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Author(s): James D. Lawrey, Javier Etayo, Manuela Dal-Forno, Kendra E. Driscoll, and Paul Diederich
Source: The Bryologist, 118(1):83-92.
Published By: The American Bryological and Lichenological Society, Inc.
DOI: http://dx.doi.org/10.1639/0007-2745-118.1.083
URL: http://www.bioone.org/doi/full/10.1639/0007-2745-118.1.083

Molecular data support establishment of a new genus for the lichenicolous species *Neobarya usneae* (Hypocreales)

James D. Lawrey[1,6], Javier Etayo[2], Manuela Dal-Forno[3], Kendra E. Driscoll[4] and Paul Diederich[5]

[1] *Department of Biology, George Mason University, Fairfax, VA 22030-4444, U.S.A.;* [2] *Navarro Villoslada 16, 3° dcha., E-31003 Pamplona, Navarra, Spain;* [3] *Department of Environmental Science and Policy, George Mason University, Fairfax, VA 22030-4444, U.S.A.;* [4] *Botany & Mycology Section, New Brunswick Museum, 277 Douglas Ave., Saint John, NB, Canada E2K 1E5;* [5] *Musée national d'histoire naturelle, 25 rue Munster, L–2160 Luxembourg, Luxembourg*

Abstract. *Neobarya usneae* Etayo is a relatively uncommon lichenicolous fungus that forms distinctive obpyriform ascomata on species of *Usnea*. The species is one of five known lichenicolous species in *Neobarya*, a genus established in the Clavicipitaceae that contains a variety of mycoparasitic species. The only molecular data for *Neobarya* species available in GenBank are for unidentified *Neobarya* species. We obtained sequences of ITS and nrLSU representing a culture and herbarium specimens of *N. usneae* from New Brunswick, Canada, and from a herbarium specimen of *N. parasitica* (Fuckel) Lowen, the type species of the genus, collected in Luxembourg, to determine the phylogenetic placement of these species. Our results indicate that *N. usneae* is not closely related to the type of *Neobarya* in the Clavicipitaceae, but is instead a member of the Hypocreaceae, the first lichenicolous species known for certain from this Hypocrealean family. Based on these results, we are now establishing a new genus, *Lichenobarya*, for *N. usneae* in the Hypocreaceae, and encouraging further study of other *Neobarya* species to establish their phylogenetic relationships, given the potential for genetic heterogeneity in the group.

Keywords. Ascomycetes, fungi, mycoparasitism, phylogenetics, nomenclature, taxonomy.

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The genus *Neobarya* Lowen was established in Eriksson & Hawksworth (1986) for fungi in the Clavicipitaceae characterized by production of soft, superficial, light-colored sessile ascomata on a pseudoparenchymatous stroma or in a subiculum, unitunicate, cylindrical or narrowly clavate asci with an enlarged thickened apical cap penetrated by a pore, and filiform ascospores, often flexuous, hyaline, guttulate, or aseptate. A variety of different asexual states are known (Candoussau et al. 2007). The genus as presently listed in Mycobank includes 13 species, and all are relatively host-specific parasites of lichens and nonlichenized fungi. Five species are lichenicolous (Lawrey & Diederich 2003; http://www.lichenicolous.net/): *N. ciliaris* Etayo on *Heterodermia*, *N. darwiniana* Etayo on *Nephroma antarcticum*, *N. lichenophila* (Ferd. & Winge) Lowen & Samuels on *Cladonia*, *N. peltigerae* Lowen, Boqueras & Gómez-Bolea on *Peltigera* and *N. usneae* Etayo on *Usnea*. The phylogeny of the genus has never been studied using molecular data, and
the placement of lichenicolous and mycoparasitic species is therefore not known. The only data representing *Neobarya* in GenBank are two sequences of unidentified species (EF160121: *Neobarya* sp. GJS 06-171; AY346293: *Neobarya* sp. *Buck 26786*), neither of which represents the type species *N. parasitica* (Fuckel) Lowen.

Etayo (2002) named *Neobarya usneae* provisionally in that genus because of some similarities with other lichenicolous species of *Neobarya*, such as the shape of perithecia, cylindrical asci, multiseptate, filiform ascospores and wall reaction with I; however, differences in ascus tip were also pointed out. Later, Candoussau et al. (2007) noted these same differences and arrived at the conclusion that "this is not a species of *Neobarya*." To test this hypothesis, we used recently collected specimens of *Neobarya usneae* from maritime Canada, from which

[6] Corresponding author's e-mail: jlawrey@gmu.edu
DOI: 10.1639/0007-2745-118.1.083

0007-2745/15/$1.15/0

we obtained one culture and several sequences. Since our preliminary analyses indicated the sequences do not cluster in the Clavicipitaceae, we furthermore studied fresh material of the type of *Neobarya*, *N. parasitica*, from which additional sequences were obtained. Our analyses of these sequences indicated that *N. usneae* is not closely related to *N. parasitica* and therefore represents a new genus, the formal description of which is provided in this paper.

Materials and Methods

Specimens studied, anatomical methods and isolation of cultures. Fresh specimens of *Neobarya usneae* were collected in New Brunswick, Canada, by one of us (KD) and by W. R. Buck. Since the publication of its description, the species has also been collected from several countries in South America, but no molecular data had been obtained from them. A single collection of *N. parasitica* from Luxembourg was provided by G. Marson. Herbarium specimens are deposited in NY, BR, LPB, NBM and in the private collections of P. Diederich and J. Etayo.

Cultures could not be obtained for *N. parasitica*, but a single culture representing *Neobarya usneae* was obtained from herbarium material (*Buck 61451*) following methods of Lawrey (2002). Ascomata were washed in 70% ethanol, dried on a glass slide and crushed in sterile water. Ascospores and ascomatal tissues were then collected in water and plated onto potato dextrose agar (PDA) or malt extract agar (ME, Difco, Detroit, Michigan, USA). Germination of ascospores, or emergence of hyphae from ascomatal tissues, was observed within days, and mycelial outgrowths were isolated after two weeks for liquid culture in ME. A sample was sent to the Fungal and Yeast Collection, Centraalbureau voor Schimmelcultures and is accessioned as CBS 137512. Approximately 2 μg dry mycelial mass was harvested from liquid cultures after two weeks and extracted for DNA analysis.

Molecular data. Genomic DNA was extracted from either a 0.5 cm^2 piece of ascomatal tissue or 2 μg dry mycelial mass using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogene, Illkirch, France) according to the manufacturer's protocol. About 10 ng of extracted DNA were subjected to a standard PCR in a 20 mL reaction volume using Taq Gold polymerase (Applied Biosystems, Foster City, CA), also according to manufacturer's protocols, with the objective of amplifying the internal transcribed spacer (ITS) and nuclear large subunit (nrLSU) rDNA. The products were purified with magnetic beads (Agencourt Bioscience, Beverly, MA) and the purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The sequencing reactions were then purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, MO), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems). The data collected were analyzed using ABI software, and the sequences were then assembled together with the software Sequencher version 5.0 (Gene Codes, Ann Arbor, MI) for manual corrections in base calling and to make contiguous alignments of overlapping fragments. The primers used were LR0R, LR3R, LR5, LR7, LR16, ITS4 and ITS5 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) for nrLSU, and ITS1F, ITS2, ITS3, ITS4 and ITS5 (Gardes & Bruns 1993; White et al, 1990) for ITS.

We obtained ITS sequences from two specimens of *Neobarya usneae* (*Buck 61451* and *Driscoll 1037*) and a culture of *Buck 61451* (CBS 137512), and nrLSU from the culture and one specimen (*Buck 61451*). For *N. parasitica*, G. Marson obtained a single combined ITS+nrLSU using the primers ITS1-F and 5.8SR (forward); LR5 and LR1 (reverse).

Phylogenetic analysis. In addition to our newly generated sequences and two sequences of *Neobarya* specimens available in GenBank (AY346293 representing *Neobarya* sp. *Buck 26786* and EF160121 representing *Neobarya* sp. GJS 06-171), we included a broad range of taxa representing families within Hypocreales (Castlebury et al. 2004; Huhndorf et al. 2004; Johnson et al. 2009; Schoch et al. 2012; Spatafora et al. 2006; Zhang et al. 2006), and especially within the Clavicipitaceae (Kepler 2010; Sung et al. 2007a,b) to establish the phylogenetic position of the genus *Neobarya* represented by the type *N. parasitica*, and the position of *N. usneae*. The final data set (**Table 1**) contained 46 species, including two identified species of *Neobarya* (*N. parasitica* and *N. usneae*) and *Neobarya* sp. *Buck 26786* from GenBank; the ITS sequence from *Neobarya* sp. GJS 06-171 was found not to cluster with any of the Hypocrealean families included in our data set and was subsequently dropped from our analyses.

Table 1. Specimens, cultures and sequences featured in the study.

Species	Country	Specimen	Substrate	Isolate	Genbank	
					ITS	nrLSU
Akanthomyces novoguineensis	—	—	—	NHJ 11923	—	EU369032
Aschersonia calendulina	Thailand	—	—	SM00186.01	JN942615	JN940909
Aschersonia luteola	Thailand	—	—	SM00098.03	JN942616	JN940907
Cladobotryum asterophorum	Tokyo, Japan	—	—	CBS 676.77	—	AJ583469
Cladobotryum sp.	Peru	—	*Schizophyllum commune*	TFC 2007-10	AM779856	AM779856
Claviceps fusiformis	—	—	—	ATCC 26019	—	CFU17402
Claviceps paspali	—	—	—	ATCC 13892	—	CPU47826
Cordyceps brongniartii	Thailand	—	—	NBRC 101395	JN943298	JN941382
Cordyceps militaris	Japan	—	—	NBRC 9787	JN943433	JN941384
Cordyceps pseudomilitaris	Thailand	—	—	NBRC 101409	JN943305	JN941393
Cordyceps tuberculata	Japan	—	—	NBRC 106957	JN943311	JN941398
Cylindrocladiella elegans	—	—	—	CBS 110801	JN943101	JN099206
Daldinia concentrica	—	—	—	ATCC 36659	—	DCU47828
Diatrype disciformis	—	—	—	CBS 197.49; AFTOL-ID 927	—	DQ470964
Hydropisphaera erubescens	—	—	—	ATCC 36093	—	AY545726
Hydropisphaera peziza	U.S.A., Alabama	—	bark	GJS92-101	—	AY489730
Hypocrea atroviridis	France	—	—	NBRC 101776	JN943356	JN941451
Hypocrea lactea	—	—	—	NBRC 8435	JN943360	JN941455
Hypocrea minutispora	U.S.A.	—	—	NBRC 101779	JN943363	JN941460
Hypocrea pulvinata	Japan	—	—	NBRC 9385	JN943376	JN941472
Hypocrea rufa	—	DAO:JBT1003	—	DAOM JBT1003	JN942883	JN938865
Hypocrea sulphurea	Japan	—	—	NBRC 8437	JN943377	JN941473
Hypocrella discoidea	Thailand	—	—	SM00552.03	JN942614	JN940910
Hypocrella luteola	Thailand	—	—	SM00098.06	JN942625	JN940908
Hypomyces aurantius	—	—	—	TFC 94-70	—	AF160230
Hypomyces polyporinus	—	—	—	ATCC 76479	—	AF543793
Ilyoneonectria radicicola	—	—	—	CBS 153.37	HQ840391	HQ840375
Lichenobarya usneae	Canada, New Brunswick	Buck 61451 (NY)	*Usnea*	CBS 137512	KP899624	KP899625
Lichenobarya usneae	Canada, New Brunswick	Driscoll 1037 (NBM)	*Usnea*	—	KP899627	—
Moelleriella oxystoma	—	—	—	CBS 129339	—	DQ384943
Nectria cinnabarina	—	—	—	GJS89-107	—	U00748
Nectriopsis violacea	—	—	—	MUCL40056	—	AF193242
Neobarya parasitica	Luxembourg	Marson s.n. (BR)	*Bertia moriformis*	—	KP899626	KP899626
Neobarya sp.	Brazil	Buck 26786 (NY)	*Bertia*	—	—	AY346293
Neonectria faginata	—	—	—	CBS 119160	HQ840384	HQ840383
Neonectria neomacrospora	—	—	—	CBS 118984	HQ840388	HQ840379
Neonectria ramulariae	—	—	—	CBS 151.29	HM054150	HM042436
Ophiocordyceps cuboidea	Japan	—	—	NBRC 100941	JN943329	JN941416
Ophiocordyceps prolifica	Japan	—	—	NBRC 103838	JN943339	JN941434
Ophiocordyceps ryogamiensis	Japan	—	—	NBRC 101751	JN943343	JN941438
Pestalotiopsis adusta	China	—	—	CGMCC 3.9103	JN943637	JN940828
Pestalotiopsis clavispora	China	—	—	CGMCC 3.9134	JN943633	JN940831
Pseudonectria rousseliana	—	—	—	AR 2716	—	PRU17416
Trichoderma aggressivum f. *aggressivum*	—	—	—	DAOM 222156	AF456924	JN939833
Trichoderma amazonicum	Peru	—	endophyte of *Hevea*	IB50	HM142358	JN939814
Viridispora diparietispora	U.S.A., New York	BPI 802202 (BPI)	*Crataegus crus-galli*	ATCC MYA 627	—	AY489735
Xylaria hypoxylon	—	—	—	ATCC 42768	—	XHU47841

The newly generated nrLSU and ITS sequences were edited in BIOEDIT 7.09 (Hall 1999) and automatically aligned with MAFFT using the –auto option (Katoh & Toh 2005). The alignments were trimmed and subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al. 2010a,b); regions aligned with low confidence (below 0.93) were removed. ITS1 and ITS2 regions exhibited the highest levels of ambiguity, so we trimmed the ITS dataset to include the 5.8S region only. This resulted in an alignment length of 170 bases for 5.8S and 1344 bases for nrLSU. Data sets for each locus were analyzed separately and evaluated for potential conflict by comparing the non-parametric bootstrap values obtained for each resolved clade in each tree. Strongly supported clades (BS higher than 70%) that are in disagreement are an indication of significant conflict that precludes combination of the data sets (Mason-Gamer & Kellogg 1996). Since no conflict was detected in our data sets, we combined them (nrLSU + 5.8S) and subjected the combined dataset to maximum likelihood (ML) searches using RAxML 7.2.6 (Stamatakis 2006; Stamatakis et al. 2005), with non-parametric bootstrapping using 500 replicates under the universal GTRGAMMA model.

A Bayesian analysis was also performed for the same combined data set using Markov chain Monte Carlo sampling (Larget & Simon 1999) in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Substitution models for each data set were selected in jModelTest 0.1.1 (Posada 2008), which employs PhyML 3.0 (Guindon & Gascuel 2003) to estimate the likelihood of the data under 24 models of evolution using a fixed topology. The AICc values under each model were compared and the model with the lowest AICc value (GTR+I+Γ model all data sets) was selected. Two parallel analyses were then run in MrBayes for 2,000,000 generations, with 4 chains each, sampling every 100 generations. The program AWTY (Nylander et al. 2008; Wilgenbusch et al. 2004) was used to assess convergence between parallel runs by creating a bivariate plot of bipartitions. Initial burn-in trees (initial 25%) were discarded for each run and a majority-rule consensus tree constructed. RAxML and MrBayes analyses were performed using the Cipres Web Portal 3.1 (Miller et al. 2010) and the University of Oslo Bioportal (http://www.bioportal.uio.no). Relationships were considered supported if they had ML bootstrap support (BS) values of 70 or greater and Bayesian posterior probabilities (PP) of 0.95 or greater.

Clustering of molecular operational taxonomic units. Initial BLAST searches of our newly generated sequences and sequences of *Neobarya* spp. from GenBank indicated that they were unusually heterogeneous, so we sought to determine how *Neobarya* sequences cluster into molecular operational taxonomic units (MOTU's) at different sequence similarity thresholds, and if any published sequences fell out of these clusters. To accomplish this, we removed the outgroup taxa and submitted the unaligned ITS (ITS1, 5.8S and ITS2) and nrLSU sequences to the CD-HIT v.4.3 server (Huang et al. 2010) where CD-HIT-EST was used to delimit MOTU's based on 5 sequence similarity thresholds (80%, 85%, 90%, 95%, 99%). We report in the paper here only clusters that included *Neobarya* sequences.

Results

Phylogenetic placement of* Neobarya *sequences in the Hypocreales. ML and Bayesian analyses of the combined nrLSU + 5.8S data set consistently resulted in a phylogeny indicating a placement of all our *Neobarya* sequences in the Hypocreales, with *N. usneae* falling within the Hypocreaceae and *N. parasitica* and *N.* sp. *Buck 26786* falling within the Clavicipitaceae (**Fig. 1**). Good support (BS 100, PP 1.0) is obtained for the Hypocreales, and moderate support is obtained for the families Hypocreaceae (BS 89, PP 0.95) that includes *N. usneae*, and Clavicipitaceae (BS 80, PP 1.0) that contains *N. parasitica* and *N.* sp. *Buck 26786*.

Assessment of molecular operational taxonomic units. Sequences, especially ITS, of many of our *Neobarya* specimens were genetically dissimilar both to each other and to sequences of *Neobarya* species deposited in GenBank. ITS is widely used as a barcoding locus for fungi (Schoch et al. 2012) and sequence similarity can frequently be used to indicate membership in taxa. We therefore clustered all *Neobarya* sequences (ITS and nrLSU separately) into molecular operational taxonomic units (MOTU's) at different sequence similarities (**Table 2**). Clusters formed at low similarity will break up into smaller clusters as the similarity threshold for membership increases, so the threshold similarity for formation (or breakup) of clusters is an indication of the genetic homogeneity of groups of sequences. Our initial

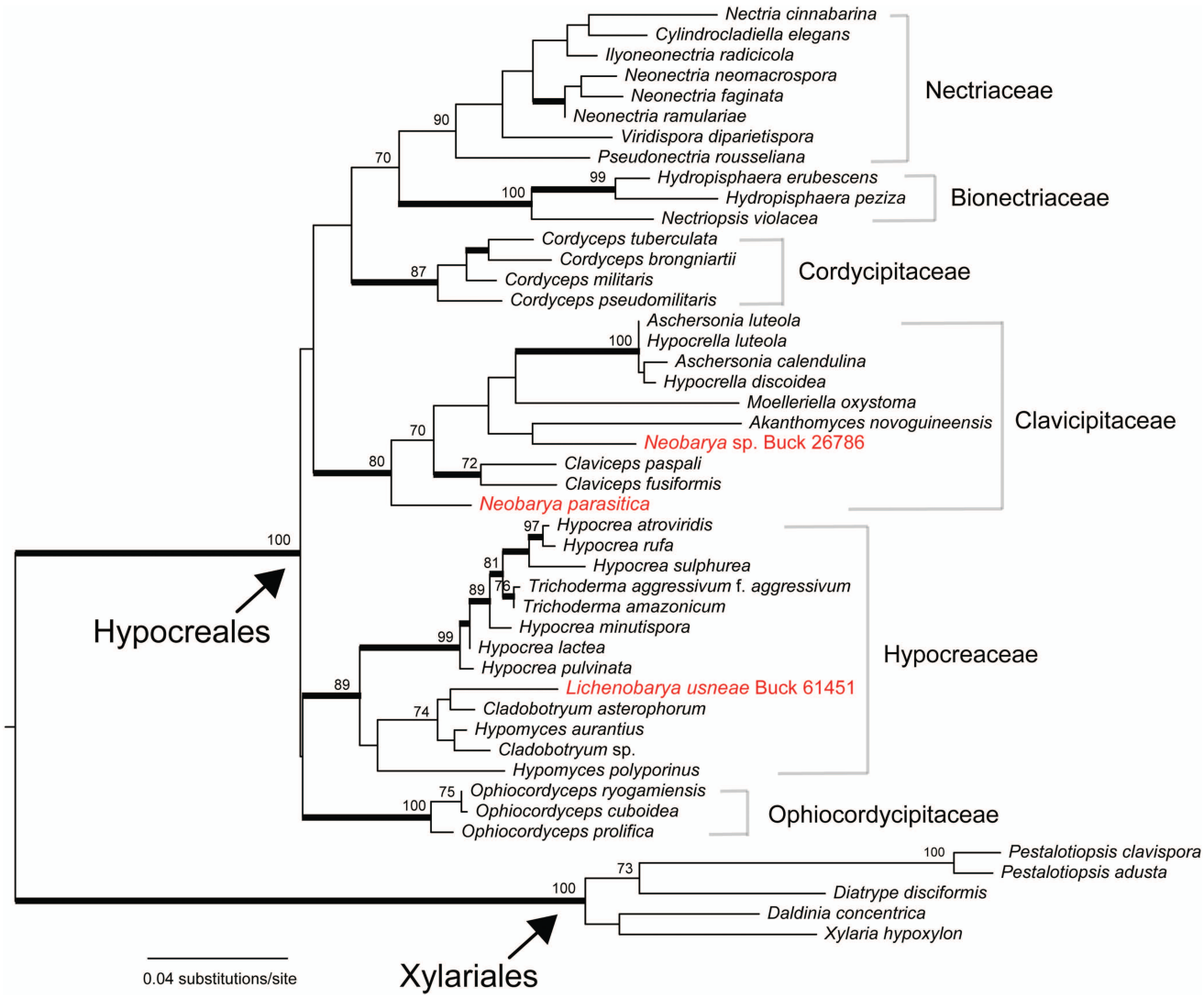

Figure 1. Best-scoring nrLSU + 5.8S RAxML phylogram of species used in the analysis, showing the placement of *Neobarya parasitica* and *Lichenobarya usneae*. Internal branches in boldface indicate posterior probabilities ≥ 0.95 and numbers are ML-BS values ≥70.

Table 2. MOTU's containing ITS and nrLSU sequences under various similarity thresholds.

	MOTU 0.99	MOTU 0.95	MOTU 0.90	MOTU 0.85	MOTU 0.80
ITS	3 clusters: *L. usneae* Driscoll 1037 *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	3 clusters: *L. usneae* Driscoll 1037 *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	3 clusters: *L. usneae* Driscoll 1037 *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	3 clusters: *L. usneae* Driscoll 1037 *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	1 cluster: *L. usneae* Driscoll 1037 *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786
nrLSU	3 clusters: *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	2 clusters: *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	1 cluster: *L. usneae* Buck 61451 *N. parasitica* *N.* sp. Buck 26786	—	—

clustering analyses found that the ITS of one GenBank sequence (*Neobarya* sp. GJS 06-171) forms clusters with other *Neobarya* sequences only at very low similarity levels (below 80%, where most other sequences in the data set also clustered), and for this reason it was deleted from the ITS data set. All *Neobarya* sequences cluster together at a lower similarity level for ITS (80%) than for nrLSU (90%), likely a reflection of the higher variation in ITS. In all cases, sequences of *N. usneae* clustered with each other (ITS), and those of *N. parasitica* and *Neobarya* sp. *Buck 26786* clustered together (nrLSU), indicating their membership in different families. ITS sequences of *N. parasitica* and *Neobarya* sp. *Buck 26786* do not form clusters except at thresholds that cluster all *Neobarya* sequences. However nrLSU sequences of these specimens do form clusters separate from *N. usneae* (at the 95% level). This along with the phylogenetic placement of these sequences in the nrLSU phylogeny (**Fig. 1**) indicates that *Buck 26786* represents a different species of *Neobarya* from *N. parasitica* in the Clavicipitaceae, an unexpected result given that they were both collected from the same host fungus (*Bertia* sp.).

Our results consistently place *Neobarya usneae* as a singular species in the Hypocreaceae, not in the Clavicipitaceae where the type *N. parasitica* is placed. Given this result, we believe there is need to establish a new genus for *N. usneae*.

Taxonomy

Neobarya Lowen. In: Eriksson & Hawksworth, System. Ascom. 5: 121, 1986.

MycoBank MB 25587

Type: *Neobarya parasitica* (Fuckel) Lowen.

Neobarya parasitica (Fuckel) Lowen. In: Eriksson & Hawksworth, System. Ascom. 5: 121, 1986. **Fig. 2A–B**

MycoBank MB 103609

Type: GERMANY. nordrhein-westfalen: Oestricher Wald, Aepfelbach, on *Bertia moriformis*, on *Fagus*, Fuckel, F. Rhenani 991 (holotype: IMI, non vid.). For a description and further illustrations, see Candoussau et al. (2007).

Comments. This is a rather common parasite of the pyrenomycete *Bertia moriformis*, having rarely been reported from other hosts. It is easily recognized by the densely agglomerated, greenish yellow perithecia surrounded by a whitish to yellow asexual state.

Specimen examined and sequenced. LUXEMBOURG. SW of Echternach, 1 km E of Michelshaff, 49°46′28.8″ N, 6°23′32.4″ E, 375 m, on dead branch lying on the ground in forest, on *Bertia moriformis*, 18 Aug 2014, *G. Marson* (BR, herb. Diederich).

Lichenobarya Etayo, Diederich & Lawrey, *gen. nov.*

MycoBank MB 811832

Characterized by superficial obpyriform perithecioid, brown ascomata immersed in a poorly developed and unapparent subiculum, elongate asci with a non- or only slightly-thickened apex, extremely long, filiform, multiseptate ascospores, and no apparent asexual state.

Type: *Lichenobarya usneae* (Etayo) Etayo, Diederich & Lawrey

Comments. Apart from being distantly related phylogenetically to *Neobarya*, *Lichenobarya* clearly differs from that genus by the non- or only slightly-thickened ascus apex (see illustrations of *L. usneae* asci in Etayo, 2002, Fig. 29, and of *N. parasitica* asci in Candoussau et al., 2007, Fig. 10), by the absent asexual state (the supposed asexual state of *Neobarya parasitica* abundantly surrounding perithecia of that species), and by the distinct brown ascomatal color, the perithecia being yellowish green in *N. parasitica*. In culture (CBS 137512), *L. usneae* is made up of a loosely arranged sterile mycelium only, white in color. The new genus is distinguished from other genera of Hypocreaceae studied by Rossman et al. (1999) by perithecia not embedded in distinct stromata and by the extremely long, filiform, not disarticulating ascospores.

Lichenobarya usneae (Etayo) Etayo, Diederich & Lawrey, *comb. nov.* **Fig. 2C**

MycoBank MB 811833

Neobarya usneae Etayo, Bibl. Lichenol. 84: 76, 2002. Type: COLOMBIA. dept. nariño: Pasto, bosque de Daza, vía Pasto-Buesaco, alt. 2750 m, on *Usnea rubicunda*, 28 Jul 1998, *J. Etayo 16450* (holotype: COL; isotype: herb. Etayo). For a description and further illustrations, see Etayo (2002).

Comments. This species was published based on material from Colombia on the thallus of *Usnea* species (Etayo 2002), but we also have samples from southern countries, including Bolivia, Ecuador and Chile (Valdivia Region). The recent discovery in Canada suggests that it is probably widespread in America. However, so far it has not been collected outside of America.

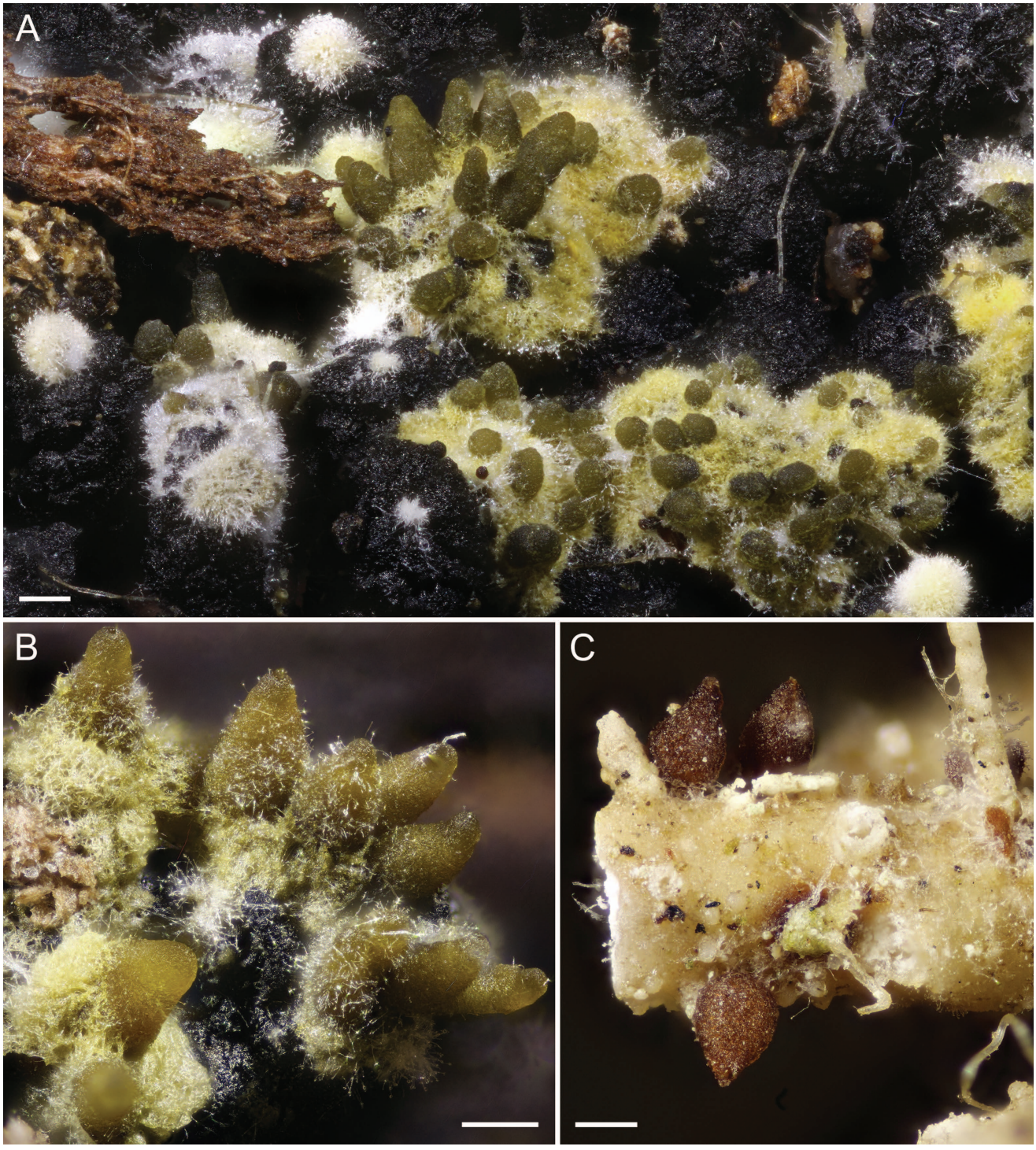

Figure 2. A–B. *Neobarya parasitica* perithecia and asexual state on *Bertia moriformis* (*Marson s.n.*, BR). **C.** *Lichenobarya usneae* perithecia on *Usnea* sp. (*Buck 61451*, NY). Scale bars: 200 μm.

Specimens examined and sequenced. CANADA. NEW BRUNSWICK: Albert County, Fundy National Park, Dickson Falls Trail, 45°35′12″ N, 64°58′20″ W, 85 m, mixed forest of *Picea rubens*, *Betula* spp. and *Acer rubrum* along rocky brook ravine, on dead *Usnea*, 23 Sep 2013, *W.R. Buck 61451* (NY). Queens County, Grand Lake Protected Natural Area, west side of Jemseg River, ca. 200 m NW of 4-lane highway (Hwy 2) bridge, 45.83020° N, 66.11777° W, 5 m, flood-plain forest of *Acer saccharinum* and *Fraxinus*

pensylvanica with understory dominated by *Onoclea*, on *Usnea subfloridana*, 16 Aug 2014, *K.E. Driscoll 1037* (NBM).

Other specimens examined. CANADA. NEW BRUNSWICK: Queens County, Canadian Forces Base Gagetown, Nerepis Hills, along Coleman Brook and northeast aspect of Watters Mountain, ca. 1.7 km N of O'Leary Lake, 45.50471° N, 66.36824° W, 165 m, forest of *Acer saccharum*, *Fagus grandifolia* and *Betula alleghaniensis*, on *Usnea scabrata* s.lat., 5 Nov 2013, *K.E. Driscoll 945* (NBM). Queens County, Grand Lake Protected Natural Area, W side of Pondstream Road, 1.8 km E of Clarks Corners, 45.94455° N, 66.11066° W, 5 m, 60 to 80 m wide roadside leave-strip of mature forest dominated by *Picea rubens*, *Pinus strobus* and *Abies balsamea*, with scattered *Pinus resinosa*, on *Usnea filipendula*, 13 Aug 2014, *K.E. Driscoll 1017* (NBM). BOLIVIA. Between La Paz and Coroico towards Chulumani, 16°18′27″ S, 67°53′48″ W, 3210 m, cloud forest, on *Usnea* growing on branches, 31 May 2011, J. Etayo 26947, A. Flakus & *M. Kukwa* (herb. Etayo); Zongo Valley, cloud forest, near metal bridge, 2450 m, 16°07′41″ S, 68°05′55″ W, on *Usnea*, 29 May 2011, *J. Etayo 26731*, *A. Flakus* & *M. Kukwa* (herb. Etayo); DEPT. LA PAZ: Prov. Larecaja, Jocollone village and 1 km further, Paramo Yungeño vegetation, open anthropogenic area, NE oriented slope, 15°37′35″ S, 68°41′21″ W, 3545 m, on *Usnea* on *Berberis*, 14 May 2011, *J. Etayo 27203*, *A. Flakus* & *M. Kukwa* (LPB); ibid., *J. Etayo 27354* (herb. Etayo). CHILE. VALDIVIA: Between the city and the airport, remnants of native forest, on *Usnea rubicunda*, 1 Dec 2013, 39°45′07.2″ S, 73°07′27.0″ W, 48 m, *J. Etayo 28332*, *L.G. Sancho* & *J. Villagrán* (herb. Etayo). ECUADOR. PROV. PICHINCHA: Sierra Central, Cráter Pululaua, Mitad del Mundo, secondary bush forest, on *Usnea* sp., 2750 m, 24 Jul 1999, *J. Etayo 17279* & *J. Santiana* (herb. Etayo, QCA). PROV. TUNGURAHUA: Between Pondoa and Tungurahua, climbing to the top, cloud forest, on reddish *Usnea*, 2400–3800 m, 29–30 Jul 1999, *J. Etayo 19969* & *Z. Palice* (herb. Etayo).

Discussion

The type of *Neobarya*, *N. parasitica*, has unitunicate asci that are cylindrical or narrowly clavate to lanceolate with an enlarged thickened apical cap typical of the Clavicipitaceae and penetrated by a pore. In the prologue, Etayo (2002) discussed this character as problematic for *N. usneae*, because asci have a thin apical cap lacking a pore; for these reasons, it was named as a *Neobarya* species provisionally (p. 74). Later in their study of *Neobarya*, Candoussau et al. (2007) corroborated this and suggested that *N. usneae* might not belong in the genus. Our results confirm their conclusion and provide anatomical support for the establishment of a separate genus for this species, which we are naming *Lichenobarya*. The lack of an apical cap is also evidence for placement of the species outside of the Clavicipitaceae, which is supported by our data.

Prior to this study, only one paper has used sequence data to place a *Neobarya* species phylogenetically (Huhndorf et al. 2004), and it placed a sequence of nrLSU from *Neobarya* sp. *Buck 26786* in an uncertain position in the Hypocreomycetidae between the Hypocreales and Coronophorales, certainly no confirmation of *Neobarya* in Clavicipitaceae. Our results demonstrate that the genus *Neobarya* is within the Clavicipitaceae and *Lichenobarya* is within the Hypocreaceae. However, relationships to other genera are uncertain at this point given the dissimilarity of ITS and nrLSU sequences to any other Clavicipitaceae (for *N. parasitica*) or Hypocreaceae (for *Lichenobarya usneae*) sequences in GenBank. Other species of *Neobarya*, including lichenicolous species, are assumed to be members of the Clavicipitaceae based on anatomy, but the placement of these in the family, including lichenicolous species, will probably require multiple loci. A multi-gene phylogeny of the Clavicipitaceae recently developed by Sung et al. (2007) indicates that the family is paraphyletic with three well-supported clades that do not represent presently recognized subfamily categories. As more specimens are sequenced, the relationships within the family will undoubtedly become more evident.

Asexual states are not known for *Lichenobarya usneae,* or at least they are not developed at the base of perithecia as in the type of *Neobarya*, and they were absent in a culture we obtained from this species. However, filamentous fungi are sometimes found on tropical *Usnea* and one of these may belong to this taxon. Several different asexual states are known for *Neobarya* species (Candoussau et al. 2007), including those associated with *N. agaricicola* (*Calcarisporium*), *N. aurantiaca* (paecilomyces-like), *N. byssicola* (*Diploospora*), *N. danica* (lecanicillium-like), *N. parasitica* (lecanicillium-like), *N. peltigerae* (acremonium-like), and *N. xylariicola* (*Calcarisporium*). The absence of a known asexual state for

Lichenobarya appears to be, at present, a distinguishing characteristic of the new genus; however, more specimens must be collected before this can be established with certainty.

Another lichenicolous species similar to *Neobarya* in having yellow, agglomerated, obpyriform perithecia with a subicule at the base is *Nectria byssophila* Rossman (Etayo & Sancho 2008). It is interesting that this species shares with *L. usneae* the thin ascus tip. So far, this common species has not been studied using molecular data, but it could belong to *Lichenobarya*.

The generic type of *Lichenobarya* was described based on material collected from *Usnea rubicunda* in Colombia, and the species has since been collected always on *Usnea* in Bolivia, Canada, Chile and Ecuador. The distribution of the species is not known at present, but it is likely to occur broadly on *Usnea* species throughout the New World. Specimens in New Brunswick were found on *Usnea* species in temperate to hemiboreal forests in regions with a humid climate. The southernmost locality where it has been collected is from Valdivia in humid, relatively temperate forests, but it disappears in the more southern, colder ones. Unhealthy *Usnea* specimens from Magallanes Region were thoroughly searched by Etayo & Sancho (2008), but no sample of *L. usneae* was collected.

Acknowledgments

Support provided by NSF grant DEB 0841405 (PI: J. Lawrey, R. Lücking and P. Gillevet). J. Etayo was partially supported by the Polish National Science Centre grant DEC-2013/11/D/NZ8/03274. K. Driscoll was supported through project funding for biological inventory projects (BioBlitz 2014 and Fundy Mycological Foray) awarded to the New Brunswick Museum by the New Brunswick Wildlife Trust Fund and the New Brunswick Environmental Trust Fund. We are grateful to Bill Buck for the loan of a specimen of *Lichenobarya usneae* from NY and to Guy Marson for the collection and sequences of a specimen of *Neobarya parasitica*.

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manuscript received January 31, 2015; accepted March 10, 2015.