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Molecular phylogeny and taxonomy of Eurasian *Neoerysiphe* species infecting *Asteraceae* and *Geranium*

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Key words

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Abstract Because Eurasian samples of *Neoerysiphe* collected on the *Asteraceae* were not identical in morphology, the molecular and morphological differences among these specimens were compared with those of the American *N. cumminsiana*. *Neoerysiphe* on *Asteraceae* was found to consist of at least four different species. Three of them are described as new species, viz. *N. hiratae*, *N. joerstadii*, and *N. nevoi*. *Neoerysiphe hiratae* is a Japanese species parasitizing hosts belonging to the genera *Cacalia* and *Ligularia* (tribe *Senecioneae*). *Neoerysiphe joerstadii* was found in Israel on *Phagnalon rupestre* (tribe *Gnaphalieae*). *Neoerysiphe nevoi* was recorded in Israel and Ukraine on a number of hosts in different genera but all belonging to tribe *Cichorieae*. Thus, Eurasian *Neoerysiphe* species infecting the *Asteraceae* are strongly specialised to particular tribes of this family. Phylogenetic analyses indicated that the three new species were not closely allied. *Neoerysiphe hiratae* is related to the American *N. cumminsiana* and species belonging to *Oidium* subg. *Striatoidium*. *Neoerysiphe nevoi* is sister to *N. geranii*, and *N. joerstadii* is allied to *N. galii*. In addition, Ukrainian *Neoerysiphe* samples on *Geranium* were phylogenetically and morphologically identical to Japanese samples of *N. geranii*, and this fungus seems to be an invasive species in Ukraine.

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INTRODUCTION

Based on the anamorph type, Heluta (1988) proposed to divide the genus *Erysiphe* into two separate genera, viz. *Erysiphe* s.str. and *Golovinomyces*. The former included all species with an anamorph of the *Pseudoidium* type (conidia formed singly on conidiophores), whereas the latter consisted of species with an anamorph of the *Oidium* s.str. type (= *Euoidium*; conidia catenate). Species belonging to two *Erysiphe* sections introduced by Braun (1978, 1981), namely *Golovinomyces* and *Galeopsidis*, were included in the genus *Golovinomyces* sensu Heluta (Heluta 1988). Only one species, *E. galeopsidis*, was contained in sect. *Galeopsidis*. This species differed from other *Erysiphe* representatives by its lobed appressoria and maturation of ascospores after wintering. It was clarified later that a few species very close to *E. galeopsidis* had to be included in sect. *Galeopsidis*, viz. *E. chelones* on *Scrophulariaceae* (USA), *E. cumminsiana* on *Asteraceae* (Asia, North America), *E. galii* on *Rubiaceae* (Europe, Asia), and *E. geranii* on *Geraniaceae* (Japan, New Zealand). However, molecular studies (Saenz & Taylor 1999, Mori et al. 2000) clearly indicated that sect. *Golovinomyces* did not group with sect. *Galeopsidis.* In addition, it was found that the conidium surface of species belonging to sect. *Galeopsidis* is unique among powdery mildew fungi enabling the creation of a new taxon for the anamorphs of this section, viz. *Oidium* subg. *Striatoidium* (Cook et al. 1997). Due to these morphological, biological, and molecular peculiarities of representatives of sect. *Galeopsidis*, Braun (1999) raised this section to genus rank and introduced the name *Neoerysiphe*. The five species in the section were transferred to this new genus with appropriate new taxonomic combinations. Later, another species, *N. rubiae*, was described on *Rubia* cf. *tinctoria* from Turkey (Bahcecioglu et al. 2006). In addition, Takamatsu et al. (2008) revealed that *Oidium aloysiae* on *Aloysia citriodora*, *O. baccharidis* on *Baccharis linearis* and *B. racemosa*, and *O. maquii* on *Aristotelia chilensis* are anamorphs of *Neoerysiphe*. Thus, at present this genus combines six teleomorph and three anamorph species, viz. *N. chelones* on the *Scrophulariaceae*, *N. cumminsiana* and *O. baccharidis* on the *Asteraceae*, *N. galii* and *N. rubiae* on the *Rubiaceae*, *N. geranii* on the *Geraniaceae*, *O. aloysiae* on the *Verbenaceae*, *O. maquii* on the *Elaeocarpaceae*, and *N. galeopsidis* parasitizing many hosts of *Lamiaceae* as the main host family but also a few species in *Acanthaceae*, *Bignoniaceae*, *Dipsacaceae*, and *Malvaceae* (Liu et al. 2005, Takamatsu et al. 2008). Each of these species has a quite different distribution. *Neoerysiphe galeopsidis* is nearly circumglobal, known from all Europe, Asia, Africa, North America, and New Zealand (Braun 1987). Distributions are rather limited for the remaining species. *Neoerysiphe chelones* is known from the USA, *N. rubiae* only from Turkey. *Neoerysiphe galii* is a Eurasian species. *Neoerysiphe geranii* was known from Japan and probably from New Zealand (Amano 1986, Nomura 1997). Heluta (2001) also reported this fungus from Ukraine. Some questions regarding the distributions of certain *Neoerysiphe* species have still to be answered. For instance, *N. geranii* seems to have a more disjunctive distribution. Furthermore, it is also possible that another species morphologically close to *N. geranii* is distributed in Ukraine. Braun (1983) described *N. cumminsiana* on *Senecio seemannii* from the USA and later reported it from North America and Japan on hosts belonging to *Cacalia*, *Eupatorium*, *Heliopsis*, and *Ligularia* (Braun 1987). Heluta (1989, 1999) first recorded a powdery mildew on *Crepis* and *Taraxacum* in Ukraine as *Golovinomyces galii*, and later changed it to *G. cumminsianus.* Voytyuk et al. (2004, 2006) reported *N. cumminsiana* from Israel on hosts of many genera of the *Asteraceae*, viz. *Carthamus*, *Crepis*, *Filago*, *Hedypnois*, *Phagnalom*, *Rhagadiolus*, *Senecio*, *Thrincia*, and *Tolpis*. According to Voytyuk et al. (2004), *N. cumminsiana* has a unique distribution being the only representative of the Erysiphales

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which must be classified as an American-African-Eurasian South Holarctic species. However, this does not correspond to the set of probable geographic and mycoflorogenetic units of powdery mildews proposed by Heluta (1993, 1995). These units consist of species having many factors in common, mainly their probable time and place of origin and current habitats. It is also not in accordance with Heluta's (1992) hypothesis on the ways of powdery mildew migration. Therefore, Voytyuk et al. (2004) assumed that this hypothesis was either not fully correct or *N. cumminsiana* is a species complex with similar morphological characteristics. In the latter case '*N. cumminsiana*' might be descended from an ancestor such as *N. galeopsidis* and might have emerged independently in several regions of North and South America, Africa, or Eurasia. In addition, specimens of Israeli '*N. cumminsiana*' are morphologically not uniform. Voytyuk et al. (2004, 2006) reported that collections on *Phagnalon rupestre* had much larger chasmothecia and smaller peridial cells than those on other host plants. Furthermore, the taxonomic status of Eurasian '*N. geranii*' and '*N. cumminsiana*' was never examined with molecular methods. The goal of this study was to clarify the origin of the European populations of *N. geranii* and the Eurasian biotypes of '*N. cumminsiana*', using mainly molecular methods.

MATERIALS AND METHODS

Molecular phylogenetic studies

The fungal species, host plants, location of collection, and accession numbers for the nucleotide sequence databases (DDBJ, EMBL and GenBank) are provided in Table 1. Isolation of whole-cell DNA was performed using the chelex method (Walsh et al. 1991) as described in Hirata & Takamatsu (1996). The 5'-end of the 28S rDNA, including the domains D1 and D2, and ITS region, including the 5.8S rDNA, were amplified by polymerase chain reaction (PCR) and then sequenced using direct sequencing as described in Takamatsu et al. (2006).

The sequences were initially aligned using the Clustal X package (Thompson et al. 1997). The alignment was then visually refined with MacClade v4.08 (Maddison & Maddison 2005). The alignments were deposited in TreeBASE (www.treebase.org/). Phylogenetic trees were obtained from the data using the maximum parsimony (MP) method in PAUP* 4.0 (Swofford 2001) and Bayesian analysis in MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001). MP analyses were performed with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analyses (Felsenstein 1985) using 1 000 replications with the stepwise addition option set as simple and maximum tree number as 100. BS values 70 % or higher are provided.

For Bayesian phylogenetic analyses, the best-fit evolutionary model was determined for each dataset by comparing different evolutionary models via the Akaike information criterion (AIC) using PAUP* and MrModeltest 2.2 (Nylander 2004). MrBayes was launched with random starting trees for 2×10^6 genera-

Table 1 Sources of *Neoerysiphe* material used for molecular analyses and their accession numbers in DNA databases.

Fungal species	Host	Location; year	Voucher no.[1]	Accession no.[2]
N. galeopsidis	*Galeopsis* sp.	Ukraine, Volhynian region; 2004	KW 33697F / MUMH4657	AB498940
	Lamium amplexicaule	Ukraine, Crimea; 2004	KW 58375F / MUMH 4673	AB498941
	Lamium purpureum	Ukraine, Crimea; 2004	KW 33682F / MUMH 4658	AB498942
	Marrubium praecox	Ukraine, Donetsk region; 2004	KW 58376F / MUMH 4674	AB498943
	Phlomis pungens	Ukraine, Donetsk region; 2004	KW 33698F / MUMH 4659	AB498944
	Phlomis pungens	Ukraine, Donetsk region; 2004	KW 58377F / MUMH 4675	AB498945
	Phlomis tuberosa	Ukraine, Cherkasy region; 2005	KW 33700F / MUMH 4660	AB498946
	Phlomis tuberosa	Ukraine, Donetsk region; 2004	KW 33699F / MUMH 4661	AB498947
	Phlomis tuberosa	Ukraine, Donetsk region; 2004	KW 58378F / MUMH 4676	AB498948
	Prasium majus	Israel, Tel-Aviv; 2004	HAI 4322 / MUMH 4680	AB498949
	Stachys distans	Israel, Carmel Mt.; 2004	HAI 4327 / MUMH 4681	AB498950
N. galii	*Galium aparine*	Israel, Jordan Valley; 2004	HAI 445 / MUMH 4682	AB498951
N. geranii	*Geranium sibiricum* var. *popovii*	Ukraine, Kyiv; 1998	KW 28118F / MUMH 4662	AB498952
	Geranium sibiricum var. *popovii*	Ukraine, Kyiv region; 1998	KW 28121F / MUMH 4663	AB498953
	Geranium sibiricum var. *popovii*	Ukraine, Kyiv region; 1998	KW 28123F / MUMH 4664	AB498954
	Geranium thunbergii	Japan, Hokkaido; 2004	KW 34781F / MUMH 3555	AB498955
	Geranium sp.	Ukraine, Kyiv; 2007	KW 33701F / MUMH 4665	AB498956
N. hiratae	*Cacalia delphiniifolia*	Japan, Ehime; 1998	KW 34784F / MUMH 567	AB498957
	Cacalia hastata ssp. *farfarifolia*	Japan, Nagano; 2004	KW 34785F / MUMH 3504	AB498958
	Ligularia fischeri	Japan, Okayama; 2006	KW 34786F / MUMH 4471	AB498959
	Ligularia stenocephala	Japan, Mie; 2004	KW 34789F / MUMH 3611	AB498960
	Ligularia stenocephala	Japan, Nagano; 2004	KW 34788F / MUMH 3505	AB498961
	Ligularia stenocephala	Japan, Shiga; 2004	KW 34787F / MUMH 3442	AB498962
N. joerstadii	*Phagnalon rupestre*	Israel, Golan Heights; 2004	HAI 4239 / MUMH 4668	AB498976
N. nevoi	*Chondrilla* sp.	Ukraine, Crimea; 2004	KW 58373F / MUMH 4672	AB498963
	Crepis aspera	Israel, Carmel Mt.; 2004	HAI 4164 / MUMH 4667	AB498964
	Crepis aspera	Israel, Northern Negev; 2004	KW 34790F / MUMH 4873	AB498965
	Crepis rhoeadifolia	Ukraine, Crimea; 1978	KW 11753F / MUMH 4655	AB498966
	Crepis rhoeadifolia	Ukraine, Crimea; 1982	KW 11755F / MUMH 4654	AB498967
	Hedypnois cretica	Israel, Northern Negev; 2004	KW 34793F / MUMH 4875	AB498968
	Picris amalecitana	Israel, Mi'ilya; 2004	HAI 4114 / MUMH 4669	AB498969
	Rhagadiolus stellatus	Israel, Northern Negev; 2004	HAI 4329 / MUMH 4670	AB498970
	Rhagadiolus stellatus	Ukraine, Crimea; 2004	KW 58374 / MUMH 4677	AB498971
	Taraxacum sp.	Ukraine, Crimea; 1981	KW 11777F / MUMH 4656	AB498972
	Thrincia tuberosa	Israel, Lower Galilee; 2004	HAI 4123 / MUMH 4678	AB498973
	Tolpis virgata	Israel, Upper Galilee; 2004	HAI 4296 / MUMH 4679	AB498974
N. nevoi var. *scolymi*	*Scolymus hispanicus*	Israel, Carmel Mt.; 2005	HAI 5195 / MUMH 4671	AB498975

[1] Sources: HAI = Haifa University, Herbarium of the Institute of Evolution, Israel; KW = National Herbarium of the M.G. Kholodny Institute of Botany, Kiev, Ukraine; MUMH = Mie University, Mycological Herbarium, Japan.

[2] The nucleotide sequence data will appear in the DDBJ, EMBL, and GenBank databases under the respective accession number.

tions and the Markov chains were sampled every 100 generations, which resulted in 2×10^4 sampled trees. To ensure that the Markov chain did not become trapped in local optima, we used the MCMCMC algorithm, performing the estimation with four incrementally heated Markov chains. Bayesian posterior probability (PP) values 0.95 or higher are shown.

Morphological analysis

Powdery mildew specimens involved in the morphological analysis are listed after each description of new taxa. Morphological features of the specimens were examined and photographed using light microscopes MBI-6 (LOMO, Russia) with objectives ×16 and ×40 (Carl Zeiss, Germany) in phase contrast and Primo Star (Carl Zeiss, Germany) with objectives ×10 and ×40. Photographs were prepared by the digital cameras EOS 350D and PowerShot A640 (both Canon, Tokyo, Japan), accordingly. Shrivelled conidiophores, conidia and superficial hyphae were restored by heating to start of boiling in 40 % lactic acid. Only dry chasmothecia on host leaves were measured. For each morphological feature 30 structures were measured and the data processed statistically. Limits of variation were determined as M ± 1.96 σ, where M is a simple average and σ is a standard deviation. The SEM micrographs were obtained with a Jeol JSM–6060LA (Tokyo, Japan) SEM microscope. Dry pieces of leaf with mycelium, conidia, and ascomata were glued to metallic stubs and gold coated under vacuum. The specimens examined are deposited at HAI, HUJ, KW, MUMH, and TNS (abbreviations according to Holmgren et al. (1990)).

RESULTS

ITS phylogeny

Thirty-five ITS sequences of *Neoerysiphe* spp. were newly determined in this study (Table 1). These sequences were aligned with 30 sequences of *Neoerysiphe* spp. and three sequences of *Arthrocladiella mougeotii* used as an outgroup taxon. The dataset consisted of 68 sequences and 523 characters. All characters were aligned unambiguously. Of the 523 characters, 119 were variable and 104 characters were phylogenetically informative for parsimony analysis. A total of 58 100 equally parsimonious trees with 187 steps (CI = 0.775, RI = 0.961, RC = 0.745) were generated by the MP analysis, when it had to be terminated due to the limit of memory size of the software. One of the trees is shown in Fig. 1. We also performed parsimony ratchet analysis (Nixon 1999) using PAUP* and PAUPRat v1 (Sikes & Lewis 2001) and confirmed the generation of almost identical tree topologies with the same tree length. Thus, we concluded that the tree shown in Fig. 1 is not the result of a local optimum. MrModeltest selected SYM+I+G model as the best for this dataset. Bayesian analysis was performed using this evolution model and yielded 2×10^4 trees. Of the trees, the first 14 130 were discarded (burn-in) because the average standard deviation of the split frequencies (ASDSF) dropped below 0.01. The remaining 5 870 trees were summarised in a majority-rule consensus tree, yielding the probability of each clade being monophyletic. The tree topology by the Bayesian analysis was almost identical to the MP tree, and thus the former tree is not shown.

The 65 sequences of *Neoerysiphe* analyzed in this study were divided into three large clades (A, B and C) clearly defined by their geographical distributions and host plants. Clade A consisted of a single species, *N. galeopsidis*, and is supported strongly with both BS and PP values (BS = 90 %; PP = 0.90). Hosts of this species mostly belong to the *Lamiaceae*, but *Acanthus* (*Acanthaceae*) and *Catalpa* (*Bignoniaceae*) are also included in this clade as hosts. Maximum genetic divergence within this clade is only 0.8 %, which suggests that *N. galeopsidis* diverged on the *Lamiaceae* and sporadically infected other plant families recently. Clades B and C formed a larger clade (BS = 80 %; PP = 0.99). Clade B (BS = 75 %; PP = 1.0) consists of hosts of the *Asteraceae*, and one specimen from both *Aloysia* (*Verbenaceae*) and *Aristotelia* (*Elaeocarpaceae*) collected in North and South America and Japan. This clade is further divided into four subclades. B1 contains *Oidium baccharidis* on *Baccharis* (tribe *Astereae*, *Asteraceae*) and B3 contains *O. aloysiae* on *Aloysia*, both collected in Argentina. B2 consists of *O. maquii* on *Aristotelia*, *N. cumminsiana* on *Bidens* (tribe *Heliantheae*, *Asteraceae*) and *Eupatorium* (tribe *Eupatorieae*, *Asteraceae*), and *Oidium* sp. on *Galinsoga* (tribe *Millerieae*, *Asteraceae*) obtained from the USA and South America (BS = 91 %; PP = 0.99). B4 (BS = 99 %; PP = 1.0) comprises seven sequences of *Neoerysiphe* on *Cacalia* and *Ligularia* (tribe *Senecioneae*, *Asteraceae*) collected in Japan. These seven sequences are identical to each other. This fungus has been identified as *N. cumminsiana* (Nomura 1997, Takamatsu et al. 2008), but the present analysis indicates that the fungus forms an independent lineage different from *N. cumminsiana* collected in North and South America. Clade C (BS = 74 %; PP = 0.89) comprises *Neoerysiphe* spp. collected in Eurasia, especially in Mediterranean and circum Mediterranean areas like the north part of Israel and the south part of Ukraine, and is further divided into four subclades. C1 consists of a single sequence of a fungus on *Phagnalon rupestre* (tribe *Gnaphalieae*, *Asteraceae*) collected in Israel. The same sequence was obtained when the sequencing of the DNA extraction from this specimen was repeated. Subclades C2 and C3 consisted of *N. galii* on *Galium* spp. (*Rubiaceae*) and *N. geranii* on *Geranium* spp. (*Geraniaceae*), respectively. Both clades were strongly supported by BS and PP values (BS = 100 %; PP = 1.0 in both C2 and C3). All these specimens were collected in Europe, except for specimens of *N. geranii* collected in Japan and one sample of *N. galii* from Israel. C4 consisted of 13 sequences from fungi on tribe *Cichorieae* of the *Asteraceae*, collected in Israel and Ukraine. There was some genetic divergence within this clade. Subclades C3 and C4 formed a clade with strong support (BS = 56 %; PP = 1.0).

28S phylogeny

Thirty-one 28S rDNA sequences including D1/D2 domains of *Neoerysiphe* spp. were newly determined in this study (Table 1). These sequences were aligned with 21 sequences of *Neoerysiphe* spp. and two sequences of *Arthrocladiella mougeotii* used as an outgroup taxon. The dataset consisted of 54 sequences and 650 characters. All characters were aligned unambiguously. Of the 650 characters, 65 were variable and 51 characters were phylogenetically informative for parsimony analysis. A total of 14 equally parsimonious trees with 87 steps (CI = 0.770, RI = 0.944, RC = 0.727) were generated by the MP analysis. Of these 14 trees, a tree with the highest likelihood value is shown in Fig. 2. MrModeltest selected GTR+I model as the best for this dataset. Bayesian analysis using this evolution model yielded 2×10^4 trees. Of these, the first 7 020 were discarded (burn-in) because ASDSF dropped below 0.01. The remaining 12 980 trees were summarised in a majority-rule consensus tree, yielding the probability of each clade being monophyletic. The tree topology by the Bayesian analysis was almost identical to the MP tree, and thus the former tree is not shown.

The tree constructed by the 28S rDNA dataset strongly supported the phylogeny of *Neoerysiphe* shown in the ITS tree. *Neoerysiphe* sequences analysed in this study were divided

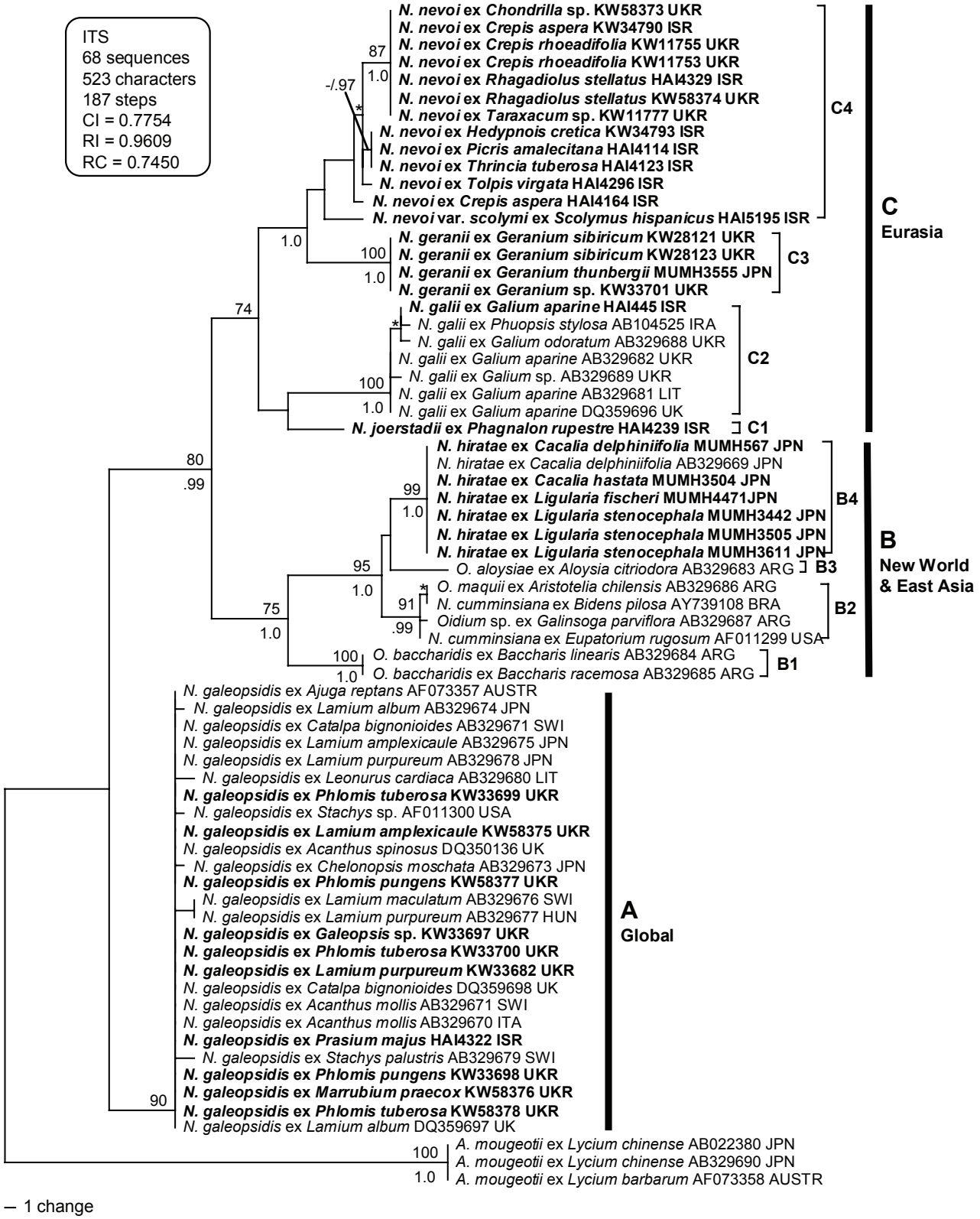

Fig. 1 Phylogenetic analysis of the nucleotide sequences of the ITS region including 5.8S rDNA for 68 sequences from *Neoerysiphe* with *Arthrocladiella* used as outgroup taxon. The tree is a phylogram of one of the 58 K MP trees with 178 steps obtained by a heuristic search employing the random stepwise addition option of PAUP*. Gaps were treated as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. Percentage BS support (1 000 replications; ≥ 70 %) and PP (≥ 0.95) are shown on and under branches, respectively. Nodes with asterisks (*) denote that the nodes collapsed in the strict consensus tree.

into three large clades (A, B, and C), although clade B was collapsed in the strict consensus tree. Subclades B1, B2, B3, B4, C1, C2, C3, and C4 are also supported in the 28S tree, although BS and PP supports were lower than those in ITS tree. Subclades C3 and C4 formed a clade with strong support (BS = 85 %; PP = 0.99).

Morphology

Mycelium of samples belonging to subclade B4 was well developed, especially along the veins of the leaves (Fig. 3a). Primary hyphae had distinct appressoria. The secondary mycelium appeared simultaneously with chasmothecium initials immediately following the sexual reproduction. This mycelium consisted of thin hyaline hyphae that later surrounded chasmothecia as an

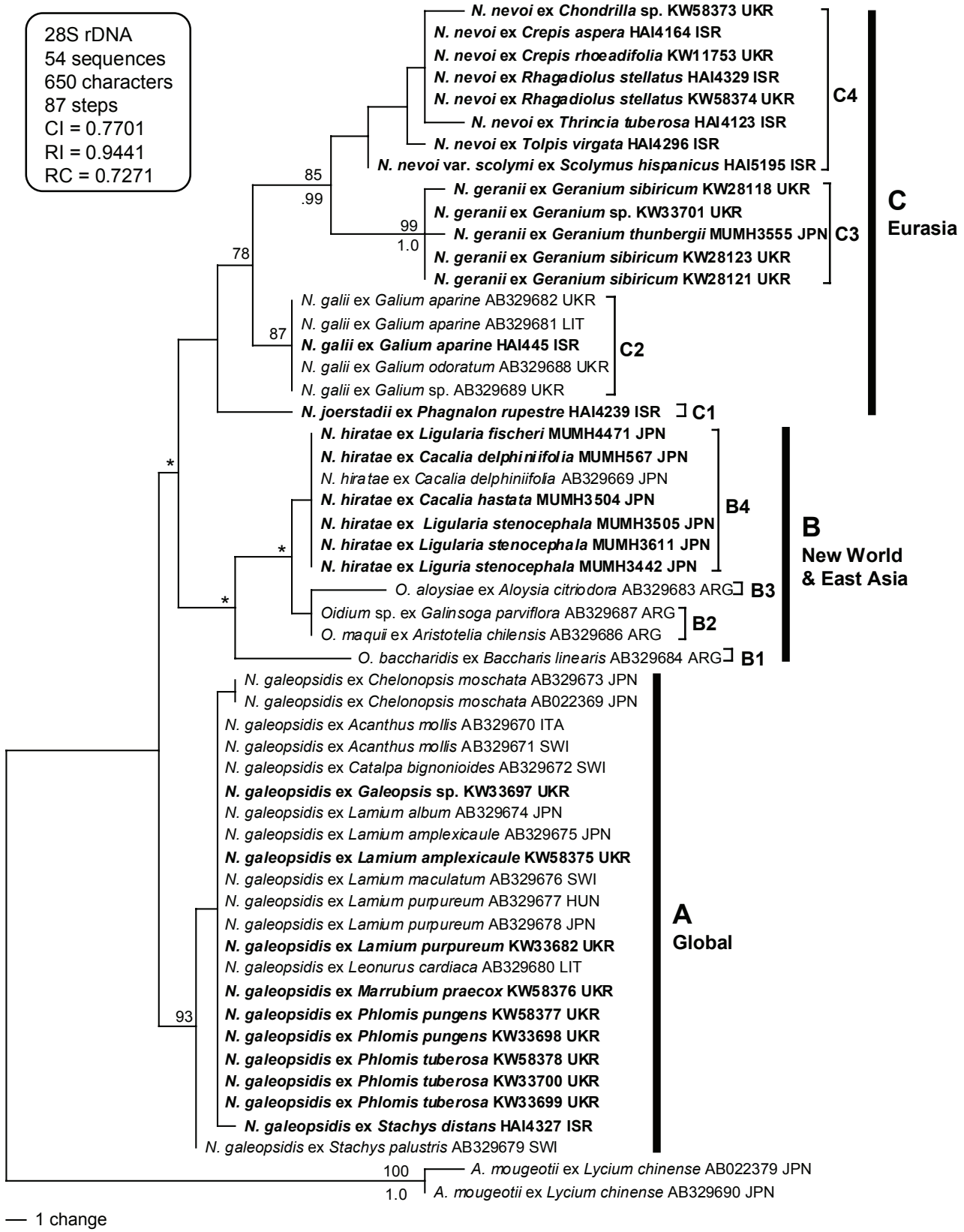

Fig. 2 Phylogenetic analysis of the divergent domains D1 and D2 sequences of the 28S rDNA for 54 sequences from *Neoerysiphe* with *Arthrocladiella* used as outgroup taxon. The tree is a phylogram of the tree with the highest likelihood score among the 14 MP trees with 87 steps, which was obtained and constructed as described for Fig. 1. **Bold** lines denote branches present in the strict consensus tree. Nodes with asterisks (*) denote that the nodes collapsed in the strict consensus tree.

interlacing delicate or somewhat denser web (Fig. 3b, g). It should be noted that both mycelia are pure white without any yellowish or brownish tints. In contrast to these fungi, samples of subclade C4 had yellowish primary mycelium which gave rise to white secondary mycelium occasionally with somewhat yellowish hyphae. Mycelium of the fungus on *Phagnalon rupestre* (C1) was very weakly developed, almost invisible, greyish and only confined to primary mycelium. *Hyphal appressoria* in all groups were variable, of similar size, unlobed or somewhat lobate (Fig. 3c, d and 5a–c). *Anamorphs* were observed only in subclades B4 and C4. *Conidiophores* were very similar morphologically, with straight, cylindrical foot-cells, frequently increasing from

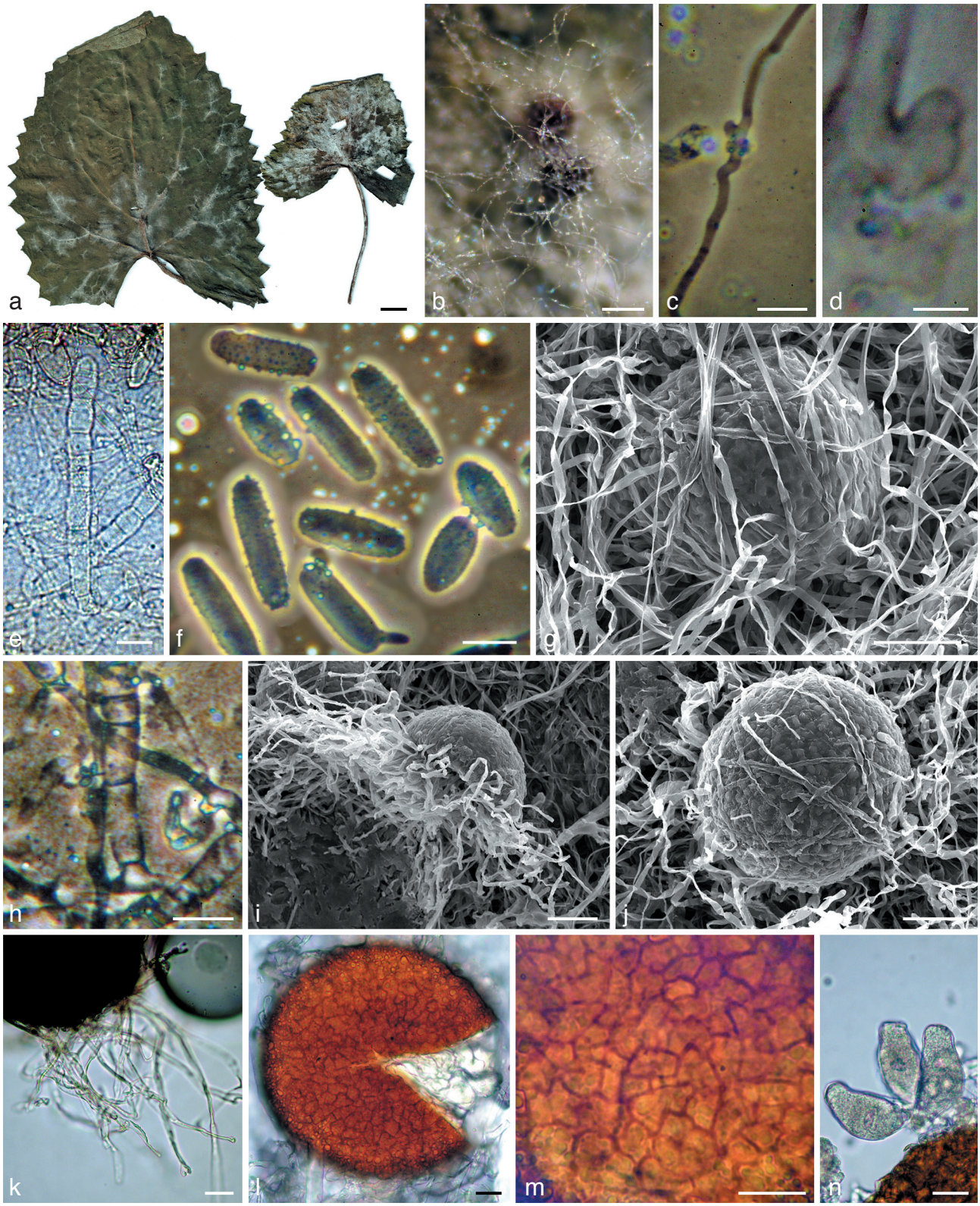

Fig. 3 Morphology within phylogenetic subclade B4. a, b, g, i–n: *Neoerysiphe hiratae* (isotype, KW 34787F) on *Ligularia stenocephala*; c–f, h: *N. hiratae* (KW 34783F) on *L. delphiniifolia*. a. The infected host; b. chasmothecia in reflected light covered by the secondary mycelium; c, d. hyphae of the primary mycelium with appressoria; e. conidiophores; f. conidia; g, i, j. chasmothecia viewed by scanning electron microscope: g, j – covered by hyphae of the secondary mycelium, i – side view; h. basal part of conidiophore; k. chasmothecial appendages; l. chasmothecium viewed by light microscope; m. peridial cells; n. asci. — Scale bars: a = 1 cm; b = 100 µm; c, e, f, h, k–n = 20 µm; d = 5 µm; g, i, j = 50 µm.

base to top, 24–45.5 × 9.5–12.5 µm, followed by 1–2 shorter cells and conidium initials (Fig. 3e, h and 5e). However, in subclade C4 the foot-cells were occasionally very long, up to 100 µm. *Conidia* in subclade B4 were catenate, mainly cylindrical with rounded ends, often oblong ellipsoidal, long, up to 48 µm (Fig. 3f), whereas in C4 they were mainly ellipsoidal or short cylindrical with rounded ends, often almost limoniform, short, up to 36 µm (Fig. 5f); the length/width ratios were 1.9–3.5 (average 2.7) and 1.5–2.5 (average 1.9), respectively. *Chasmothecia* of all specimens studied were hemispherical, depressed or even concave in the lower part (Fig. 3i, 4c, and 5i) but on *Phagnalon rupestre* (C1) they were more flattened and distinctly larger, up to 200 µm diam. This contrasted with the other specimens, e.g. subclades B4 and C4 having chasmothecial diameters

mainly up to 157 µm and 148 µm, respectively. The fungus on *Phagnalon* also had a rather transparent *peridium*, enabling the number of asci to be easily viewed and counted whilst still within the chasmothecium (Fig. 4d). Peridial cells of this fungus were obscure, polygonal or irregular in shape and small, 11–17(–33) × 10–14(–16) µm. The peridial surface was indistinctly close-meshed or knobbly (Fig. 4b, c), whilst in subclades B4 and C4 the peridium was less transparent with asci invisible within the chasmothecium. Peridial cells in subclade B4 were, however, more visible and more regular in shape than those of the *Phagnalon* parasite, but similarly small. Peridial cells in subclade C4 were distinguished from those in C1 and B4 by being distinct and perceptibly larger, up to 30 × 17 µm. The peridial surface also differed in being distinctly meshed and similar to the other samples in this group (Fig. 5i, j) with the exception of the fungus on *Scolymus hispanicus*. This fungus has chasmothecia with a deeply pitted peridium where cell junctions formed conspicuous ridges (Fig. 5k). *Appendages* of all specimens studied were well developed but very short and hyaline in chasmothecia on *Phagnalon rupestre*. In subclade B4 appendages were also hyaline and only in one specimen they were somewhat yellowish. All those in C4 were more or less pigmented. *Asci* in all three clades B4, C1, and C4 were similar in shape, mainly obpyriform, stipitate, and immature in the current season but they were more oblong on *Phagnalon* and more numerous, 16–32 per chasmothecium, in contrast to subclades B4 and C4 where the number of asci did not exceed 12 (compare Fig. 4g with Fig. 3n and 5n–q).

Morphological analysis indicated that the fungi belonging to subclades B4, C1 and C4 had obvious differences and must be treated as separate species. This conclusion fully agreed with the results of our phylogenetic analysis. The type specimen of *N. cumminsiana*, another *Neoerysiphe* species parasitizing the *Asteraceae*, was also included in the morphological analysis. In this specimen secondary mycelium was also formed, but it is barely visible and appressed to the substrate. Chasmothecia were large like those on *Phagnalon* but differed in having a unique structure, characterised by a clearly visible basal evagination up to 27 µm height (Fig. 4h–j). Such a feature is unknown in any other *Neoerysiphe* species. Although the peridium of *N. cumminsiana* was also transparent and the chasmothecia were large like those on *Phagnalon*, the asci were fewer, mainly 10, and notably larger, 50–57 × 31–35.5 µm. Thus, all the studied samples of Eurasian *Neoerysiphe* parasitizing *Asteraceae* differed from *N. cumminsiana* morphologically and so do undoubtedly not belong to this species. An attempt to sequence the type specimen of *N. cumminsiana* failed.

Results of the phylogenetic analysis indicated that subclades C4 and C3 (*N. geranii*) were sister groups. Their propinquity was confirmed by morphological examinations of these fungi, including the type specimen of *N. geranii*. However, subclade C4 had a more developed secondary mycelium, a conidial surface with larger number of lengthwise striations, a less transparent peridium, and asci more ellipsoidal than obpyriform (compare Fig. 5h–q with 4l–o).

Neoerysiphe on *Phagnalon* (C1) differs strongly from all known *Neoerysiphe* species, first of all, by its large chasmothecia with numerous somewhat elongated asci. Powdery mildews in subclade B4 are allied to American *N. cumminsiana* but they differ in having smaller fruiting bodies, chasmothecium being concave in the lower half, without evagination and aerial secondary mycelium, not appressed to the substrate. Morphologically, this group seems to be closer to subclade C4 but is distinguished by long cylindrical conidia, white secondary mycelium, and a less sculptured chasmothecial surface.

Taxonomy

Phylogenetic and morphological analyses have indicated that all three groups of Eurasian *Neoerysiphe* specimens parasitizing *Asteraceae* do not correspond to *N. cumminsiana* or any other known species of this genus, i.e. they have to be considered separate, new species, which are described as *N. hiratae* from Japan, *N. joerstadii* from Israel, and *N. nevoi*, including its variety *scolymi*, mainly from the Mediterranean region.

Neoerysiphe hiratae Heluta & S. Takam., *sp. nov.* — MycoBank MB513278; Fig. 3

Anamorph. Oidium subgenus *Striatoidium.*

Species nostra *Neoerysiphe cumminsianae* affinis est tamen mycelio secundario albo et tomentoso, conidiis longis, chasmotheciis minoribus et basi chasmothecii protuberatione carens bene differt.

Etymology. Named in honour of the famous Japanese mycologist Koji Amano (Hirata).

Mycelium amphigenous, often more developed along the veins of leaves, also caulicolous and on petioles, at first forming patches, then confluent. *Primary mycelium* thin, greyish, hyphal diam 5–6(–11) µm. *Secondary mycelium* arising from primary hyphae, pure white, hyphae smooth, without appressoria, 5–6 µm diam, forming a delicate or thick and tomentose web around ascomata. *Hyphal appressoria* very variable in shape and size, distinct, unlobed or slightly lobate, frequently in pairs, 7–10 × 4.5–6.5 µm. *Conidiophores* straight or sometimes arcuate, 112–154 µm, foot-cells cylindrical, 24–41 × 11–12 µm, frequently increasing in width towards the tip, followed by one shorter cell and conidial initials. *Conidia* catenate, mainly cylindrical with rounded ends, often oblong ellipsoidal, 27–48 × 11–17.5 µm, length/width ratio 1.9–3.5 (average 2.7). *Chasmothecia* scattered, hemispherical, depressed in the lower part, with an indistinct close-meshed or often knobbly peridial surface, (102–)105–153(–157) µm diam. Peridial cells polygonal or irregular, small, 9–20 × 5–12 µm. *Appendages* numerous, in the basal part of the chasmothecium, mycelioid, well developed, 0.5–2 times as long as the chasmothecial diam, 5–6 µm wide, hyaline, rarely somewhat brownish. *Asci* 7–12 per chasmothecium, immature, oblong ellipsoid, obpyriform, with an irregular outline, wide in the lower part and abruptly narrowed in the upper part, 46–57 × 21–30 µm, short-stalked, ascospores not developed before overwintering.

Specimens examined. Japan, Shiga, Mt Ibuki, on *Ligularia stenocephala* (Maxim.) Matsum. & Koidz. (*Asteraceae*), 7 Nov. 2004, S. *Takamatsu*, holotype TNS F-25684, isotype KW 34787F, MUMH 3442, rDNA sequence ex-type AB498962; Echime, Mt Ishiduchi, on *Cacalia delphiniifolia* Siebold & Zucc., 9 Nov. 1998, S. *Takamatsu*, MUMH 552, KW 34783F; Mt Bingamori, on *C. delphiniifolia*, 10 Nov. 1998, S. *Takamatsu*, MUMH 567, KW 34784F; Nagano, Kamikouchi, on *C. hastata* L. ssp. *farfaraefolia*, 3 Sept. 2004, *S. Takamatsu*, MUMH 3504, KW 34785F; Okayama, Kagamino Town, Forest Park, on *Ligularia fischeri* Turcz., 2 Nov. 2006, S. *Takamatsu*, MUMH 4471, KW 34786F; Mie, Mt Nonobori, on *L. stenocephala*, 21 Nov. 2004, S. *Takamatsu*, MUMH 3611, KW 34789F; Nagano, Kamikouchi, on *L. stenocephala*, 4 Sept. 2004, S. *Takamatsu*, MUMH 3505, KW 34788F.

Neoerysiphe joerstadii Heluta & S. Takam., *sp. nov.* — MycoBank MB513279; Fig. 4a–g

Anamorph. Not observed.

Species nostra *Neoerysiphe cumminsianae* similis est tamen absentia mycelii secundarii, numero ascorum majoribus, 16–32 in chasmothecio, ascis longioribus et basi chasmothecii protuberatione carens bene differt.

Etymology. Named in honour of the famous Norwegian mycologist Ivar Jørstad.

Primary mycelium amphigenous, very sparse, inconspicuous. *Secondary mycelium* absent. *Appressoria* obscure, unlobed or

slightly lobate. *Anamorph* not observed. *Chasmothecia* scattered, hemispherical, very depressed in the lower part, with an indistinct close-meshed or knobbly peridial surface, (118–)125–171(–200) µm diam and 92–97 µm high. Peridial cells obscure, polygonal or irregular, small, 11–17(–33) × 10–14(–16) µm. *Appendages* numerous, in the basal part of the chasmothecium, mycelioid, 0.5–1 times as long as the chasmothecial diam, 4–6 µm wide, always hyaline, somewhat rough, interlaced with host fibres. *Asci* numerous, 16–32 per chasmothecium, oblong ellipsoid, with irregular outline when immature, increased in the lower part and narrowed in the upper part, 42–60 × 20–31 µm, stalked, ascospores not developed before overwintering.

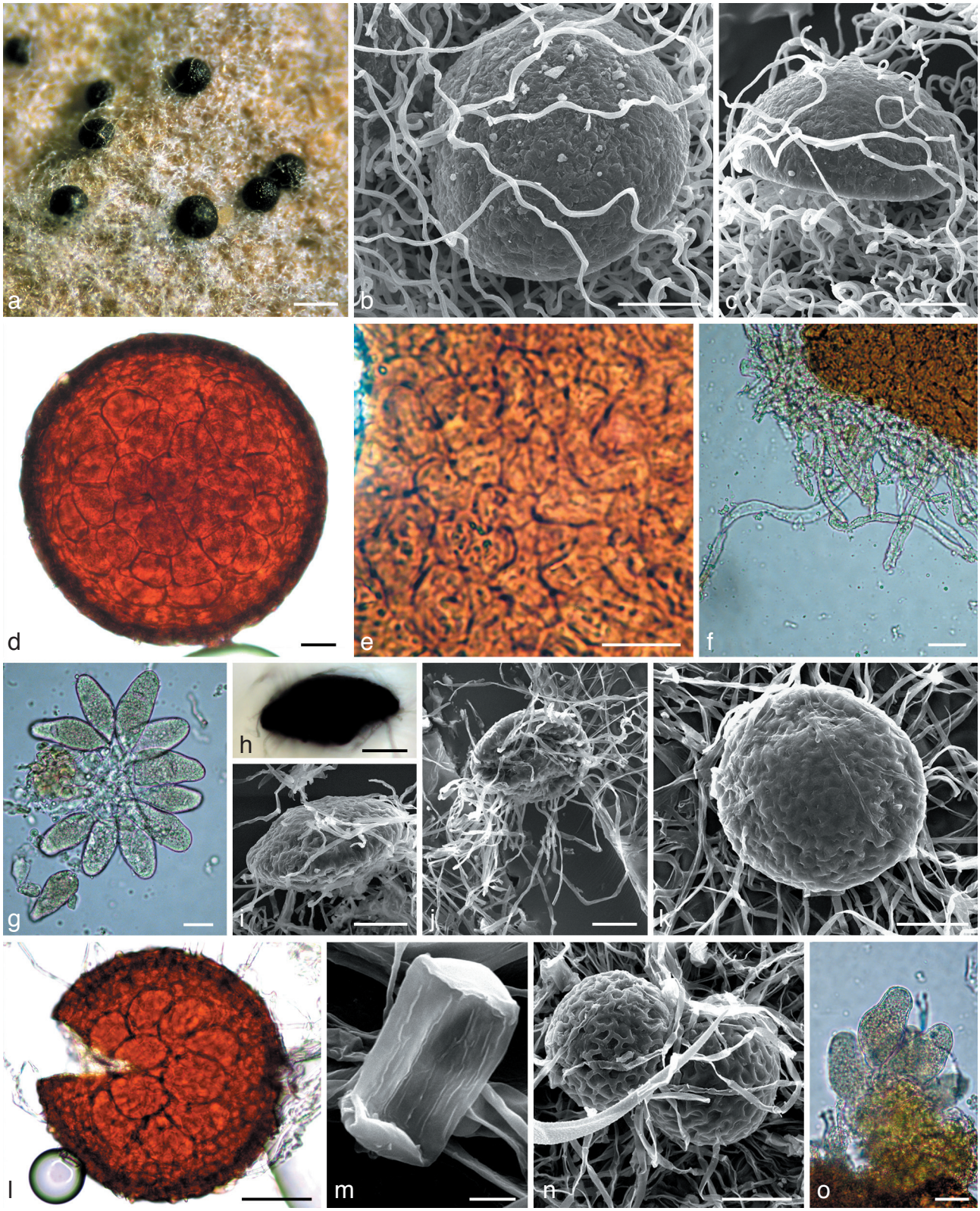

Fig. 4 Morphology within phylogenetic subclades C1, B2 and C3. a–g: *Neoerysiphe joerstadii* (holotype, KW 35717F, subclade C1) on *Phagnalon rupestre*; h–l: *N. cumminsiana* (isotype, HAL 1462F, subclade B2) on *Senecio seemannii*; m–o: *N. geranii* (KW 34782F, subclade C3) on *Geranium* sp. a. Chasmothecia in reflected light; b, c. chasmothecia viewed by scanning electron microscope: c – side view; d. chasmothecium in transmitted light; e. peridial cells; f. chasmothecial appendages; g. asci; h. chasmothecium with evagination on the lower side, side view; i–k. chasmothecia viewed by scanning electron microscope: i – side view, j – bottom view; l. chasmothecium in transmitted light; m. conidium with longitudinal ridges; n. chasmothecia; o. asci. — Scale bars: a = 200 µm; b–d, h–l, n = 50 µm; e–g, o = 20 µm; m = 5 µm.

Specimens examined. Israel, Golan Heights, Yehudiyya, 32°56'N, 35°41'E, on *Phagnalon rupestre* (L.) DC. (*Asteraceae*), 17 May 2004, *S. Voytyuk*, holotype KW 35717F, isotypes HAI 4239, 4245, KW 34794F, 34795F, MUMH 4668, rDNA sequence ex-type AB498976; Upper Galilee, Mt Meron, Nahal Keziv, on *Phagnalon rupestre*, 18 Mar. 2002, *T. Andrianova*, KW 35716F; Zefat (= Safed), 22 Aug. 1953, T. *Rayss*, HUJ 301/111 147S.

Neoerysiphe nevoi Heluta & S. Takam., *sp. nov.* — MycoBank MB513280; Fig. 5

Anamorph. Oidium subgenus *Striatoidium*.

Species nostra *Neoerysiphe geranii* affinis est tamen mycelio secundario bene evoluto, conidiis magis striatulis, peridio minus translucenti, ascis magis ellipsoideis et minus pyriformibus differt.

Etymology. Named in honour of the famous Israeli biologist Eviatar Nevo.

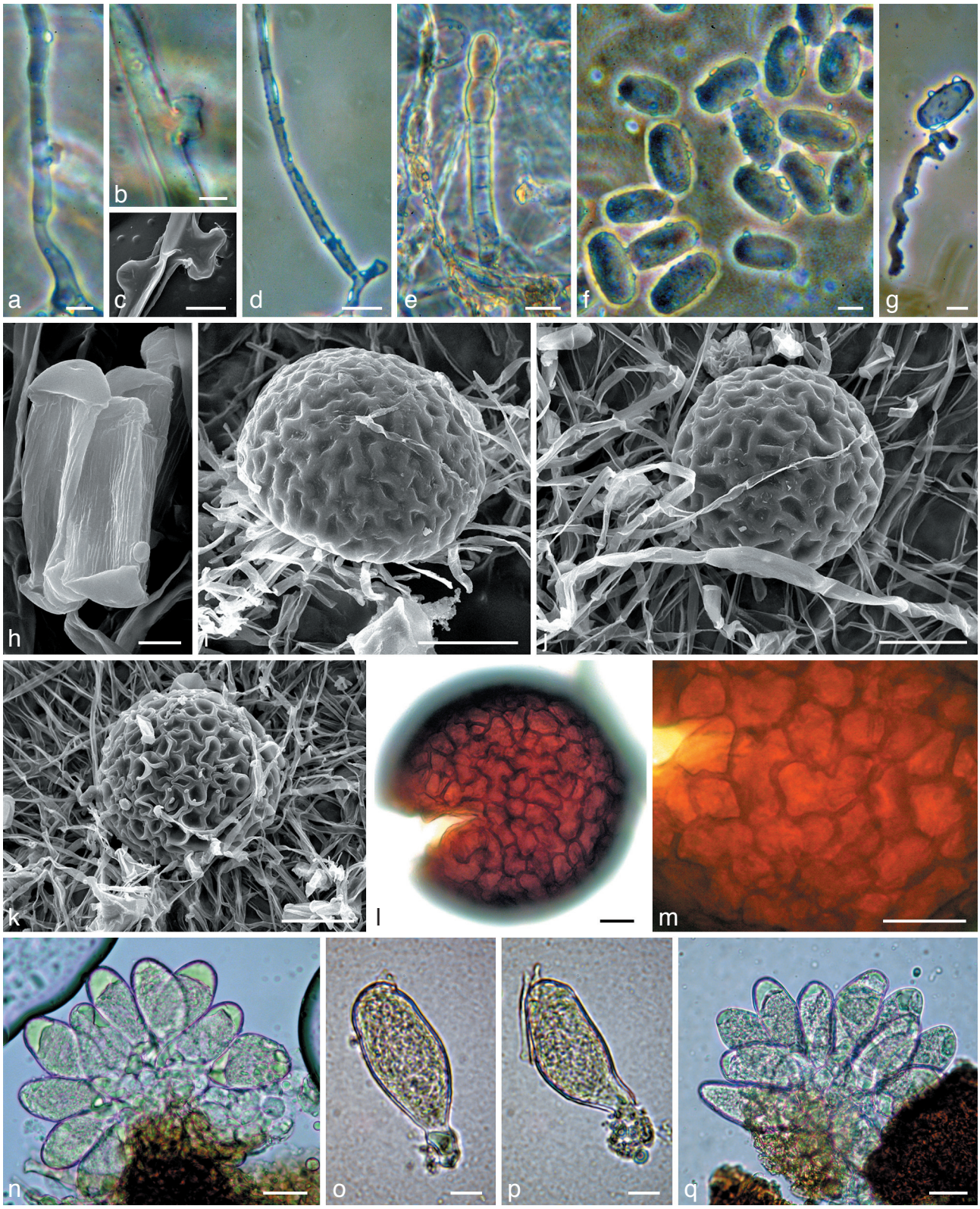

Fig. 5 Morphology within phylogenetic subclade C4. a, b, d–g, q: *Neoerysiphe nevoi* (KW 35726F) on *Thrincia tuberosa*; c, h–j, l–p: *N. nevoi* (holotype, KW 34802F) on *Tolpis virgata*; k: *N. nevoi* var. *scolymi* (holotype, KW 34800F) on *Scolymus hispanicus*. a–c. Hyphae of the primary mycelium with appressoria; d. secondary hypha arisen from the primary hypha; e. conidiophore; f–h. conidia; g. germinated conidium with a hypha extending from a lobed appressorium of the *Striatoidium* type; i–k. chasmothecia viewed by scanning electron microscope; l. chasmothecium viewed by light microscope; m. peridial cells; n–q. asci. — Scale bars: a, f, g, o, p = 10 µm; b, c, h = 5 µm; d, e, l–n, q = 20 µm; i–k = 50 µm.

Mycelium amphigenous, also caulicolous, effuse. *Primary mycelium* thin, yellowish, hyphal diam 4.5–7.5 µm. *Secondary mycelium* arising from primary hyphae, white to brownish, hyphae smooth, without appressoria, 4.5–6.5 µm diam, surrounding ascomata mainly as a delicate web, persistent or evanescent. *Hyphal appressoria* on primary mycelium variable in shape and size, distinct, unlobed or slightly lobate, 6.5–8.5 × 4–5 µm. *Conidiophores* straight or somewhat arcuate, 125–165 µm, foot-cells cylindrical, 28.5–45.5(–100) × 9.5–12.5 µm, frequently increasing in width towards the tip, followed by 1–2 shorter cells and conidial initials. *Conidia* catenate, mainly ellipsoidal or short cylindrical with rounded ends, often almost limoniform, 23.5–36 × 11–17.5 µm, length/width ratio 1.5–2.5 (average 1.9). Conidial germ tube with a lobed appressorium typical of the *Striatoidium* type as defined by Cook & Braun (2009). *Chasmothecia* scattered, hemispherical, depressed in the lower part, with a distinctly meshed peridial surface, (90–)94–138(–148) µm diam. Peridial cells rather distinct, polygonal, rounded or irregular in shape, 11–30 × 11–17 µm. *Appendages* numerous, in the basal part of the chasmothecium, mycelioid, well developed or occasionally poorly developed, 0.5–2 times as long as the chasmothecial diam, 5.5–6.5(–8) µm wide, somewhat rough, brownish, yellow, rarely hyaline. *Asci* 6–12 per chasmothecium, immature, oblong ovoid to obpyriform, with irregular outline, wide in the lower part and narrowed in the upper part, 40.5–51.5 × 21–25 µm, stalked, ascospores not developed before overwintering.

Specimens examined. Israel, Upper Galilee, near 'En Kamonnim, 32°54'N, 35°26'E, on *Tolpis virgata* (Desf.) Bertol. (*Asteraceae*), 2 May 2004, *S. Voytyuk*, holotype KW 34802F; isotypes KW 34803F, MUMH 4679, rDNA sequence ex-type AB498974; Haifa, Mt Carmel, near Institute of Evolution, on *Crepis aspera* L., 22 Apr. 2004, *S. Voytyuk*, HAI 4164, KW 34791F, MUMH 4667; Atlit near Haifa, on *C. aspera*, 5 Apr. 2004, *S. Voytyuk*, HAI 4235, KW 34790F, MUMH 4873; Lower Halilee, Mi'ilya near Ma'a lot, on *C. sancta* (L.) Babc., 25 Mar. 2004, *S. Voytyuk*, HAI 4238, KW 34792F, MUMH *4874*; Carmel Coast, near Atlit, on *Crepis* sp., 19 Mar. 2002, *V. Heluta*, anamorph, KW 35721F; Haifa, on *Crepis* sp., 15 Mar. 2002, *V. Heluta*, KW 35722F; Pardes Hana, on *Crepis* sp., 12 Apr. 2002, *E. Nevo*, KW 35723F; Carmel Coast, near Atlit, on *Hedypnois cretica* (L.) Willd., 27 Apr. 2004, *S. Voytyuk*, HAI 4101, 4102, KW 34793F, 35724F, MUMH 4875; Golan Heights, Avné Etan near Ramat Magshimim, on *Picris altissima* Ledeb. ex Rchb., 2 Mar. 2004, *S. Voytyuk*, anamorph, HAI 4110, 4112, KW 34796F, 34797F; Mi'ilya, on *P. amalecitana* (Boiss.) Eig, 25 Mar. 2004, *S. Voytyuk*, HAI 4113, 4114, KW 34798F, 35725F, MUMH 4669; Northern Negev, near Lahav, on *Rhagadiolus stellatus* Gaertn., 21 Mar. 2004, *S. Voytyuk*, KW 34799F, MUMH 4670; Lower Galilee, Alloné Abba near Qirat Tiv'on, on *Thrincia tuberosa* DC., 18 Apr. 2004, *S. Voytyuk*, HAI 4123, KW 34801F, MUMH 4678; Mt Carmel, Muchraka, on *T. tuberosa*, 17 Mar. 2004, *S. Voytyuk*, HAI 4124, KW 35726F; Upper Galilee, near 'En Kamonnim, on *Tolpis virgata*, 2 May 2004, *S. Voytyuk*, KW 34802F, 34803F, MUMH 4679; Lower Galilee, near Alloné Abba near Qirat Tiv'on, on *T. virgata*, 18 Apr. 2004, *S. Voytyuk*, HAI 4182, KW 35727F. – Ukraine, Autonomous Republic of Crimea, Yalta, Livadia, on *Chondrilla* sp., 22 Aug. 2004, *V. Heluta*, KW 58373F, MUMH 4672; Alupka, on *Crepis micrantha* Czerep., 17 July 1982, *V. Heluta*, KW 11752F; Miskhor, on *C. micrantha*, 8 July 1959, *M. Sokolova*, KW 35720F; Chornomorske urban village, on *C. rhoeadifolia* M. Bieb., 13 July 1982, *V. Heluta*, anamorph, KW 11754F; Chornomorske distr., Daleke village, on *C. rhoeadifolia*, 12 July 1982, *V. Heluta*, KW 11755F; Chornomorske distr., Olenivka village, on *C. rhoeadifolia*, 16 July 1982, *V. Heluta*, KW 11756F; Saky, on *C. rhoeadifolia*, 15 July 1978, *V. Heluta*, anamorph, KW *11753F*; Kherson region, Hola Prystan distr., Black Sea Biosphere reserve, on *C. rhoeadifolia*, 5 July 1978, *V. Heluta*, anamorph, KW 11757F; Odessa region, Mykolayiv distr., Nastasiyivka village, on *C. rhoeadifolia*, 26 Sept. 1977, *V. Heluta*, anamorph, KW 11758F; Autonomous Republic of Crimea, Yalta Nature reserve, Mt Ai-Petri, on *Taraxacum* sp., 27 July 1981, *V. Heluta*, KW 11777, MUMH 4656.

Neoerysiphe nevoi var. ***scolymi*** Heluta & S. Takam., *var. nov.* — MycoBank MB513281; Fig. 5k

Anamorph. *Oidium* subgenus *Striatoidium*.

A typo peridio profunde scrobiculato differt.

Etymology. Named from host plant genus.

This variety differs from the type by a deeply pitted peridial surface, i.e. a clearly visible mesh is formed by high ridges surrounding cell margins.

Specimen examined. Israel, Haifa, Mt Carmel, Nahal Oren, on *Scolymus hispanicus* L. (*Asteraceae*), 31 May 2005, *S. Voytyuk*, holotype KW 34800F, isotype MUMH 4671, rDNA sequence ex-type AB498975.

KEY FOR IDENTIFICATION OF *NEOERYSIPHE* SPECIES PARASITIZING *ASTERACEAE*

1. Mycelium inconspicuous, secondary mycelium absent; chasmothecia rather large, mainly 125–170 µm, up to 200 µm; asci 16–32 per chasmothecium; on *Phagnalon*; in the Mediterranean region . . . . . *N. joerstadii*
1. Mycelium well developed, secondary mycelium present; chasmothecia smaller, usually 95–150 µm, if larger then only about 10 asci per chasmothecium; on other hosts . 2
2. Chasmothecia large, mainly 125–165 µm, with visible evagination on the lower side; on *Senecio* and probably other hosts; in North and South America . . . . . *N. cumminsiana*
2. Chasmothecia smaller, concave in the lower part 3
3. Both primary and secondary mycelia pure white; conidia mainly cylindrical with rounded ends, often oblong ellipsoidal, average length/width ratio of 2.7; chasmothecial appendages hyaline, occasionally somewhat brownish; peridial surface with indistinct meshes or even knobbly; on *Cacalia* and *Ligularia*; in Japan . . . . . *N. hiratae*
3. Primary mycelium yellowish, even somewhat brownish, secondary mycelium mainly white or sometimes faintly pigmented; conidia ellipsoidal or short cylindrical with rounded ends, often somewhat limoniform, average length/width ratio 1.9; appendages brownish, occasionally hyaline; peridial surface with a clear meshes or even deeply pitted; on different members of the *Asteraceae*; mainly in the Mediterranean region . . . . . *N. nevoi*

DISCUSSION

Based on the ITS sequences as well as the 28S rDNA sequences, Takamatsu et al. (2008) reported that *Neoerysiphe* specimens of hosts belonging to the *Asteraceae* are divided into three subgroups, each of which corresponds to different host tribes, viz. *Heliantheae* and *Eupatorieae* (Group 2a), *Senecioneae* (2c), and *Astereae* (2d) (see Fig. 2 in Takamatsu et al. (2008) and Table 2). American and Japanese samples of '*N. cumminsiana*' appeared in different subgroups. Consequently, the authors concluded that '*N. cumminsiana*' could be divided into two different species. The present study confirmed this assumption. Morphological analysis demonstrated that Japanese samples are uniform, differ clearly from the true *N. cumminsiana*, and belong to a separate species here named *N. hiratae*. This species parasitizes *Cacalia* and *Ligularia* (tribe *Senecioneae*, subfamily *Asteroideae*) and is known only from Japan (East Asia). Other new species analysed here were *N. joerstadii* collected on *Phagnalon rupestre* (tribe *Gnaphalieae* in *Asteroideae*) in Israel (West Asia) and *N. nevoi* infecting several hosts belonging to different genera in the tribe *Cichorieae* (in *Cichorioideae*), viz. *Chondrilla*, *Crepis*, *Hedypnois*, *Picris*, *Rhagadiolus*, *Scolymus*, *Taraxacum*, *Thrincia* (= *Leontodon*), and *Tolpis* (Table 2). Thus, Eurasian *Neoerysiphe* species are clearly connected with specific tribes of the *Asteraceae*. Similar close relationships between powdery mildews and their host tribes of the *Asteraceae* were also reported in *Golovinomyces* (Matsuda & Takamatsu 2003).

As mentioned above, Voytyuk et al. (2004) expressed doubt on the correctness of Heluta's (1992) hypothesis about migrations

Table 2 *Neoerysiphe* and *Oidium* subgenus *Striatoidium* species aligned with their hosts in the *Asteraceae* and their geographical regions.

Fungal species	Subfamily	Tribe	Genus	Geographical region
Neoerysiphe				
cumminsiana	*Asteroideae*	*Coreopsideae*	*Bidens*	South America
		Eupatorieae	*Eupatorium*	Norh America
		Senecioneae	*Senecio*	North America
hiratae	*Asteroideae*	*Senecioneae*	*Cacalia* (=*Parasenecio*), *Ligularia*	Eastern Asia
joerstadii	*Asteroideae*	*Gnaphalieae*	*Phagnalon*	Mediterranean
nevoi	*Cichorioideae*	*Cichorieae*	*Chondrilla*, *Crepis*, *Hedypnois*, *Picris*, *Rhagadiolus*, *Scolymus*, *Taraxacum*, *Thrincia* (= *Leontodon*), *Tolpis*	Mediterranean
Oidium				
baccharidis	*Asteroideae*	*Astereae*	*Baccharis*	South America
sp.		*Millerieae*	*Galinsoga*	South America

of powdery mildew fungi because the natural distribution of *N. cumminsiana sensu* Heluta was not in accordance with this hypothesis. However, it is now clear that these authors dealt with a species complex composed of four different species, namely the American genuine *N. cumminsiana*, the Japanese *N. hiratae*, and the Mediterranean taxa *N. joerstadii* and *N. nevoi*. We should note that the description of *N. cumminsiana* in the monograph of Braun (1987) combined characteristics of *N. cumminsiana* and *N. hiratae*. Therefore, only the original description published by Braun (1983) refers to *N. cumminsiana* s.str. *Neoerysiphe nevoi* is currently only known from Israel and Ukraine but this species has probably a much wider distribution. It is very likely that all collections formerly reported as *Erysiphe cumminsiana*, *E. galeopsidis* or *E. galii* on *Asteraceae* from European countries and Africa (Amano 1986, Braun 1987, Gorter 1987) belong to this species. A few years ago we examined all specimens of powdery mildews from the herbarium of Jerusalem University (HUJ, Israel). Many specimens originally identified as *E. cichoracearum* actually belonged to *N. nevoi*. It is possible that numerous records of '*E. cichoracearum'* on hosts belonging to the genera *Crepis*, *Filago*, *Hedypnois*, *Hypochaeris*, *Picris*, and *Rhagadiolus* on the Canary and the Balearic Islands (Jørstad 1962a, b), in Portugal (de Mendonça & de Sequeira 1963, de Sequeira & de Mendonça 1965, de Sequeira 1969, 1975, 1978, 1981), Romania (Sandu-Ville 1967, Bontea 1986), Spain (Durrieu & Mercé 1972), and central Europe (Blumer 1967) also belong to *N. nevoi*. De Sequeira (1978) reported that fruiting bodies of '*Erysiphe cichoracearum*' on *Hedypnois cretica*, *Picris echioides*, *P. hieracioides*, and *Tolpis barbata* collected in Portugal were immature, and the descriptions of other characters of these fungi agree well with those of *N. nevoi.*

We collected *N. joerstadii* only on *Phagnalon rupestre*, but according to Amano (1986), *E. cichoracearum* was recorded on this host and *Ph. saxatile* in France, on the Balearic and the Canary Islands, and in the Spanish Sahara. These specimens very likely belong to *N. joerstadii*. Furthermore, the chasmothecia of this fungus on *Ph. saxatile* from the Balearic and the Canary Islands measured 130–200 µm diam (Jørstad 1962a, b). Such a size range fully conforms to *N. joerstadii*.

As explained above, a powdery mildew on hosts belonging to *Geranium* in Ukraine was identified as *N. geranii* by Heluta (2001). However, since this species was known only from Japan and New Zealand (Amano 1986, Nomura 1997), we compared Japanese and Ukrainian samples including the type specimen. Phylogenetically and morphologically, all specimens were found to be uniform. Thus, the true *N. geranii* was correctly recorded in Ukraine as an invasive species.

In conclusion, molecular and morphological evidence revealed that at least four *Neoerysiphe* species, viz. *N. cumminsiana*, *N. hiratae*, *N. joerstadii*, and *N. nevoi*, are able to infect *Asteraceae*. Some of these fungi, above all *N. joerstadii* and *N. nevoi*, are probably common in the Mediterranean region but have been formerly identified and reported mainly as *E. cichoracearum*. Thus, the identity of powdery mildews collected on the *Asteraceae* in the Mediterranean and adjacent regions needs re-examination in the light of these findings.

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