culture CBS 713.97 = G.J.S. 91-111.

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 anamorph 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. nigrescens*, *N. cinnabarina* is distinguished in culture by abundant budding mature conidia that are ellipsoidal and strongly constricted in the centre (Fig. 6O, 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.) Kuntze, 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.) Mussat 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

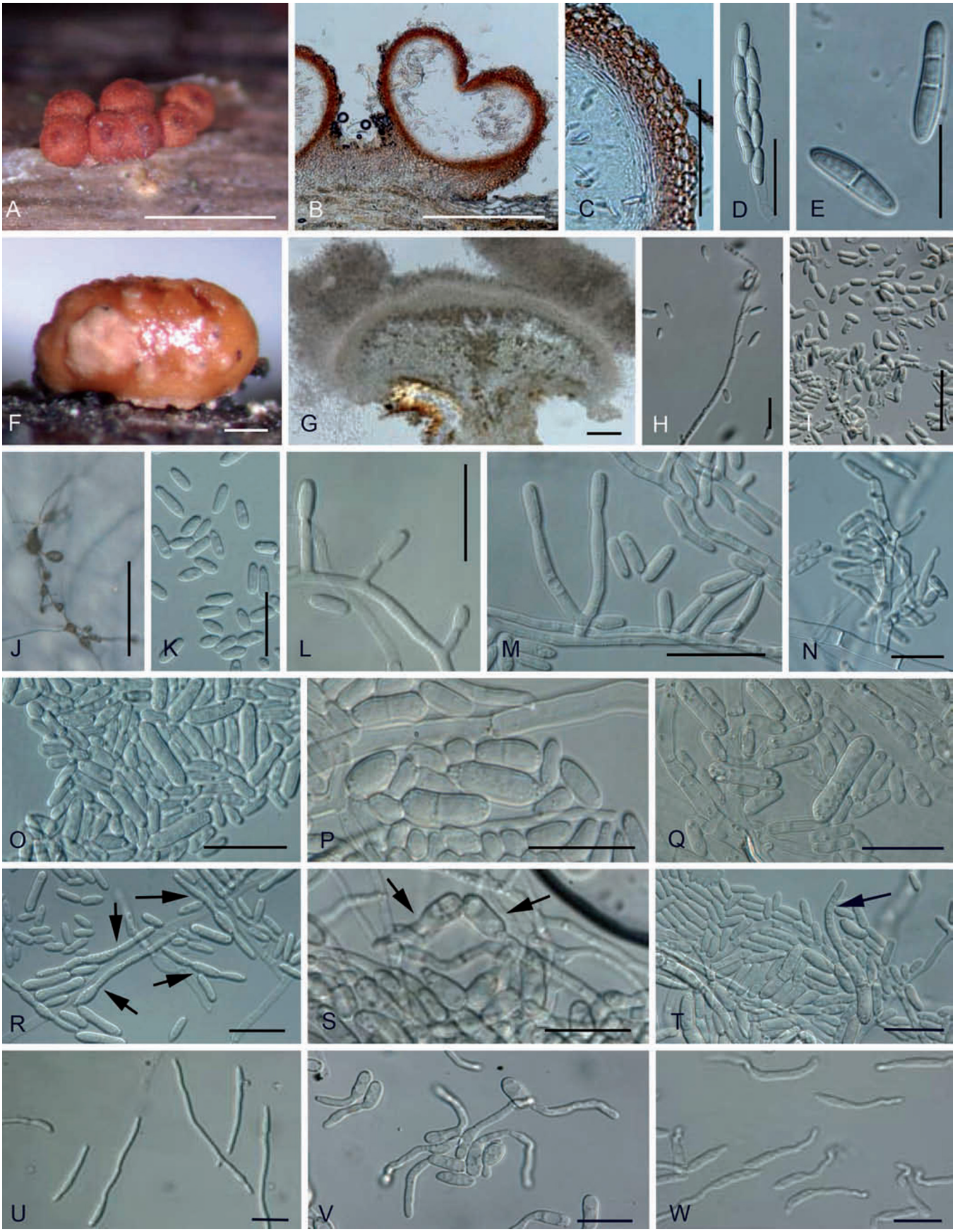

Fig. 7.A–W. *Nectria dematiosa* species complex. A. Perithecia in the natural environment. B. Median section of perithecium. C. Median section of perithecial wall. D. Ascus. E. 1–2 septates ascospores. F. Astipitate sporodochium in the natural environment. G. Median section of stipitate sporodochium. 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, F, G = 100 µm; D, H, I, R = 30 µm; E, K, L–W = 15 µm.

diam, erumpent through epidermis, orange to bay, sometimes darker red, KOH+ dark red, LA+ yellow, pseudoparenchymatous, cells forming *textura angularis* to *t. prismatica* with cells oriented more or less vertically; cells 3–10 µm diam, with 1–1.5 µm thick walls, intergrading with the ascomatal wall. *Perithecia* 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, 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.* 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*. *Asci* unitunicate, (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* ellipsoidal 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. *Sporodochial conidiomata* without stipe, superficial on well-developed stroma, smooth, cerebriform or tubercularoid, scattered, solitary, rarely caespitose, astipitate, sessile, pustular, discoid or cylindrical-capitate, up to 200–700 mm high, 250–1000 mm wide, white, whitish yellow to orange, sometimes brown. *Hymenium* arising directly from *textura prismatica* elongating from *textura angularis*, up to 90 µm long, of cells 2.0–7.5 µm wide, not curved at margin. *Conidiophores* monoverticillate or sometimes bi-verticillate, then developing acropleurogenously for 3–6 levels, straight, curved hyaline. *Phialides* intercalary occurring below each septum, or rarely terminal; intercalary phialides monophialidic, 2.5–8.5 µm long, 1.3–2.4 µm wide at base; terminal cells monophialidic, sometimes sterile, no collarettes, 10.5–15 µm long, 2.3–2.8 µm wide at base. *Conidia* hyaline, narrowly long ellipsoidal to cylindrical, straight or slightly curved, non-septate, (4.5–)5.7–7.1(–8.8) × (1.7–)2.2–2.8(–3.1) µm (*n* = 60). Subclade A: (4.5–)5.5–7.1(–8.8) × (2.0–)2.2–2.6(–2.9) µm (*n* = 30), subclade B: (5.2–)5.8–7.0(–7.8) × (1.7–)2.3–2.9(–3.1) µm (*n* = 30), subclade C: none present.

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 cottony with aerial mycelium, white to whitish saffron; aerial mycelium developing in a few isolates (CBS 125127, CBS 126570), white to whitish yellow sporodochial conidial masses produced after 2 wk; *reverse* white to slightly whitish yellow. *Odour* slightly fruity. Sporulation on SNA from lateral phialidic pegs on submerged or aerial hyphae common, 2.5–4.5 µm long, 1.5–3.0 µm wide at base. *Aerial conidiophores* occasionally developing on aerial hyphae, unbranched, sometimes verticillate, 1–2-branched, becoming loosely to moderately densely branched, 6.0–34 µm long, 2.1–4.5 µm wide at base. *Conidiogenous cells* monophialidic, cylindrical, slightly tapering toward tip or narrowly flask-shaped with widest point in middle, 8–26 µm long, 2.5–3.5 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 round at both ends, (4.1–)6.0–10.6(–17.3) × (1.6–)2.4–3.4(–5.1) µm (*n* = 496); subclade A: (4.6–) 5.9–10.1(–14.0) × (1.6–)2.3–3.1(–4.0) µm (*n* = 200); subclade B: (4.1–)6.0–10.6(–16.8) × (1.6–)2.4–3.6(–5.1) µm (*n* = 213); subclade C: (5.0–)6.5–11.5(–17.3) × (2.2–)2.6–3.4(–4.0) µm (*n* = 83). *Mature conidia* swollen, mostly 0-, rarely 1-septate, ellipsoidal, oblong or allantoid, rarely ellipsoidal, straight or slightly curved, rounded at both ends, germinating, never budding secondary conidia on media, (7.1–)10.0–17.4(–29.3) × (2.8–)3.8–5.6(–7.9) µm (*n* = 429); subclade A: (8.2–)10.7–19.1(–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.

Distribution: Asia (China, Japan), Europe (Finland, Poland), New Zealand, North America (Canada, USA).

Habitat: On dead woody substrata including *Acer macrophyllum*, *A. pseudoplatanus*, *Acer* sp., *Morus* sp., *Prunus tenella, Ribes* sp., *Rosa* sp., *Sambucus nigra* ssp. *canadensis*, and *Weigela coraeensis*.

Lectotype of Nectria dematiosa *designated here*: **USA**, Pennsylvania, on *Morus* sp., Bethlehem, Schweinitz, lectotype BPI 799536, isolectotype 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 *designated 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 00927949. Holotype of *Nectria cinnabarina* subsp. *amygdalina*: **Finland**, Mustiala, on dead branch of *Amygdalus nana*, now considered to be *Prunus tenella*, 28 May 1889, P.A. Karsten. Holotype H 6009374.

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. Zhuang, 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 241416; Tokyo, Okutama-gun, on twig, Nov. 2003, Y. Hirooka, BPI 879985, culture MAFF 241430. **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 twig, 21 May 2006, D. Karasinski and D. Ronikier, BPI 878308, culture CBS 125159 = A.R. 4380. **Unknown**: on *Acer pseudoplatanus*, culture CBS 279.48; on *Ribes* sp., culture CBS 278.48.

Notes: *Nectria dematiosa* is distinguished from other species of the NCSC by sessile sporodochia and ascospores that are up to 2-septate. Care must be taken in observing these characters, because the short stipitate 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. 7R–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 differences 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 recognise 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.

Jøgensen (1952) found morphological differences between typical *N. cinnabarina* and *N. cinnabarina* on *Ribes*. Jøgensen (1952) also mentioned that the fungus grew faster than *N. cinnabarina* from other hosts. One isolate was obtained of *N.* '*cinnabarina*' on *Ribes* sp. (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 rhenani 2657. 1874.
≡ *Nectria cinnabarina* var. *dendroidea* (Fuckel) Wollenw., Angew. Bot. 8: 186. 1926.
= *Nectria cinnabarina* var. *minor* Wollenw., Angew. Bot. 8: 185. 1926.
= *Nectria meliae* Earle, Bull. Torrey Bot. Club 25: 364. 1898.
= *Nectria fuscopurpurea* Wakef., Kew Bull., p. 232. 1918.

Anamorph: *Tuberculariа vulgaris*-like.

Teleomorph on natural substrata: *Mycelium* rarely visible around perithecia and on host. *Stromata* up to 2.0 mm high and 4 mm diam, erumpent through epidermis, whitish yellow to bay, sometimes darker red, KOH+ dark red, LA+ yellow, pseudoparenchymatous, cells forming textura *angularis* to *t. prismatica* with cells oriented more or less vertically; cells 4–17 µm diam, with 1–1.5 µm thick walls, intergrading with the ascomatal wall. *Perithecia* superficial on well-developed stroma, solitary or caespitose, up to 20 on an erumpent stroma, rarely clustered around base of stipitate sporodochia, subglobose to globose, 265–420 µm high × 236–410 µm diam (*n* = 38), 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* 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* unitunicate, (62–)70–98(–113) × (6.5–)7.5–10.0(–11.5) µm (*n* = 63), cylindrical to 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 tubercularoid, 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* monophialidic, up to 3.0–5.0 µm long, 1.0–2.0 µm wide; *terminal cells* monophialidic, 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, 1–1.9 µm wide near aperture. *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. *Conidiogenous cells* monophialidic, 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–)7.6–14.6(–24.3) × (2.3–)3.5–4.9(–6.6) µm (*n* = 180). *Chlamydospores* rare, globose, subglobose, broadly ellipsoidal, 0(–1)-septate, solitary or chains, 8.0–13.0 µm wide. *Perithecia* not produced in culture.

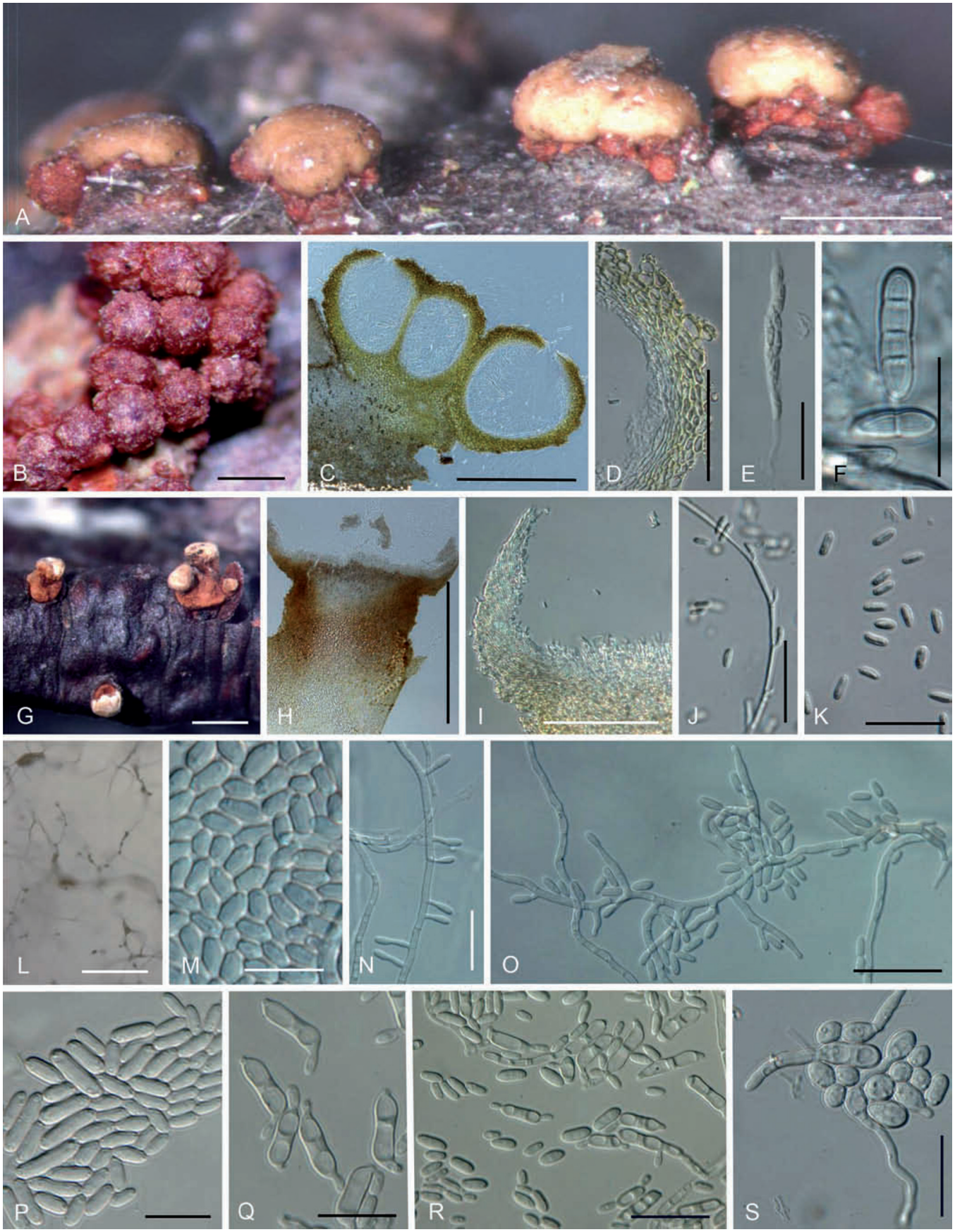

Fig. 8.A–S. *Nectria nigrescens*. A. Perithecia and short stipitate sporodochia in the natural environment. B. Peritheica in the natural environment. C. Median section of perithecia. D. Median section of perithecial wall. E. Ascus. F. One and three septatas ascospores. G. Long stipitate sporodochia in 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.

Epitype 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 dictyledonous tree, 7 Sep. 2005, A.Y. Rossman, epitype BPI 871083, ex-epitype culture CBS 125148 = A.R. 4211.

Additional type specimens examined. Holotype of *Nectria cinnabarina* f. *dendroidea*: **Germany**, Fungi Rehnani 2657, FH. Holotype of *Nectria fuscopurpurea*: **UK**, Wisbech, on dead branch of *Prunus domestica* L., 1917, J.C.F. Fryer or A.D. Cotton, K 98615. Neotype of *Nectria meliae* designated here: **USA**, Alabama, on *Melia* sp., 1 Dec. 1896, C.F. Baker, BPI 552588.

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, Puymardier, on dead twig of *Acer* sp., 18 May 2006, C. Lechat, BPI 878455A = C.L.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*, 8 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. 4565.

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 1985). 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 Wollenwever (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 topotype, 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 f., 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***
1. Ascospores up to 1-, rarely 2-septate; sporodochia sessile or stipitate; Asia, Europe or North America **2**

2. Ascospores up to 1-septate; sporodochia less than 800 µm high, short stipitate; Asia ..... ***N. asiatica***
2. Ascospores up to 1- or rarely 2-septate (3 %); sporodochia sessile or long stipitate; Asia, Europe or North America **3**

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 *Tubercularia* 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 *Tubercularia vulgaris*. Many synonyms are known for *T. vulgaris* (Jøgensen 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 *Tubercularia 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. asiastica, 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, Jøgensen 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, Jøgensen (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 "coral 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). Jøgensen (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 *Hypocrea*/*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.

ACKNOWLEDGEMENTS

The authors thank Dr Gary J. Samuels (USDA-ARS, Beltsville, Maryland, USA) for his contributions to the systematics of the hypocrealean fungi including discussions about the NCSC and other *Nectria*-like fungi. It is a distinct honour to publish our paper here. We thank the many mycologists who shared their fresh specimens and/or cultures of these fungi with us, specifically Alex Akulov, Margaret Barr, Alex Chlebicki, Walter Gams, Tom Gräfenhan, Walter Jaklitsch, Thomas Laessoe, Gaston Laflamme, Christian Lechat, Yasunori Ono, Gary Samuels, Hans-Josef Schroers, Keith Seifert, and Wen-ying Zhuang. We are indebted to Sato Toyozo, Takayuki Aoki, and Keisuke Tomioka (NIAS Genebank, National Institute of Agrobiological Sciences, Tukuba, Ibaraki, Japan), and Keiko T. Natuaki (Tokyo University of Agriculture) for sending and quickly depositing Japanese cultures. We also thank Orlando Petrini (Repubblica e Cantone Ticino, Bellinzona, Switzerland) for giving many ideas and advice about statistical analyses. We express sincere thanks to Andrew Minnis for providing nomenclatural advice and to our excellent technicians, Sasha Allen, Aimee Hyten and Tunesha Phillips. We also gratefully acknowledge the assistance of the curators and staff of the BPI, FH, H, TNS, K, NY, PH, SPA, and UPS. This study was supported in part by the United States National Science Foundation (NSF) PEET grant DEB-0731510 "Monographic Studies in the *Nectriaceae, Hypocreales*: *Nectria, Cosmospora*, and *Neonectria"* to University of Maryland (PIs: P. Chaverri, A.Y. Rossman, G.J. Samuels).

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