

## *Nephroma squamigerum* (Nephromataceae, Lichenized Ascomycota) Is a Distinct Species

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We evaluated the taxonomic status of *Nephroma squamigerum* in Japan, a taxon currently ascribed as a forma to *N. bellum*. Using an extensive data set of herbarium and freshly collected specimens we confirm that *N. squamigerum* differs morphologically, chemically and genetically from *N. bellum* and other *Nephroma* species, and should be treated as a distinct species. *Nephroma squamigerum* is morphologically similar to *N. bellum* and to glabrous morphotypes of *N. helveticum* and may have been frequently overlooked, especially in other parts of eastern Asia. However, the absence of lichen substances in *N. squamigerum* always allows a clear identification. Also, *N. squamigerum* can be clearly distinguished from all other *Nephroma* species based on the sequences of ITS rDNA and mtSSU rDNA. Neotypification of *N. squamigerum* is made. A key to the Japanese taxa of *Nephroma* is provided.

**Keywords:** Asia, ITS rDNA, mtSSU, neotype, *Peltigerales*, triterpenoids.

The lichen genus *Nephroma* Ach. (*Nephromataceae*, lichenized *Ascomycota*) is almost cosmopolitan in its distribution. The highest species diversity can be found especially in oceanic to boreal-montane areas of both hemispheres (James and White 1987, White and James 1988, Smith et al. 2009). The genus includes 36 species worldwide (Kirk et al. 2008), and nine species including four infraspecific taxa have been reported from Japan (Kurokawa and Kashiwadani 2006).

Despite extensive research on the genus *Nephroma* (e.g., Inumaru 1940, Wetmore 1960, Asahina 1962, James and White 1987,

White and James 1988), the morphological delimitations and taxonomy of some variable and widespread taxa like *N. bellum*, *N. helveticum*, *N. laevigatum*, *N. resupinatum*, and *N. tropicum* are unresolved to date (Asahina 1962, Lohtander et al. 2002, Piercey-Normore et al. 2006, Louwhoff 2009, Sérusiaux et al. 2011, Fedrowitz et al. 2012a). Such inadequate knowledge of species may mask species' endangered status and put them at extinction risk since their occurrences cannot be reliably monitored (Stork 1993). *Nephroma* species are sensitive to sulfur dioxide pollution and often negatively affected by intensive forestry, and the

genus includes several indicator species for areas of high conservation value, such as old-growth forest habitats (e.g. Kuusinen 1996, Nitare 2000, Smith et al. 2009, Fedrowitz et al. 2012b). In Europe, several *Nephroma* species are red-listed or locally extinct (Wirth 1995, Vitikainen 2007, Gärdenfors 2010, Rassi et al. 2010), while none is red-listed in Japan to date.

*Nephroma squamigerum* was originally described as an independent taxon at species level by Inumaru (1940). After that, the taxon was treated as a forma in *N. bellum* [= *N. bellum* f. *squamigerum* (Inumaru) Asahina] by Asahina (1962), and has been recognized as such by Japanese lichenologists (Kurokawa and Kashiwadani 2006). However, already James and White (1987) suggested the possibility of *N. squamigerum* as a distinct taxon based on their examination of Japanese materials. The taxon has been characterized by having a glabrous lower thallus surface, isidial lobules on the thallus surface and margins, often markedly reticulate lobes, a more or less smooth dorsal surface of the apothecia, and a lack of detectable lichen substances by Thin-Layer Chromatography (TLC) (Inumaru 1940, Asahina 1962, James and White 1987). However, a taxon ascribed to *N. squamigerum* is sometimes morphologically undistinguishable with some morphotypes of *N. bellum* or *N. helveticum* and may have been frequently overlooked in the field. Genetic differences based on the Internal Transcribed Spacer region of ribosomal DNA (ITS rDNA) between *N. bellum* and a taxon provisionally assigned to *N. squamigerum* have previously been shown but only few rather small specimens were available for the study (Fedrowitz et al. 2012a). The holotype of *N. squamigerum* should have been placed in the herbarium of Hiroshima University (HIRO) in Hiroshima, Japan, but it has been missing for a long time and was probably destroyed during World War II (Harada et al. 2004). This makes it additionally difficult to delimit the identity of *N. squamigerum*.

The aim of this study was to examine the taxonomy of *Nephroma squamigerum* and its phylogenetic position among the related species based on freshly collected materials from the type locality of *N. squamigerum* on Mt. Kanmuri, Hiroshima Prefecture and other localities on Honshu and Hokkaido in Japan as well as thorough investigation of herbarium specimens. A key to the Japanese taxa of *Nephroma* is also provided.

## Materials and Methods

### *Specimens*

Fresh specimens of *Nephroma bellum*, *N. helveticum*, *N. parile*, *N. resupinatum*, *N. squamigerum*, and *N. tropicum* were collected in 2010 and 2012 at the type locality of *N. squamigerum* on Mt. Kanmuri, Hiroshima Prefecture, and other localities on Honshu and Hokkaido in Japan (Table 1). These specimens are deposited in the National Museum of Nature and Science (TNS), Japan, or at the Museum of Evolution at Uppsala University (UPS), Sweden. In addition, 67 herbarium specimens preserved in TNS were examined that were identified as *N. squamigerum* or *N. bellum*. The related taxa of Inumaru's collections deposited in HIRO were also examined.

### *Morphological and chemical analyses*

Morphological observations were made using a dissecting microscope and a bright field microscope. Sections of thallus and apothecium were cut by hand with a razor blade, and observed after mounting in GAW (glycerin: ethanol: water, 1: 1: 1). Chemical analyses were performed by means of color spot tests and TLC in solvent systems B', C and G (Orange et al. 2001).

### *Molecular analyses*

DNA was extracted from thallus fragments of all fresh lichen specimens and the fungal ITS rDNA and the gene coding for the fungal mitochondrial small subunit ribosomal DNA

Table 1. Samples of *Nephroma* species used in this study

Species	Voucher number, herbarium	Locality (collection year)	Genotypes		GenBank accession numbers		
			ITS	mtSSU	ITS	mtSSU	
<i>N. bellum</i>							
	GT24040 (= KU494b), UPS	Shiretoko, Hokkaido (2010)	NB1	----	<b>JN857210*</b>	----	
	GT24041 (= KU519; KF9), UPS	Shiretoko, Hokkaido (2010)	NB1	NB	<b>JN857211</b>	KJ150406	
	GT400A4/2nb (= KU518), UPS	Shiretoko, Hokkaido (2010)	NB2	----	<b>JN857212</b>	----	
	GT24333 (= KU559; KF10), UPS	Shiretoko, Hokkaido (2010)	NB2	NB	<b>JN857213</b>	KJ150407	
	GT24131 (= KU557a; KF8), UPS	Shiretoko, Hokkaido (2010)	NB3	NB	<b>JN857214</b>	KJ150408	
	GT24213 (= KU558; KF16), UPS	Shiretoko, Hokkaido (2010)	NB3	NB	<b>JN857215</b>	KJ150409	
	AF10/Jp3 (= KU523a), TNS	Shiretoko, Hokkaido (2010)	NB4	----	<b>JN857216</b>	----	
	AF10/Jp5 (= KU525a), TNS	Shiretoko, Hokkaido (2010)	NB5	----	<b>JN857217</b>	----	
	GT24356 (= KU560), UPS	Shiretoko, Hokkaido (2010)	NB6	----	<b>JN857218</b>	----	
	KFJp14, TNS	Mt. Akadake, Nagano (2012)	NB 6	NB	KJ150331	KJ150410	
	KFJp15, TNS	Mt. Akadake, Nagano (2012)	NB 6	NB	KJ150332	KJ150411	
	KFJp16B, TNS	Mt. Akadake, Nagano (2012)	NB 15	NB	KJ150333	KJ150412	
	KF21.2, TNS	Mt. Akadake, Nagano (2012)	NB 16	NB	KJ150334	KJ150413	
	KFJp16A, TNS	Mt. Akadake, Nagano (2012)	NB 16	NB	KJ150335	KJ150414	
	KFJp20, TNS	Mt. Akadake, Nagano (2012)	NB 16	NB	KJ150336	KJ150415	
	KFJp22, TNS	Mt. Akadake, Nagano (2012)	NB 16	NB	KJ150337	KJ150416	
	KF21.6, TNS	Mt. Akadake, Nagano (2012)	NB 17	NB	KJ150338	KJ150417	
	KF21.9, TNS	Mt. Akadake, Nagano (2012)	NB 17	NB	KJ150339	KJ150418	
	KFJp25, TNS	Mt. Akadake, Nagano (2012)	NB 18	NB	KJ150340	KJ150419	
<i>N. helveticum</i>							
	AF12/Jp308 (= KF6), TNS	Minami-Alps, Nagano (2012)	NH2	NH typ1	KJ150341	KJ150420	
	AF12/Jp356 (= KF7), TNS	Mt. Fuji, Shizuoka (2012)	NH2	NH typ1	KJ150342	KJ150421	
	KFJp38B, TNS	Mt. Kanmuri, Hiroshima (2012)	NH3	NH typ1	KJ150343	KJ150422	
	KFJp31, TNS	Mt. Kanmuri, Hiroshima (2012)	NH3	NH typ1	KJ150344	KJ150423	
	KFJp5, TNS	Mt. Akadake, Nagano (2012)	NH3	NH typ1	KJ150345	KJ150424	
	KFJp38, TNS	Mt. Kanmuri, Hiroshima (2012)	NH3	NH typ1	KJ150346	KJ150425	
	KFJp30, TNS	Mt. Kanmuri, Hiroshima (2012)	NH3	NH typ1	KJ150347	KJ150426	
	KFJp30B, TNS	Mt. Kanmuri, Hiroshima (2012)	NH3	----	KJ150348	----	
	YO8955, TNS	Minami-Alps, Nagano (2012)	NH4	NH typ1	KJ150349	KJ150427	
	KF21.1, TNS	Mt. Akadake, Nagano (2012)	NH4	NH typ1	KJ150350	KJ150428	
	KF21.5, TNS	Mt. Akadake, Nagano (2012)	NH4	----	KJ150351	----	
	KFJp19, TNS	Mt. Akadake, Nagano (2012)	NH4	NH typ1	KJ150352	KJ150429	
	KFJp2, TNS	Mt. Akadake, Nagano (2012)	NH4	NH typ1	KJ150353	KJ150430	
	KFJp9, TNS	Mt. Akadake, Nagano (2012)	NH4	----	KJ150354	----	
	KF21.7, TNS	Mt. Akadake, Nagano (2012)	NH4	----	KJ150355	----	
	KFJp11, TNS	Mt. Akadake, Nagano (2012)	NH4	----	KJ150356	----	
	KF21.3, TNS	Mt. Akadake, Nagano (2012)	NH23	----	KJ150358	----	
	KF21.10, TNS	Mt. Akadake, Nagano (2012)	NH23	----	KJ150359	----	
	KFJp17C, TNS	Mt. Akadake, Nagano (2012)	NH23	NH typ1	KJ150360	KJ150432	
	KFJp28, TNS	Mt. Kanmuri, Hiroshima (2012)	NH24	NH typ1	KJ150361	KJ150433	
	KFJp29D, TNS	Mt. Kanmuri, Hiroshima (2012)	NH24	NH typ1	KJ150362	KJ150434	
	KFJp29B, TNS	Mt. Kanmuri, Hiroshima (2012)	NH24	----	KJ150363	----	
	KFJp29E, TNS	Mt. Kanmuri, Hiroshima (2012)	NH24	----	KJ150364	----	
	AF12/Jp300 (= KF5), TNS	Minami-Alps, Nagano (2012)	NH25	NH typ1	KJ150365	KJ150435	
	KFJp6A, TNS	Mt. Akadake, Nagano (2012)	NH26	NH typ1	KJ150366	KJ150436	
	KFJp35, TNS	Mt. Kanmuri, Hiroshima (2012)	NH27	NH typ1	KJ150367	KJ150437	
	KFJp26, TNS	Mt. Akadake, Nagano (2012)	NH28	NH typ1	KJ150368	KJ150438	
	KFJp4, TNS	Mt. Akadake, Nagano (2012)	NH29	NH typ1	KJ150369	KJ150439	
	KFJp6B, TNS	Mt. Akadake, Nagano (2012)	NH29	NH typ1	KJ150370	KJ150440	
	KFJp39, TNS	Mt. Kanmuri, Hiroshima (2012)	NH30	----	KJ150371	----	
	KFJp39B, TNS	Mt. Kanmuri, Hiroshima (2012)	NH30	----	KJ150372	----	

Table 1. Continued

Species	Voucher number, herbarium	Locality (collection year)	Genotypes		GenBank accession numbers		
			ITS	mtSSU	ITS	mtSSU	
<i>N. parile</i>							
	GT23856 (= KU492a), UPS	Shiretoko, Hokkaido (2010)	NP1	----	<b>JN857253</b>	----	
	AF10/Jp3 (= KU523b), TNS	Shiretoko, Hokkaido (2010)	NP4	----	<b>JN857277</b>	----	
	AF10/Jp5 (= KU525b), TNS	Shiretoko, Hokkaido (2010)	NP4	----	<b>JN857278</b>	----	
	GT24040 (= KU494a), UPS	Shiretoko, Hokkaido (2010)	NP4	----	<b>JN857279</b>	----	
	KFJp13A, TNS	Mt. Akadake, Nagano (2012)	NP1	NP	KJ150373	KJ150441	
	KFJp24, TNS	Mt. Akadake, Nagano (2012)	NP1	----	KJ150374	----	
	KFJp8, TNS	Mt. Akadake, Nagano (2012)	NP1	----	KJ150375	----	
	YO8948, TNS	Minami-Alps, Nagano (2012)	NP6	NP	KJ150376	KJ150442	
	KFJp13B, TNS	Mt. Akadake, Nagano (2012)	NP9	----	KJ150377	----	
<i>N. resupinatum</i>							
	GT24097 (= KU495), UPS	Shiretoko, Hokkaido (2010)	NR5	----	<b>JN857287</b>	----	
	GT24175 (= KU496a), UPS	Shiretoko, Hokkaido (2010)	NR5	----	<b>JN857288</b>	----	
	AF10/Jp2 (= KU522a), TNS	Shiretoko, Hokkaido (2010)	NR6	----	<b>JN857289</b>	----	
	KFJp3, TNS	Mt. Akadake, Nagano (2012)	NR5	NR	KJ150378	KJ150443	
	KFJp10, TNS	Mt. Akadake, Nagano (2012)	NR5	----	KJ150379	----	
	KFJp7, TNS	Mt. Akadake, Nagano (2012)	NR5	----	KJ150380	----	
	KFJp35B, TNS	Mt. Kanmuri, Hiroshima (2012)	NR5	----	KJ150381	----	
	KFJp29C, TNS	Mt. Kanmuri, Hiroshima (2012)	NR5	----	KJ150382	----	
	KFJp1, TNS	Mt. Akadake, Nagano (2012)	NR6	NR	KJ150383	KJ150444	
	KFJp12, TNS	Mt. Akadake, Nagano (2012)	NR6	----	KJ150384	----	
	KFJp23, TNS	Mt. Akadake, Nagano (2012)	NR6	----	KJ150385	----	
	KFJp27, TNS	Mt. Akadake, Nagano (2012)	NR6	----	KJ150386	----	
	YO9470_KF A, TNS	Mt. Kanmuri, Hiroshima (2012)	NR6	----	KJ150387	----	
	YO9470_KF B, TNS	Mt. Kanmuri, Hiroshima (2012)	NR6	----	KJ150388	----	
	KFJp34 C, TNS	Mt. Kanmuri, Hiroshima (2012)	NR11	NR	KJ150389	KJ150445	
	KFJp34, TNS	Mt. Kanmuri, Hiroshima (2012)	NR11	----	KJ150390	----	
<i>N. squamigerum</i>							
	AF10/Jp12 (= KU526, KF11), TNS	Shiretoko, Hokkaido (2010)	NS1	NSq	<b>JN857292</b>	KJ150446	
	AF10/Jp4 (= KU524a, KF12), TNS	Shiretoko, Hokkaido (2010)	NS2	NSq	<b>JN857293</b>	KJ150447	
	GT23855 (= KU517a, KF13), TNS	Shiretoko, Hokkaido (2010)	NS3	NSq	<b>JN857294</b>	KJ150448	
	GT600A2/3 (= KU556), TNS	Shiretoko, Hokkaido (2010)	NS4	----	<b>JN857295</b>	----	
	AF10/Jp1 (=KU521a, KF14), TNS	Shiretoko, Hokkaido (2010)	NS4	NSq	<b>JN857296</b>	KJ150449	
	GT24506 (=KU516, KF15), TNS	Shiretoko, Hokkaido (2010)	NS4	NSq	<b>JN857297</b>	KJ150450	
	YO7230, TNS	Mt. Oginosen, Tottori (2010)	NS2	NSq	KJ150391	KJ150451	
	KFJp33, TNS	Mt. Kanmuri, Hiroshima (2012)	NS3	NSq	KJ150392	KJ150452	
	KFJp33B, TNS	Mt. Kanmuri, Hiroshima (2012)	NS3	NSq	KJ150393	KJ150453	
	KFJp33C, TNS	Mt. Kanmuri, Hiroshima (2012)	NS3	NSq	KJ150394	KJ150454	
	KFJp37, TNS	Mt. Kanmuri, Hiroshima (2012)	NS3	NSq	KJ150395	KJ150455	
	KFJp37C, TNS	Mt. Kanmuri, Hiroshima (2012)	NS3	NSq	KJ150396	KJ150456	
	KFJp29F, TNS	Mt. Kanmuri, Hiroshima (2012)	NS3	NSq	KJ150397	KJ150457	
	KFJp36B, TNS	Mt. Kanmuri, Hiroshima (2012)	NS6	NSq	KJ150398	KJ150458	
	KFJp37B, TNS	Mt. Kanmuri, Hiroshima (2012)	NS6	NSq	KJ150399	KJ150459	
	KFJp34B, TNS	Mt. Kanmuri, Hiroshima (2012)	NS6	NSq	KJ150400	KJ150460	
	KF21.4, TNS	Mt. Akadake, Nagano (2012)	NS7	NSq	KJ150401	KJ150461	
	KFJp32, TNS	Mt. Kanmuri, Hiroshima (2012)	NS8	NSq	KJ150402	KJ150462	
	KFJp36, TNS	Mt. Kanmuri, Hiroshima (2012)	NS8	NSq	KJ150403	KJ150463	
	KFJp36C, TNS	Mt. Kanmuri, Hiroshima (2012)	NS8	NSq	KJ150404	KJ150464	
	KFJp29, TNS	Mt. Kanmuri, Hiroshima (2012)	NS8	NSq	KJ150405	KJ150465	
<i>N. tropicum</i>							
	YO8952, TNS	Minami-Alps, Nagano (2012)	NT1	NT	KJ150357	KJ150431	

\*Sequences obtained from GenBank are in bold.

Table 2. Morphological and chemical differences among *Nephroma bellum*, *N. helveticum*, *N. squamigerum*, and *N. tropicum*

Species	Lower thallus surface	Lobules	Fertile lobes	Hopane triterpenoids (T1–T5: see James and White 1987)
<i>N. bellum</i>	glabrous to slightly patchily distributed tomentose	±at thallus margins	entire to ±lobulate	T3, T5, ±T2
<i>N. helveticum</i>	tomentose to densely pubescent (sparse or almost absent in young or depauperate thalli)	on thallus surface and/or margins	±lobulate, sharp serration	T4, ±T1
<i>N. squamigerum</i>	glabrous	on thallus surface and/or margins	entire to ±lobulate	nil
<i>N. tropicum</i>	tomentose to densely pubescent	on thallus surface and/or margins	lobulate, obtuse indentation	T4, ±T1

(mtSSU rDNA) were amplified. The primer sets were ITS1F (White et al. 1990) and LR1 (Vilgalys and Hester 1990) for ITS rDNA, and mrSSU1 and mrSSU3R (Zoller et al. 1999) for mtSSU rDNA. The obtained sequences were checked and manually edited. They were aligned using the programs BioEdit version 7.1.9 (Hall 1999) and PhyDE<sup>®</sup> v1.0.

Phylogenetic analyses on the sequences were performed with MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001) using an alignment of 1322 nt including 778 nt mtSSU rDNA and 544 nt ITS1-5.8S-ITS2. *Nephroma resupinatum* was used as outgroup based on the preliminary mtSSU analysis and previous results (Lohtander et al. 2002, Fedrowitz et al. 2012a). To allow possible deviating substitution models for the different regions, the data sets were divided into sequence partitions, and for mtSSU rDNA and 5.8S the model GTR+ $\Gamma$ +I was applied and for ITS1 and ITS2 GTR+ $\Gamma$ . The a priori probabilities supplied were those specified in the default settings of the program. Posterior probability distributions of trees were calculated using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method and the search strategies suggested by Huelsenbeck et al. (2001, 2002). Four runs with four chains ( $1 \times 10^7$  generations each) were run simultaneously with default settings. Chains were sampled every 1000 generations and consensus trees and posterior probabilities for the clades were calculated after

the chains converged ( $>$  generation 2 500 000).

## Results

### Morphology

The morphological differences observed in *Nephroma bellum*, *N. helveticum*, *N. squamigerum*, and *N. tropicum* are shown in Table 2.

When well developed, *N. squamigerum* can be characterized by the frequent occurrence of laminal and marginal lobules, the lack of a tomentose lower surface and a more or less smooth dorsal surface of the apothecia. In comparison, *N. bellum* has usually no or only few marginal lobules and a glabrous to slightly patchily distributed tomentose lower thallus surface. The latter characteristic, glabrous lower surface, also distinguishes *N. squamigerum* from *N. helveticum* and *N. tropicum* which typically bear numerous marginal and occasional laminal isidial lobules but usually have a tomentose to densely pubescent lower surface. However, young or depauperate specimens of *N. helveticum*, which are often found in mixed populations, are sometimes difficult to identify when the tomentum on the lower surface is sparse or almost absent. Such specimens could be misidentified as *N. bellum* or *N. squamigerum*. Furthermore, specimens having the same chemistry as *N. bellum* sometimes had a rather large amount of laminal lobules and were therefore often wrongly identified as

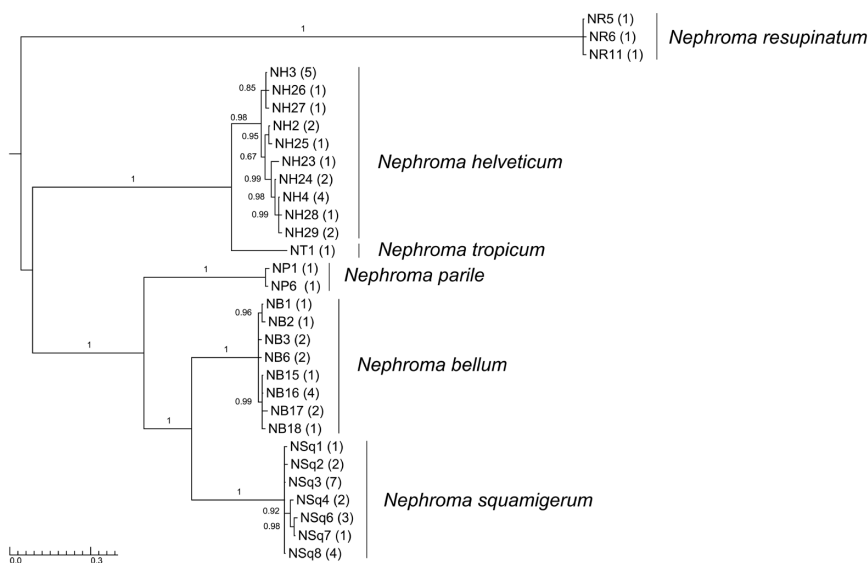


Fig. 1. Molecular phylogeny of *Nephroma squamigerum* and the related species in Japan. The Bayesian tree was inferred from ITS rDNA and mtSSU rDNA sequences of Japanese *Nephroma* specimens. Scale bar represents the number of substitutions per site. Number in brackets indicates the number of specimens from which both ITS rDNA and mtSSU rDNA sequences were obtained.

*N. squamigerum* based on thallus morphology alone.

#### Chemistry

Using TLC, specimens assigned to *N. squamigerum* by thallus morphology or genetic markers show no demonstrable lichen substances. In comparison, the related species *N. bellum*, *N. helveticum* and *N. tropicum* contain one to several hopane triterpenoids (Table 2) in addition to other accessory substances.

#### Phylogenetic analysis

All 21 freshly collected specimens that were identified as *N. squamigerum* had different genotypes of ITS rDNA and mtSSU rDNA from all other investigated *Nephroma* species (Table 1). All Japanese specimens of *N. bellum*, *N. helveticum*, *N. parile*, *N. resupinatum*, *N. squamigerum*, and *N. tropicum* had a single species-specific mtSSU rDNA genotype. In comparison, one to several ITS rDNA genotypes were identified for each of the six *Nephroma*

species collected from Japan.

The phylogenetic tree (Fig. 1) was consistent with the recent phylogenies of *Nephroma* based on mtSSU rDNA, ITS rDNA and/or nuclear large subunit (nLSU) sequences (Lohtander et al. 2002, Sérusiaux et al. 2011, Fedrowitz et al. 2012a). We could show that *N. squamigerum* forms a well-supported monophyletic clade which is a sister clade to *N. bellum*. The clade of *N. squamigerum* was not related to those of *N. helveticum* and *N. tropicum* which are closely related to each other.

#### Discussion

Our study shows that *Nephroma squamigerum* should be treated as a distinct taxon separate from *N. bellum* and its related taxa, and thus confirms an earlier assumption by James and White (1987) and preliminary results by Fedrowitz et al. (2012a). In addition to Asahina (1962) and James and White (1987) who considered *N. squamigerum* as being morphologically similar to *N. bellum*, we point



Fig. 2. Neotype of *Nephroma squamigerum* (S. Inumaru 418, HIRO).

out that *N. squamigerum* can also be confused with *N. helveticum* in particular with those specimens that lack well developed apothecia and show only few or no tomentum on the lower surface. Such specimens were regularly collected during this study and appear to be rather common in Japan. Although morphology alone is often not sufficient for a reliable identification, thallus chemistry and molecular markers show a consistent result.

Since *N. squamigerum* has long been treated as a forma of *N. bellum*, and specimens can easily be misidentified as *N. bellum* and *N. helveticum*, it is likely that this species has frequently been overlooked in the past. To date, *N. squamigerum* is known only from Japan, but it is likely that this species also occurs in adjacent parts of eastern Asia. One indication for this are two ITS rDNA sequences from South Korea in GenBank (DQ066700 and DQ066701) that are assigned to *N. bellum* but belong to the genotypes of *N. squamigerum*. Further investigation into these specimens is necessary

in order to confirm that *N. squamigerum* is not endemic to Japan.

#### Typification of *Nephroma squamigerum*

*Nephroma squamigerum* Inumaru in Acta Phytotax. Geobot. **9**: 51 (1940).

*N. bellum* f. *squamigerum* (Inumaru) Asahina in J. Jap. Bot. **37**: 262 (1962).

**NEOTYPE** (designated here): **JAPAN**. Honshu, Nagano Pref., Mt. Harinoki, 18 July 1948, S. Inumaru 418 (HIRO). Chemistry: nil (annotated by F. J. White and P. James 1987).

[Fig. 2]

The holotype of *N. squamigerum* in HIRO has been missing for a long time. It is thought that most of Inumaru's collections were destroyed during World War II (Harada et al. 2004). There is a specimen annotated as "neotype" (S. Inumaru, no. 418, HIRO) chosen by F. J. White and P. James in 1987, but this neotypification has not been published yet. Since this specimen agrees well with the protologue we design it here as the neotype.

### Key to Japanese taxa of the genus *Nephroma*

Although *Nephroma javanicum* Gyeln. var. *japonicum* (Räsänen) M. Sato, an endemic taxon to Japan, is listed in Kurokawa and Kashiwadani (2006), it is not included in this current key. The characters described in the protologue (J. Jpn. Bot. 16: 144, 1940) of the taxon fall within the ranges of morphological variations in *N. bellum* and *N. helveticum*. Further taxonomic research including the examination of type material is needed to clarify the identity of this taxon.

1. Photobiont green; thallus yellow-green; cephalodia present ..... *N. arcticum* (L.) Torss.
1. Photobiont blue green; thallus brown; cephalodia absent ..... 2
2. Soredia present ..... *N. parile* (Ach.) Ach.
2. Soredia absent ..... 3
3. Lower surface papillate .....  
..... *N. resupinatum* (L.) Ach.
3. Lower surface not papillate ..... 4
4. Lower surface glabrous to slightly patchily distributed tomentose ..... 5
4. Lower surface tomentose to densely pubescent ..... 7
5. Lobules laminal and/or marginal; no lichen substance ..... *N. squamigerum* Inumaru
5. Lobules ± marginal; terpenoids (T3, T5, ±T2) present ..... 6
6. Medulla P- ..... *N. bellum* (Spreng.) Tuck.
6. Medulla P+ yellow .....  
..... *N. bellum* f. *flavoreagens* Asahina
7. Medulla yellow to pale yellow, K+ purple (anthraquinones present) ..... 8
7. Medulla white, K- (anthraquinones absent)... 9
8. Hymenium I+ blue (I: 0.6% of Lugol's iodine solution) ..... *N. laevigatum* Ach.
8. Hymenium I+ orange-red .....  
..... *N. servitianum* Gyeln.
9. Lobules caespitously present on the upper surface of thallus; thallus thin 0.10-0.12 mm in thickness .....  
..... *N. helveticum* f. *caespitosum* Asahina
9. Lobule not caespitously distributed on the upper surface of thallus; thallus more than 0.15 mm in thickness ..... 10
10. Fertile lobes with sharp serration (0.6–1.0 × ca. 0.1 mm); thallus 0.15–0.20 mm in thickness; spore 14–18 × 6–7 μm ..... *N. helveticum* Ach.
10. Fertile lobes with obtuse indentation (0.6–1.0 × ca. 0.3 mm); thallus more than 0.20 mm in thickness; spore 20–24 × 5.5–6.0 μm .....  
..... *N. tropicum* (Müll. Arg.) Zahlbr.

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### References

- Asahina Y. 1962. Lichenologische Notizen (185). Preliminary arrangement of Japanese *Nephroma* species. J. Jap. Bot. **37**: 257–262.
- Fedrowitz K., Kaasalainen U. and Rikkinen J. 2012a. Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. Ecol. Evol. **2**: 2291–2303.
- Fedrowitz K., Kuusinen M. and Snäll T. 2012b. Metapopulation dynamics and future persistence of epiphytic cyanolichens in a European boreal forest ecosystem. J. Appl. Ecol. **49**: 493–502.
- Gärdenfors U. 2010. The 2010 Red List of Swedish Species. ArtDatabanken SLU, Uppsala.
- Hall T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95–98.
- Harada, H., Okamoto, T. and Yoshimura, I. 2004. A checklist of lichens and lichen-allies of Japan. Lichenology **2**: 47–165.
- Huelsensbeck J. P., Larget B., Miller R. E. and Ronquist F. 2002. Potential applications and pitfalls of bayesian inference of phylogeny. Syst. Biol. **51**: 673–688.
- Huelsensbeck J. P. and Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics **17**: 754–755.
- Huelsensbeck J. P., Ronquist F., Nielsen R. and Bollback J. P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science **294**: 2310–



- 2314.
- Inumaru S. 1940. Studia Licheneum Japoniae II. *Peltigeraceae* Japoniae II. *Nephroma* Acharius Grex 2. *Glabrae*. Acta Phytotax. Geobot. **9**: 51–63.
- James P. W. and White F. J. 1987. Studies on the genus *Nephroma* I. The European and Macaronesian species. Lichenologist **19**: 215–268.
- Kirk M., Cannon P. F., Minter D. W. and Stalpers J. A. (eds.) 2008. Ainsworth & Bisby's Dictionary of the Fungi, 10th Edition. CAB International, Wallingford.
- Kurokawa S. and Kashiwadani H. 2006. Checklist of Japanese Lichens and Allied Fungi. National Science Museum Monographs No. 33. National Science Museum, Tokyo.
- Kuusinen M. 1996. Cyanobacterial macrolichens on *Populus tremula* as indicators of forest continuity in Finland. Biol. Cons. **75**: 43–49.
- Lohtander K., Oksanen I. and Rikkinen J. 2002. A phylogenetic study of *Nephroma* (lichen-forming Ascomycota). Mycol. Res. **106**: 777–787.
- Louwhoff S. H. J. 2009. *Nephromataceae*. In: McCarthy P. M., Flora of Australia vol. 57. Lichens 5. pp. 423–427. ABRS and CSIRO Publishing, Canberra.
- Nitare J. (ed.) 2000. Indicator Species for Assessing the Nature Conservation Value of Woodland Sites: A Flora of Selected Cryptogams. Skogsstyrelsen Förlag, Jönköping.
- Orange A., James P. W. and White F. J. 2001. Microchemical Methods for the Identification of Lichens. 101 pp. British Lichen Society.
- Piercey-Normore M. D., Coxson D., Goward T. and Goffinet B. 2006. Phylogenetic position of a Pacific Northwest North American endemic cyanolichen, *Nephroma oculatum* (Ascomycota, Peltigerales). Lichenologist **38**: 441–456.
- Rassi P., Hyvärinen E., Juslén A. and Mannerkoski I. (eds.) 2010. The 2010 Red List of Finnish Species. Ministry of the Environment and Finnish Environment Institute, Helsinki.
- Sérusiaux E., Villarreal A. J. C., Wheeler T. and Goffinet B. 2011. Recent origin, active speciation and dispersal for the lichen genus *Nephroma* (*Peltigerales*) in Macaronesia. J. Biogeogr. **38**: 1138–1151.
- Smith C. W., Aptroot A., Coppins B. J., Fletcher A., Gilbert O. L., James P. W. and Wolseley P. A. (eds.) 2009. The Lichens of Great Britain and Ireland. London: The British Lichen Society.
- Stork N. E. 1993. How many species are there? Biodivers. Conserv. **2**: 215–232.
- Vilgalys R. and Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. **172**: 4238–4246.
- Vitikainen O. 2007. *Nephromataceae*. Nordic Lichen Flora **3**: 91–95. Uddevalla.
- Wetmore C. 1960. The lichen genus *Nephroma* in North and Middle America. Publ. Mus. Michigan State Univ., Biol. Ser. **1**: 369–452.
- White F. J. and James P. W. 1988. Studies on the Genus *Nephroma* II. The Southern Temperate Species. Lichenologist **20**: 103–166.
- White T. J., Bruns T., Lee S. and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M. A., Gelfand D. H., Sninsky J. J. and White T. J. (eds.), PCR Protocols: A Guide to Methods and Applications. pp. 315–322. Academic Press, San Diego.
- Wirth V. 1995. Die Flechten Baden-Württembergs, Teil 1 & 2. Eugen Ulmer GmbH & Co., Stuttgart.
- Zoller S., Scheidegger C. and Sperisen C. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist **31**: 511–516.

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 コモチウラムイゴケ (ウラムイゴケ科, 地衣化子嚢菌) は独立種である

コモチウラムイゴケ *Nephroma squamigerum* は, 地衣体腹面にトメントが無く, 小裂片を地衣体背面および周縁につけ, 子器を有する裂片には小裂片ができるかまたは全縁となる. ナメラウラムイゴケ *N. bellum* やウラムイゴケモドキ *N. helveticum* と形態的に中間的な個体もしばしば見られるが, それらの種からはテルペン類などの地衣成分が含まれないことで区別できる. コモチウラムイゴケは独立種とする見解がある一方で, 形態的連続性からナメラウラムイゴケの品種とする見解もある. 本研究では, コモチウラムイゴケの分類学的位置を明らかにするために, 関連種との形態および化学成分の比較に加えて,

核リボゾーム遺伝子 ITS 領域および mtSSU に基づく分子系統解析を行った. コモチウラムイゴケは単一クレードを形成し, ナメラウラムイゴケと姉妹群となったが, ウラムイゴケモドキとの直接的な系統関係は示されなかった. 以上, 形態的, 化学的, 分子系統学的結果より, コモチウラムイゴケは独立種として扱うのが妥当であると結論づけた.

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