A REVISED GENERIC CLASSIFICATION FOR THE RHODOCYBE-CLITOPILUS CLADE (ENTOLOMATACEAE, AGARICALES) INCLUDING THE DESCRIPTION OF A NEW GENUS, *CLITOCELLA* GEN. NOV.

by

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ABSTRACT

Morphological and phylogenetic analyses in previous studies have led to conflicting hypotheses regarding the generic classification for the taxa within the Rhodocybe-Clitopilus clade of the Entolomataceae Kotl. & Pouzar (Agaricales, Basidiomycota). The majority of species in the Rhodocybe-Clitopilus clade are classified as either *Clitopilus* (Fr. ex Rabenh.) P. Kumm. (basidiospores ornamented with longitudinal ridges) or *Rhodocybe* Maire (undulate-pustulate basidiospore wall ornamentation). Recent phylogenetic analyses have revealed that taxa with basidiospores that are ornamented with pustules (*Rhodocybe*) do not form a monophyletic lineage. Here, the systematics of the Rhodocybe-Clitopilus clade are evaluated using a multi-locus phylogenetic analysis of three partial protein coding gene regions: (i) the mitochondrial ATPase subunit 6 (*atp6*), (ii) the nuclear RNA polymerase second largest subunit (*rpb2*) and (iii) the nuclear translation elongation factor subunit 1-α (*tef1*). The results of this analysis provide support for five internal clades: (i) a Clitopilus clade, (ii) a Clitocella clade, (iii) a Clitopilopsis clade, (iv) a Rhodocybe s. str. clade and (v) a Rhodophana clade. Based on these results, a revised classification recognizing five monophyletic genera is proposed and the morphological characters used to distinguish them are outlined. *Clitopilopsis* Maire and *Rhodophana* Kühner are resurrected, the boundaries of *Rhodocybe* are amended and *Clitocella* is described as a new genus with *Clitocella popinalis* designated as the type species.

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CHAPTER ONE: INTRODUCTION

The Entolomataceae Kotl. & Pouzar is one of the three largest euagaric families containing nearly 1,500 described species (Co-David et al. 2009, Baroni et al. 2011). The species within this group are recognized by the presence of attached lamellae and basidiospores that are pinkish in deposit, have evenly cyanophilic walls (absorb cotton blue evenly) and appear angular in polar view (Baroni 1981, Singer 1986, Largent 1994, Co-David et al. 2009). Within the Entolomataceae, two major clades are recognized (herein referred to as the Entoloma and the Rhodocybe-Clitopilus clades) (Matheny et al. 2006, Co-David et al. 2009, Baroni and Matheny 2011, Baroni et al. 2011). The largest group, the Entoloma clade, includes over 1000 species (Baroni et al. 2011) described as either *Entoloma s.l.* (Noordeloos 1981) or as segregate genera (Largent 1994) and is differentiated from the Rhodocybe-Clitopilus clade by basidiospores that appear angular in face and profile views in addition to polar view. The Rhodocybe-Clitopilus clade includes approximately 300 described species, the majority of which are placed in one of two genera, *Rhodocybe* Maire or *Clitopilus* (Fr. ex Rabenh.) P. Kumm. (indexfungorum.org). The ornamentations on the wall of the basidiospore is an important character used to differentiate between *Rhodocybe* and *Clitopilus* species. Species either possess basidiospores ornamented with longitudinal ridges (*Clitopilus* spp.) or unorganized pustules (*Rhodocybe* spp.) visible in face and profile views (Singer 1975, Baroni 1981, Singer 1986).

The focus of this study is to evaluate generic boundaries for the species within the Rhodocybe-Clitopilus clade. The lack of agreement concerning the number of genera within this clade is a major obstacle for proposing a consistent generic classification. Co-

David et al. (2009) proposed that only a single genus, *Clitopilus*, should be recognized based on their interpretation of a three-gene phylogenetic analysis that included 12 *Rhodocybe* and three *Clitopilus* species. Recognizing only a single genus, while taxonomically valid, may deemphasize the breadth of morphological and evolutionary diversity within the Rhodocybe-Clitopilus clade and is in contrast to the infrageneric classification defined by macro- and micro-morphological analysis (Baroni 1981, Singer 1986) and the internal clades resolved in recent phylogenetic analyses (Baroni et al. 2011, Baroni and Matheny 2011).

A three-gene phylogenetic analysis of the Rhodocybe-Clitopilus clade is conducted to understand the evolution and diversification of species recognized as *Clitopilus* and *Rhodocybe*. Based on the results of this analysis, a classification recognizing five monophyletic genera is proposed. The previously described macro- and micro-morphological characters used to support these new generic delimitations are outlined.

CHAPTER TWO: METHODS

Taxon Sampling.— Two hundred and forty-six sequences from 25 collections traditionally classified within *Clitopilus*, and 65 collections traditionally placed within *Rhodocybe* (Table 1) were generated for this study. The ingroup taxa sampled represent all infrageneric sections of *Clitopilus* based on differences in basidiocarp size and stipe attachment (Singer 1986). This sampling includes species collected from Africa, Asia, Australia, Europe, North America, and South America. Sampled *Rhodocybe* taxa included species from the majority of infrageneric sections as identified by Baroni (1981) and Singer (1986), including taxa with and without abundant clamp connections, pleurotoid taxa with and without brightly colored hymenial pseudocystidia and centrally stipitate taxa with and without hymenial pseudocystidia. It was not possible to represent one group of *Rhodocybe* taxa (*Rhodocybe* section *Tomentosi*) defined by Baroni (1981) with tomentose pileal surfaces because of rarity of the samples. In addition, 12 sequences from four outgroup collections (*Tricholoma flavovirens, Mycena* aff. *pura, Panellus stipticus*, and *Catathelasma imperiale*) were generated for this study (Table 1). Outgroup taxa were selected based on evolutionary relationships inferred from previous phylogenetic analyses. Taxa classified in genera identified as closely related and basal to the Entolomataceae within the Tricholomatoid clade *sensu* Matheny et al. (2006) were used as outgroup taxa.

Molecular Sampling.— Partial protein-coding gene regions were targeted because of the sufficient levels of nucleotide polymorphism for resolving phylogenies of the Entolomataceae and other Basidiomycetes (Kretzer and Bruns 1999, Matheny 2005, Matheny et al. 2007).

Table 1. Voucher specimen collection information and GenBank accession numbers for sequences used in phylogenetic analyses.

Species	Collection Identifier	Herbarium Accession No.	Collector(s), Location and Year	GenBank Accession No.		
				atp6	*rpb2*	*tef1*
Clitopilus apalus	26394 Watling	WAT26394[c]	R. Watling, Kepong, Forest Research Institute, Malaysia, 1995	KC816738	KC816906	KC816822
C. cf. *argentinus*	Klaus Siepe Geeste 133 - D-46342	MTB4804/2[c]	H. Bender, Mönchengladbach, Germany, 2011	KC816739	KC816907	KC816823
C. "*cinerascens*"	8024 TJB	TB8024[c]	T.J. Baroni, Alachua Co., Florida, USA, 1996	KC816740	KC816908	KC816824
C. "*cinerascens*"	8133 TJB	TB8133[c]	M. Blackwell, West Feliciana Parish, Louisiana, USA, 1996	KC816741	KC816909	KC816825
C. crispus	10027 TJB	TB10027[c]	T.J. Baroni, Chaing Mai Prov., Thailand, 2006	KC816743	KC816911	KC816827
C. crispus	9982 TJB	TB9982[c]	T.J. Baroni, Chiang Mai Prov., Thailand, 2006	KC816742	KC816910	KC816826
C. hobsonii	DL Largent 9779		D.L. Largent, Danbulla National Park, Kauri Creek Track, rainforest section, Queensland, Australia, 2010	KC816747	KC816916	KC816831
C. hobsonii	5967 TJB	TB5967[c]	T.J. Baroni, Hamilton Co, Raquette Lake, New York, USA, 1988	KC816748	KC816917	———
C. aff. *hobsonii*	TDB-3667	UC1860830[a]	N. Nguyen, Mariposa Grove Area, Yosemite National Park, Mariposa County, California, USA, 2011	KC816759	KC816928	KC816841
C. hobsonii	DL Largent 9586		D.L. Largent, Crater Lakes National Park, Lake Barrine, Queensland, Australia, 2009	———	KC816912	KC816828
C. hobsonii	DL Largent 9635		D.L. Largent, Mt. Hypipamee National Park, Queensland, Australia, 2009	KC816744	KC816913	KC816829
C. hobsonii	DL Largent 9643		D.L. Largent, Mt. Hypipamee National Park, Queensland, Australia, 2009	KC816745	KC816914	———
C. hobsonii	DL Largent 9746		D.L. Largent, Daintree National Park, Tribulation Section, Emmagen Creek Track, Queensland, Australia, 2010	KC816746	KC816915	KC816830
C. hobsonii grp.	7051 TJB	TB7051[c]	T.J. Baroni, Macon Co., Coweeta, North Carolina, USA, 1993	KC816749	KC816918	———
C. paxilloides	5809 TJB	TB5809[c]	T.J. Baroni, Mendocino Co., Little River, California, USA, 1987	KC816750	KC816919	KC816832
C. peri	10040 TJB	TB10040[c]	T.J. Baroni, Chiang Mai Prov., Doi Suthep National Park, Thailand, 2006	KC816752	KC816921	KC816834
C. peri	10033 TJB	TB10033[c]	T.J. Baroni, Chiang Mai Prov., above Ban Pha Deng Village, Thailand, 2006	KC816751	KC816920	KC816833
C. peri	10041 TJB	TB10041[c]	T.J. Baroni, Chiang Mai Prov., Doi Suthep National Park, Thailand, 2006	KC816753	KC816922	KC816835
C. cf. *prunulus*	E226 Gates	E226[c]	G.M. Gates, Kermandie Track, Tasmania, 1999	KC816758	KC816927	KC816840
C. prunulus	11CA012		K.L. Kluting, Big Lagoon Elementery School, Trinidad, Humboldt County, California, USA, 2011	KC816757	KC816926	KC816839
C. prunulus	8456 R.E. Halling	REH8456[c]	R.E. Halling, Novgorod Region, Valdai District, Valdaiski National Park, Russia, 2003	KC816754	KC816923	KC816836
C. prunulus	6805 TJB	TB6805[c]	T.J. Baroni, Erie Co., Orchard Park Township, Chestnut Ridge Park, New York, USA, 1982	KC816755	KC816924	KC816837

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				atp6	*rpb2*	*tef1*
C. prunulus	7003 TJB	TB7003[c]	T.J. Baroni, Macon Co., Coweeta Hydrological Research Station, North Carolina, USA, 1992	KC816756	KC816925	———
C. sp.	7130 TJB	TB7130[c]	T.J. Baroni, Hamilton Co., SUNY Cortland Outdoor Education Facility, Camp Huntington, New York, USA, 1993	KC816760	KC816929	———
C. venososulcatus	8111 TJB	TB8111[c]	M. Blackwell, East Baton Rouge Parish, Louisiana, 1996	KC816761	KC816930	———
Rhodocybe alutacea	5726 TJB	TB5726[c]	T.J. Baroni, Haywood Co., Cherokee National Forest, North Carolina, USA, 1987	KC816762	KC816931	KC816842
R. caelata	6919 TJB	TB6919[c]	T.J. Baroni, Macon Co., Coweeta Hydrological Research Station, North Carolina, USA, 1992	KC816764	KC816933	KC816843
R. caelata	J. Parkin	J. Parkin[c]	J. Parkin, York County, Ontario, Canada, 1988	KC816765	KC816934	———
R. caelata	3569 R.E. Halling	REH3569[c]	R.E. Halling, Jurmala, Latvia, 1982	KC816763	KC816932	———
R. caelata		K(M): 158060[d]	R.G. Betts, Tyntesfield, Wraxall, North Somerset, England, 2006	KC816802	KC816978	KC816885
R. collybioides	10417 TJB	TB10417[c]	T.J. Baroni, Jujuy Province, Parque Nacional Calilegua, Argentina, 2011	KC816766	KC816935	KC816844
R. fallax	136 LP	K(M): 116541[d]	P. Leonard, Camino Real, La Palma, Canary Islands, Spain, 1997	KC816769	KC816938	KC816847
R. fallax	52/85	O-F88953[e]	A. Hov and P. Marstad, Valtersborg, Vale, Vestfold County, Norway, 1985	KC816767	KC816936	KC816845
R. fallax	25668OKM	25668OKM[c]	O.K. Miller, Jr., Malheur Co., Malheur National Forest, Oregon, USA, 1993	KC816768	KC816937	KC816846
R. formosa	1061015-6		F. Caballero and J. Vila, Spain, 2006	———	KC816939	KC816849
R. fuliginea	E537 Gates	E537[c]	G.M. Gates and D. Ratkowsky, Waverly Flora Park, Bellerive, Tasmania, 1999	KC816770	KC816940	KC816850
R. hirneola	8490 R.E. Halling	REH8490[c]	R.E. Halling, Novgorod Region, Valdai District Valdaiski National Park, Russia, 2003	———	KC816904	KC816820
R. hirneola	155 SC	155 SC[c]	S. Carpenter, Mt. St. Helens, Polar Star Mine, Washington, USA, 1982	———	KC816905	KC816821
R. hirneola	PM 247-08	Artsobs. 1376857[e]	P. Marstad, Konglungen, Asker, Akershus County, Norway, 2008	———	KC816977	KC816883
R. hondensis	6103 TJB	TB6103[c]	T.J. Baroni, Humboldt Co., Largent Property, California, USA, 1988	KC816771	KC816941	KC816851
R. lateritia	E1589 Gates (ISOTYPE)	E1589[c]	G.M. Gates and D. Ratkowsky, Waterworks Reserve, Hobart, Tasmania, 2002	KC816772	KC816942	KC816852
R. luteocinnamomea	Lodge G-162	GUA241[c]	D.J. Lodge, Guana Island, Quail Dove Ghut Trail, lower Tamarind orchard, British Virgin Islands, 1999	KC816773	KC816943	KC816853
R. mellea	6883 TJB	TB6883[c]	T.J. Baroni, Alachua Co., Sugar Foot Hammock, Florida, USA, 1992	KC816774	KC816944	KC816854

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				atp6	*rpb2*	*tef1*
R. melleopallens		K(M): 143160[d]	A Henrici, Pembrey, Tywyn Burrows, Carmarthenshire [Dyfed] County, Wales, 2006	KC816775	KC816945	KC816855
R. melleopallens	415/83	O-F172919[e]	G. Gulden, Bonn, Frogn, Akershus County, Norway, 1983	KC816776	KC816946	KC816856
R. minutispora	1071101-4		F. Caballero and J. Vila, Spain, 2007	KC816777	KC816947	KC816857
R. mundula	7161 TJB	TB7161[c]	T.J. Baroni, Essex Co., Upper Jay, Styles Brook Rd., New York, USA, 1993	KC816782	KC816952	KC816862
R. mundula	20894	O-F19454[e]	J.K. Stordal, Hensvoll, Ostre Toten, Oppland County, Norway, 1980	KC816784	KC816954	KC816864
R. mundula	PM 67-95	O-F71544[e]	G. Mathiassen and P. Marstad, Lulle, Skibotndalen, Storfjord, Troms County, ,Norway, 1995	KC816780	KC816950	KC816860
R. mundula	7599 TJB /AFTOLID 521	TB7599[c]	T.J. Baroni, Tompkins Co., Ringwood Preserve, New York, USA, 1994	KC816783	KC816953	KC816863
R. mundula	7115 TJB	TB7115[c]	T.J. Baroni, Hamilton Co., SUNY Cortland Outdoor Education Center, Camp Marion Swamp, Long Point, New York, USA, 1993	KC816781	KC816951	KC816861
R. mundula		K(M): 164736[d]	N. Mahler, Minsmere RSPB Nature Reserve, East Suffolk, Suffolk County England, 2009	KC816779	KC816949	KC816859
R. mundula		K(M): 49620[d]	J.R. Hawes, Near St. Helier, Jersey, Channel Islands, 1996	KC816778	KC816948	KC816858
R. nitellina		K(M): 132700[d]	N.W. Legon, Mildenhall Woods, Mildenhall, West Suffolk, Suffolk County England, 2004	———	KC816960	KC816867
R. nitellina		Artsobs. 1541959[e]	P.G. Larsen, More og Romsdal, Seljeneset, Stordal, Norway, 2009	KC816790	KC816961	KC816868
R. nitellina		Artsobs. 1553208[e]	R. Braathen and E.W. Hanssen, Ormtjern, NedreEiker, Buskerud County, Norway, 2009	———	KC816966	KC816873
R. nitellina		O-F291457[e]	O. Forland and J.B. Jordal, Hjelmeland, Rogaland County, Norway, 2009	KC816787	KC816957	———
R. nitellina	6404 TJB	TB6404[c]	T.J. Baroni, Graubunden Canton, Switzerland, 1990	———	KC816963	KC816870
R. nitellina	6740 TJB	TB6740[c]	T.J. Baroni, Mendocino Co., Navarro River, California, USA, 1992	———	KC816964	KC816871
R. nitellina	7861 TJB	TB7861[c]	T.J. Baroni, Mendocino Co, Rt. 20 near Chamberlain Creek west of Willits, California, USA, 1996	KC816789	KC816959	KC816866
R. nitellina	11CA025		unknown, California, USA, 2011	KC816792	KC816965	KC816872
R. nitellina	HH74/10	O-F293352[e]	H. Holien and T.E. Brandrud, Kvam, Steinkjer, Nord-Trondelag County, Norway, 2010	KC816788	KC816958	KC816865
R. nitellina	I-LF08-48	O-F285851[e]	I.-L. Fonneland and D. Pettersen, Askeroya, Tvedestrand, Aust-Agder County Norway, 2008	KC816786	KC816956	———
R. nitellina	MC3-CAR		M. Contu, Italy, 1995	KC816785	KC816955	———

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Species	Collection Identifier	Herbarium Accession No.	Collector(s), Location and Year	GenBank Accession No.		
				atp6	*rpb2*	*tef1*
R. aff. *nitellina*	5528 TJB	TB5528[c]	T.J. Baroni, Sevier Co., Cherokee Orchard, Great Smoky Mountain National Park, Tennessee, USA, 1987	KC816791	KC816962	KC816869
R. aff. *nitellina*	DL Largent 10199		D.L. Largent, Barrington Tops National Park, Williams River Day Use Area, New South Wales, Australia, 2011	———	KC816967	KC816874
R. pallidogrisea	E652 Gates	E652[c]	G.M. Gates and D. Ratkowsky, Mt. Field, Tasmania, 1999	KC816793	KC816968	KC816875
R. paurii	99/233 Moncalvo (ISOTYPE)	JM99/233[c]	J.-M. Moncalvo, Garhwal Himalaya, Pauri, Nagdev, Uttaranchal, India, 1999	KC816794	KC816969	KC816876
R. popinalis		K(M): 143166[d]	R.G. Betts, Bamburgh, Northumberland County, England, 2004	KC816796	KC816971	KC816878
R. popinalis		K(M): 167017[d]	E.W. Brown, Palace Lawn, Kew, Royal Botanic Gardens, Surrey County, England, 2010	KC816797	KC816972	KC816879
R. popinalis		O-F63376[e]	J.I. Johnsen, Brusand, Ha, Rogaland County, Norway, 1997	KC816799	KC816974	KC816880
R. popinalis	6378 TJB	TB6378[c]	T.J. Baroni, Graubunden Canton, Fetan, Switzerland, 1990	KC816801	KC816976	KC816882
R. popinalis	116-2000	O-F105360[e]	P. Marstad, Skallvold, Tonsberg, Vestfold County, Norway, 2000	KC816800	KC816975	KC816881
R. popinalis	648/06	K(M): 146162[d]	D.J. Savage, Invernaver raised beach, Bettyhill area, West Sutherland, Scotland, 2006	KC816795	KC816970	KC816877
R. popinalis	MC2-TRENT		L. Pennone, Trentino, Italy, 2003	KC816798	KC816973	———
R. pseudopiperita	E1159 Gates	E1159[c]	G.M. Gates and D. Ratkowsky, Mt. Wellington, Myrtle Gully, Tasmania, 2001	KC816803	KC816979	KC816886
R. reticulata	E2183 Gates	E2183[c]	G.M. Gates and D. Ratkowsky, North West Bay River, Tasmania, 2005	KC816804	KC816980	KC816887
R. rhizogena	5551 TJB (ISOTYPE)	TB5551[c]	T.J. Baroni, Macon Co., Ellicot Rock Trail, Ammons Creek Area, North Carolina, USA, 1987	KC816805	KC816981	KC816888
R. roseiavellanea	8130 TJB	TB8130[c]	T.J. Baroni, Louisiana, USA	KC816806	KC816982	KC816889
R. sp.	DL Largent 9851		D.L. Largent, Myall Lakes National Park, Seal Rocks road, New South Wales, Australia, 2010	KC816809	KC816986	KC816893
R. sp.	DL Largent 9846		D.L. Largent, Barrington Tops National Park, Jerusalem Creek track, New South Wales, Australia, 2010	KC816808	KC816985	KC816892
R. sp.	DL Largent 9860		D.L. Largent, Barrington Tops National Park, Jerusalem Creek, bottom end of track, New South Wales, Australia, 2010	KC816810	KC816987	KC816894
R. sp.	DL Largent 9952		D.L. Largent, Barrington Tops National Park, Williams River Day Use Area, end of blue gum track to lion's rock, New South Wales, Australia, 2010	KC816811	KC816988	KC816895
R. sp.	DL Largent 9957		D.L. Largent, Myall Lakes National Park, Mungo Brush track, New South Wales, Australia, 2010	KC816812	KC816989	KC816896

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Species	Collection Identifier	Herbarium Accession No.	Collector(s), Location and Year	GenBank Accession No.		
				atp6	*rpb2*	*tef1*
R. sp.	DL Largent 10218		D.L. Largent, Barrington Tops National Park, Jerusalem Creek, lower parking lot, New South Wales, Australia, 2011	KC816813	KC816990	KC816897
R. sp.	DL Largent 10032		D.L. Largent, Yorkies Knob Beach Forest, northern end, Queensland, Australia, 2011	KC816814	KC816991	KC816898
R. stangliana	2073 T. Laessoe	2073TL[c]	T. Læssøe, East Jutland, Mariager, Hou Skov, Denmark, 1989	———	KC816992	KC816899
R. stipitata	5523 TJB	TB5523[c]	T.J. Baroni, Sevier Co., Cherokee Orchard Trail, Great Smoky Mountain National Park, Tennessee, USA, 1987	KC816815	KC816993	———
Rhodophana "*sienna*"	6167 TJB	TB6167[c]	T.J. Baroni, Essex Co., Wilmington, New York, USA, 1989	KC816807	KC816983	KC816890
Rhodophana sp.	434 HAMA	COFC5029[b]	O. Hama, Tamou, Parque Nacional de W. Mekrou, Niger, 2010	———	KC816984	KC816891
Catathelasma imperiale	11CA01A		K.L. Kluting, Redwood National Park, Orick, Humboldt County, California, USA, 2011	KC816816	KC816994	KC816900
Mycena aff. *pura*	11CA007		K.L. Kluting, Redwood National Park, Orick, Humboldt County, California, USA, 2011	KC816817	KC816995	KC816901
Panellus stipticus	11CA052		K.L. Kluting, Grays Falls Campground, Trinity County, California, USA, 2011	KC816818	KC816996	KC816902
Tricholoma flavovirens	11CA038		K.L. Kluting, Humboldt County, California, USA, 2011	KC816819	KC816997	KC816903
Tricholoma aurantium	LCG2308		L.C. Grubisha, British Columbia, Canada	JN019434	JN019705	JN019386

[a]JEPS = Jepson Herbarium, University of California, Berkeley, California, USA (JEPS); [b]Herbario, Departamento de Biología Vegetal, Facultad de Ciencias, Universidad de Córdoba, 14071 Córdoba, Spain (COFC); [c]CORT = State University of New York College at Cortland Herbarium, Cortland, New York, USA (CORT); [d]KEW = The Royal Herbarium , Royal Botanic Gardens, Kew, Richmond, Surrey, England (KEW); [e]The Mycological Herbarium, Botanical Museum, University of Oslo, Oslo, Norway (OSLO).

The three partial gene regions sampled for this study were the mitochondrial ATP synthase subunit 6 (*atp6* ca. 450 bp), the nuclear RNA polymerase subunit II (*rpb2* ca. 600 bp) and the nuclear translation elongation factor subunit 1-α (*tef1* ca. 1000 bp). Multi-copy nuclear rDNA internal transcribed spacer (ITS) region and the large subunit (28S) and mitochondrial small subunit (mtSSU) were not used because of the high frequency of indels, associated alignment ambiguity and the lack of resolution among clades in other phylogenetic analyses (Moncalvo et al. 2000, Hofstetter et al. 2002, Moncalvo et al. 2002, Matheny et al. 2006, Co-David et al. 2009).

DNA isolation, amplification, and sequencing.— Tissues for each sample were excised from preserved collections using the protocol outlined in Baumgartner et al. (2010). Tissues were pulverized using 6.35mm glass beads in an FP120 FastPrep Instrument (QBiogene, Carlsbad CA USA) after lyophilization for at least 30 min and up to 2 h. DNA was extracted using 2× CTAB (cetyl-trimethyl-ammonium-bromide) buffer followed by isolation using phenol-chloroform-isoamyl alcohol (25:24:1). After extraction, supernatants were suspended in Turbo GeneClean GNomic Salt (MP Biomedicals, Solon OH USA) and bound to GeneClean Turbo Columns (MP Biomedicals, Solon OH USA). The columns were washed with 70% EtOH and DNA was eluted from the column with and buffered in 0.1× Tris-EDTA (TE).

Polymerase Chain Reactions (PCRs) were conducted to amplify partial sequences from the three gene regions (*atp6*, *rpb2*, and *tef1*) with previously published primer sets and taxon-specific primers designed for this study using the default parameters in PRIMER3 (Rozen and Skaletsky 2000) (Table 2). All *atp6* sequences were amplified using primers ATP6-3 (Kretzer and Bruns 1999) and ATP6-6r (Binder and Hibbett 2003).

Table 2. Sequences of primers (using IUPAC ambiguity codes), citations for previously published primers and annealing temperature used for PCR amplification of sequences generated for this study. Internal primers are denoted.

Gene Region	Forward Primer	Primer Sequence (5'-3')	Reverse Primer	Primer Sequence (5'-3')	PCR Annealing Temperature (°C)[e]
atp6	ATP6-3[a] (Krezter and Bruns 1999)	TCTCCTTTAGAACAATTTGA	ATP6-6r[a] (Binder and Hibbett 2003)	AACTAATARAGGAACTAAAGCTA	40, 42[f] 44 or 50
rpb2	rpb2-i6f[c] (Co-David et al. 2009)	GAAGGYCAAGCYTGYGGTCT	rpb2-i7r[c] (Co-David et al. 2009)	ATCATRCTNGGATGRATYTC	(touchdown protocol)
rpb2	rpb2-i6f-RhoF1[d]	GAAGGNCARGCWTGYGGTCT	rpb2-RhoR1[d]	GTGRATYTCRCARTGTGTCCA	56, 58[f] or 60
rpb2	rpb2-i6f-RhoF1[d]	(see above)	rpb2-RhoR1b[d]	ATGRATYTCRCARTGTGTCCA	56[f] or 58
rpb2	rpb2-i6f-RhoF1[d]	(see above)	rpb2-RhoR3[d]	TGRATYTCRCARTGCGTCCA	56
rpb2	rpb2-i6f-RhoF2[d]	GAAGGNCARGCWTGYGGCCT	rpb2-RhoR1b[d]	(see above)	50
rpb2	RPB2-5F (Liu, Whelen and Hall 1999)	GAYGAYMGWGATCAYTTYGG	bRPB2-7R[b] (Matheny 2005)	GAYTGRTTRTGRTCTGGGAAVGG	(touchdown protocol)
rpb2	RPB2-5F[a]	(see above)	bRPB2-7R2[b] (Matheny et al. 2007)	ACYTGRTTRTCNGGRAANGG	(touchdown protocol)
rpb2	bRPB2-6F[b] (Matheny 2005)	TGGGGYATGGTNTGYCCYGC	bRPB2-7.1R[b] (Matheny 2005)	CCCATRGCYTGYTTMCCCATDGC	52
tef1	EFA-RhoF1[d]	GCYTCRAATTCACCRGTRCC	EFA-RhoR1[d] (internal)	GNCCARCCYTTRTACCANG	(touchdown protocol)
tef1	EFA-RhoF1[d]	(see above)	EFA-RhoR2[d]	CAARCCYATGTGTGTYGGT	(touchdown protocol)
tef1	EFA-RhoF2[d] (internal)	CNTGGTAYAARGGYTGGNC	EFA-RhoR2[d]	(see above)	54 or 56 (or touchdown protocol[f])
tef1	EFA-RhoF3[d]	GGTGAATTYGARGCYGGTATYT	EFA-RhoR2[d]	(see above)	58
tef1	EFA-RhoF4[d]	GCYGGTATYTCNAARGAYGG	EFA-RhoR2[d]	(see above)	58
tef1	EF1-983F[a] (Rehner 2001)	GCYCCYGGHCAYCGTGAYTTYAT	EF1-1953R[a] (Rehner 2001)	CCRGCRACRGTRTGTCTCAT	48, 50 or 52[f]
tef1	EF1-983F[a]	(see above)	Efgr[a] (Rehner 2001)	GCAATGTGGGCRGTRTGRCARTC	(touchdown protocol)
tef1	EF595F[a] (Kauserud and Schumacher 2001)	CGTGACTTCATCAAGAACATG	Efgr[a]	(see above)	(touchdown protocol)

[a]Primer specific to Fungi. [b]Primer specific to Basidiomycota. [c]Primer specific to the Entolomataceae. [d]Primer specific to the Rhodocybe-Clitopilus clade designed for this study. [e]See METHODS section for touchdown protocol thermal cycling details. [f]Annealing temperature or cycling protocol most frequently used when more than one has been used for a primer combination.

The majority of the *rpb2* sequences were amplified using primers rpb2-i6f-RhoF1 and either rpb2-i7r-RhoR1 or rpb2-i7r-RhoR1b designed for this study (Table 2). Eight additional primer combinations used in rare instances to amplify *rpb2* sequences are outlined in Table 2. The majority of the *tef1* sequences were obtained by two amplifying the region in two separate segments using primers EFA-RhoF1 with EFA-RhoR1 and EFA-RhoF2 with EFA-RhoR2 designed for specific amplification of the *tef1* gene region from *Clitopilus* and *Rhodocybe* (see Table 2 for internal primers). In some instances, PCR amplification of the *tef1* was performed using previously published forward primer EF-983F in conjunction with the reverse primer EF-1953R (Rehner 2001). Five additional primer combinations used very infrequently to amplify the *tef1* region are outlined in Table 2.

PCR amplifications were performed in 25 µL reactions using 1× GoTaq Buffer (Promega, Madison WI USA), 2 mM magnesium chloride, 0.2µM dNTPs, 1-1.5 µM each forward and reverse primer, 0.025 U Taq polymerase (Promega, Madison WI USA), 0.2 mg/mL bovine serum albumin and 0.5-4.0µL of genomic template DNA. *Rpb2* sequences were amplified using a touchdown protocol adapted from Co-David et al. (2009) with an initial incubation of 94 C for 5 min, followed by 12 cycles of 94 C for 1 min, 67 C for 1 min, decreasing 1 C each cycle to a final annealing temperature of 55 C and 72 C 1.5 min, followed by 36 cycles of 94 C for 45 sec, 55 C for 1 min, 72 C for 1.5 min, and followed by a final extension period at 72 C for 7 min. An alternative protocol with an optimized annealing temperature was also utilized: 95 C for 4 min, 35 cycles of 95 C for 30 sec, an annealing temperature dependent on primer combination used (see Table 2) for 1 min, 72 C for 1 min, and a final extension period of 72 C for 7 min. *Tef1*

sequences were amplified using either the touchdown protocol described above or a cycling protocol of 95 C for 3 min, followed by 35 cycles of 95 C for 1 min, an annealing temperature dependent on primer combination used (see Table 2) for 1.5 min, 72 C for 1 min and a final extension period of 72 C for 10 min. Amplification of *atp6* sequences utilized a cycling protocol of 95 C for 5 min, followed by 40 cycles of 95 C for 30 sec, an annealing temperature of 40 C, 42 C or 44 C for 2 min and 72 C for 1 min and a final extension at 72 C for 10 min. For only a few sequences, an annealing temperature of 50 C was used. Annealing temperatures appropriate for each primer combination is given in Table 2. For primer combinations where different annealing temperatures were used for different collections, the most frequently used annealing temperature for the primer combination is also denoted. For sequences with overlapping chromatograms due to indels, weak signal strength or contaminants, PCR amplicons were sub-cloned using the TOPO TA cloning kit version U (Invitrogen, Carlsbad CA USA) following the procedure described in Bergemann and Garbelotto (2006) with two exceptions: (i) isolated colonies were grown overnight in tryptic soy broth and, (ii) sequences of one to eight PCR clones were generated using primers T7 and M13R. Sequences were generated on an ABI3130*xl* at Middle Tennessee State University using sequencing protocols described in Largent et al. (2011). All sequence alignments can be found deposited in TreeBASE (http:/purl.org/phylo/treebase/phylows/study/TB2:S14164) and GenBank accession numbers for sequences generated for this study and voucher information for associated collections are provided in Table 1.

Phylogenetic analysis.— Sequences were assembled and edited using Sequencher ver. 4.2.2 (Gene Codes Corporation, Ann Arbor, MI) and multiple sequence

alignments were generated manually using Se-Al ver. 2.0a11 Carbon (Rambaut 2002). Introns were delimited from the *tef1* sequences using Augustus ver. 2.4 web server (Stanke et al. 2008) and excluded from the alignment prior to phylogenetic analyses. For phylogenetic analyses, a supermatrix of sequences from the three genes was assembled with missing sequence data (12 *atp6* and 13 *tef1* sequences). Alignment lengths of *atp6*, *rpb2* and *tef1* were 471, 906, and 898 bp, respectively.

Prior to phylogenetic analysis, the best-fit model of DNA substitution for the dataset according to Akaike information criterion (AIC) and Bayesian information criterion (BIC) was selected using TOPALi ver. 2.5 (Milne et al. 2008). TOPALi ver. 2.5 uses a ModelTest approach but differs by computing a PhyML tree estimation for each model (Milne et al. 2008). This method tests the 24 and 56 nucleotide models available for MrBayes and PhyML, respectively. The PhyML model test results were only used to determine appropriate rate heterogeneity and substitution rates for ML analyses since RAxML only has one available substitution model, a general time reversible model (GTR). The GTR model with a gamma distribution (GTR+Γ) was used for all partitions in ML analyses. For Bayesian analyses, a symmetric model (A GTR model with fixed equal base frequencies and a gamma distribution (SYM+Γ) was selected for *tef1* and *rpb2*, and a model of GTR+Γ was selected for the *atp6* analysis.

Phylogenetic analyses and levels of support were inferred using a Maximum Likelihood (ML) analysis and ML bootstrap (MLBS) values generated using RAxML-HPC v. 7.2.8 ALPHA (Stamatakis 2006, Stamatakis et al. 2008) for each gene region. All trees were visualized using FigTree v1.4.0 (Rambaut 2012). Gene regions were then analyzed individually to test for topological incongruence using the program compat.py

(Kauff and Lutzoni 2002) following the criteria used by Hofstetter et al. (2007). In the absence of significant incongruence, the combined three-locus dataset was analyzed using a partitioned model. Each dataset was partitioned across gene region and codon position (nine total partitions for the combined matrix, three total partitions for the individual gene regions). ML analyses were conducted using 1000 replications and 1000 MLBS replications. *Panellus stipticus* was used to root the tree since it was inferred as the most distantly related and basal outgroup taxon sampled and produced the highest bootstrap values of the sampled outgroup taxa when used for rooting purposes.

Further branch supports were obtained using a Bayesian approach in MrBayes v.3.2.1 (Ronquist et al. 2012). Analyses of individual gene regions were conducted with two separate runs using one cold and ten heated chains with default temperatures for two concurrent runs of 2,500,000 generations (*atp6*), 1,000,000 generations (*rpb2*), 1,000,000 generations (*tef1*) or 5,000,000 generations (combined three-locus analysis) using the same partitions as in the ML analysis. Trees were sampled every 100 generations and swapping between four chains each generation (number of chains = 11, number of swaps = 3). The substitution rates, transition/transversion rate ratios, character state (stationary nucleotide) frequencies, and the alpha shape parameter were unlinked so that each parameter was estimated independently for each partition. For all analyses, the two runs converged on the same tree topology (standard deviation split frequencies being ≤ 0.01). Scatterplots were generated to determine stationarity (n = 25,000 for *atp6*, 10,000 for *rpb2* and *tef1*, and 50,000 for the combined matrix). A burnin of 50,000 generations, 20,000 generations, 30,000 generations, and 30,000 generations was used for *atp6*, *rpb2*, *tef1*, and the combined matrix, respectively. Parameter values for the remaining samples

after burn-in samples were discarded and Bayesian Posterior probabilities (BPP) and branch lengths were calculated for a 50% majority rule consensus tree.

CHAPTER THREE: RESULTS

Since there was no strong indication of topological incongruence between the three gene regions when analyzed individually, a combined phylogenetic analysis (*atp6+rpb2+tef1*) is presented. This analysis provides strong support values (MLBS ≥ 70 and BPP ≥ 0.95) for all nodes that constitute the backbone of the phylogeny as well as five well-resolved internal clades (Figure 1).

The terminal Clitopilus clade (MLBS = 91, BPP = 0.9999) comprises two internal supported clades (Figure 1, node A). One clade (MLBS = 97, BPP = 1.0) includes the pleurotoid *Clitopilus* species. *C. venososulcatus, C. hobsonii,* unidentified collections that are closely related to *C. argentinus* (referred to here as *C.* cf. *argentinus*) and a currently undescribed taxon tentatively referred to as *C.* "*cinerascens*" (Figure 1, node A1). The second internal clade (MLBS = 100, BPP = 1.0) comprises centrally stipitate *Clitopilus* species *C. apalus, C. peri, C. prunulus,* and *C. paxilloides* (Figure 1, node A2). The Clitocella clade (MLBS = 100, BPP = 1.0) includes species classified as *Rhodocybe* (*R. popinalis, R. fallax,* and *R. mundula*) and this clade is sister to the Clitopilus clade (Figure 1, node B). The Clitopilopsis clade (MLBS = 100, BPP = 1.0) contains multiple isolates of *R. hirneola* and is sister to a clade containing the Clitopilus and Clitocella clades (Figure 1, node C). The Rhodocybe s. str. clade (MLBS = 99 BPP = 1.0) comprises the type species for the genus, *R. caelata*, as well as a diverse assemblage of many taxa classified as *Rhodocybe* species (Figure 1, node D). Lastly, the basal Rhodophana clade (MLBS = 100, BPP = 1.0) includes *Rhodocybe nitellina*, *R. melleopallens*, *R. stangliana*, two undescribed species with abundant clamp connections, tentatively identified as

Rhodophana "*sienna*" and an unidentified *Rhodophana* sp. from Africa (Figure 1, node E).

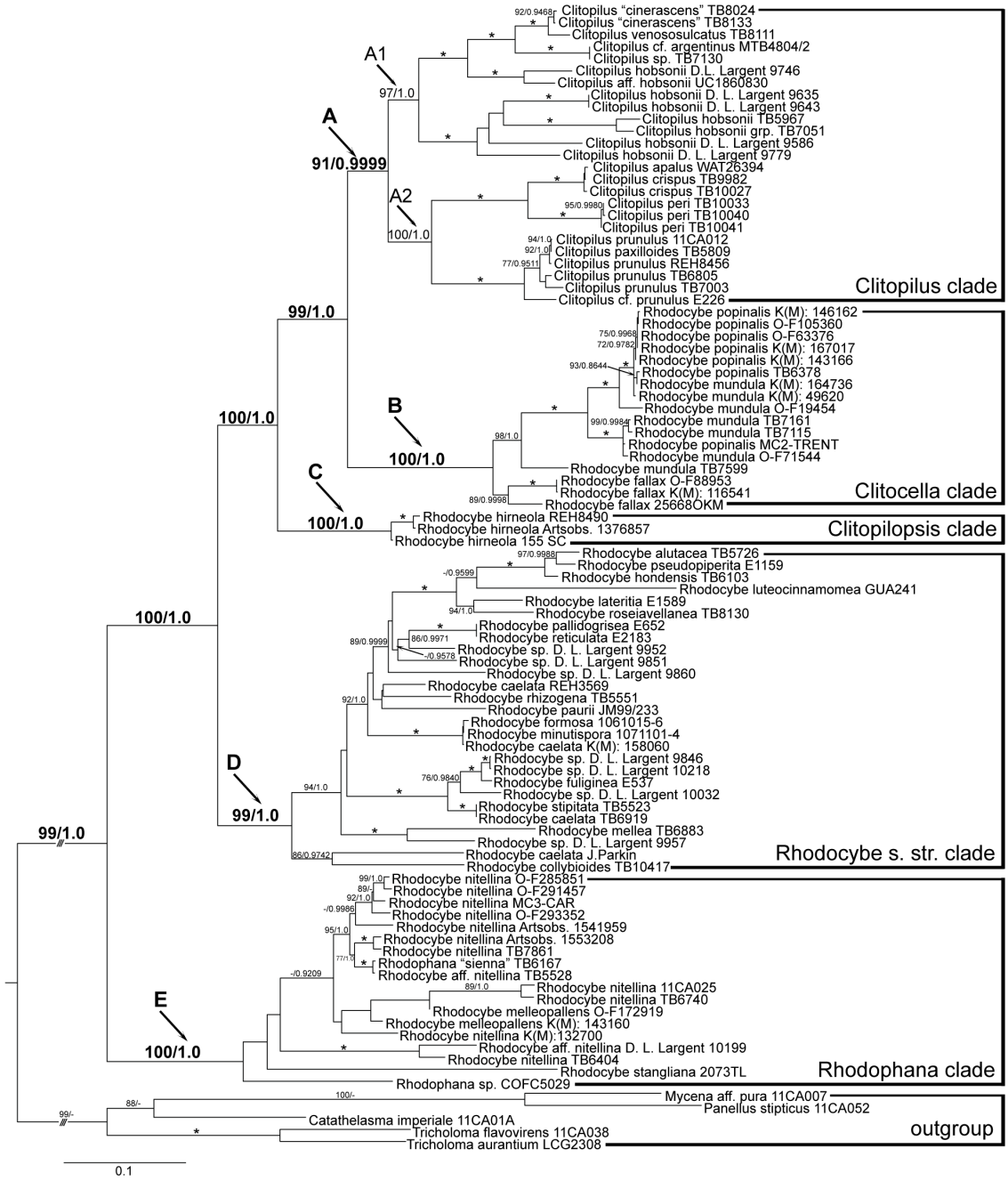

Figure 1. Maximum Likelihood phylogram of the Rhodocybe-Clitopilus clade based on combined sequences from *atp6*, *rpb2* and *tef1*. Branches with an asterisk (*) represent those with support values of both 100 and 1.0 for MLBS and BPP, respectively. Both MLBS and BPP support values are reported for selected nodes (nodes A-E and nodes along the backbone of the inferred phylogenetic tree) and for nodes with high branch support values (MLBS ≥ 70, BPP ≥ 0.95) but MLBS < 100 and/or BPP < 1.0. Truncated branches are denoted with three slanted lines.

Each of the five internal clades of the Rhodocybe-Clitopilus clade can be diagnosed using a combination of micro- and macro-morphological characters. The Clitopilus clade contains taxa with longitudinally ridged basidiospores and either stipitate forms with long-decurrent, narrow, crowded lamellae in stipitate forms or pleurotoid forms (Figure 2, A, B and G). The Clitocella clade comprises taxa that have pustulate-undulate basidiospores and long-decurrent, narrow, crowded lamellae (Figure 2, C and H). The Clitopilopsis clade contains collections of a single morphospecies described as *Rhodocybe hirneola* that have subdecurrent to decurrent lamellae and basidiospores that appear slightly roughened or obscurely ornamented because of slightly thickened walls (Figure 2, D and I). The Rhodocybe s. str. clade contains taxa with variable lamellar attachment ranging from adnexed to adnate and basidiospores with well-defined pustulate-undulate ornamentations (Figure 2, E and J). The basal Rhodophana clade also comprises species that exhibit variable lamellar attachment ranging from adnexed to adnate and prominent undulate-pustulate ornamentations on the walls of the basidiospores, but exhibit abundant hyphal clamp connections, unlike the taxa within the Rhodocybe s. str. clade (Figure 2, F, K and L). Based on the presence of five monophyletic clades with morphological features that support them, *Clitopilopsis* and *Rhodophana* are resurrected, the boundaries of *Rhodocybe* are redefined, and *Clitocella* is described as a new genus with *Clitocella popinalis* designated as the type species.

Figure 2. Macro- and microscopic characters used in the delineation of generic boundaries within the Rhodocybe-Clitopilus clade. Scale bars for A-F = 10 mm. Scale bars for G-L = 5 µm. A. *Clitopilus prunulus* basidiocarp, 9425 T.J. Baroni. B. *Clitopilus hobsonii* basidiocarps, 8490 T.J. Baroni. C. *Clitocella mundula* basidiocarps, 2737 T.J. Baroni. D. *Clitopilopsis hirneola* basidiocarps, 2370 T.J. Baroni. E. *Rhodocybe caelata* basidiocarps, 3843 T.J. Baroni. F. *Rhodophana nitellina* basidiocarps, 7861 T.J. Baroni. G. *Clitopilus prunulus* basidiospores, 3213 T.J. Baroni. H. *Clitocella mundula* basidiospores, 7161 T.J. Baroni. I. *Clitopilopsis hirneola* basidiospores, 8490 R.E. Halling. J. *Rhodocybe caelata* basidiospores, 3569 R.E. Halling. K. *Rhodophana nitellina* basidiospores, 6740 T.J. Baroni. L. *Rhodophana nitellina* clamp connections (at arrows) 6740 T.J. Baroni.

CHAPTER FOUR: TAXONOMY

The five genera now recognized in the Rhodocybe-Clitopilus clade are compared morphologically and the necessary combinations are provided.

Clitocella K. L. Kluting, T. J. Baroni & S. E. Bergemann, gen. nov.

Etymology. *Clitocella* reflects the morphological similarities and phylogenetic placement in relation to *Clitopilus* and *Clitopilopsis*.

Description. Basidiocarps centrally stipitate. Lamellae are long-decurrent, narrow or very narrow (≤ 3mm) and close to crowded or very crowded with a smooth lamellar edge. Basidiospores are flesh-pinkish in deposit and have thin (≤ 0.5µm), evenly cyanophilic walls. Basidiospores are angular in polar view, although often vaguely, and are ornamented with obscurely defined undulating pustules or minute bumps that are visible in profile and face views. Most *Clitocella* species lack cystidia, but if cystidia are present then they are rare and arranged in small bunches along the lamellar edge. If present, cystidia are never as pseudocystidia containing brightly colored contents. Clamp connections are absent.

Type. Clitocella popinalis (Fr.) K. L. Kluting, T. J. Baroni & S. E. Bergemann

Notes. Macroscopically, *Clitocella* closely resembles centrally stipitate *Clitopilus* forms, but differs by the presence of undulate-pustulate basidiospore ornamentation in contrast to the longitudinally ridged basidiospore ornamentation of *Clitopilus*. *Clitocella* differs from *Clitopilopsis* by its presence of close, narrow, long-decurrent lamellae, and *Clitopilopsis* is characterized by having basidiospores with thickened spore walls (≥ 0.5µm), a character not found in *Clitocella*. *Clitocella* can easily be

distinguished from *Rhodocybe* and *Rhodophana* because both *Rhodocybe* and *Rhodophana* possess basidiospores with well-defined and isolated pustules that are distinctly angular in polar view, as opposed to the only obscurely or minutely ornamented spores found in *Clitocella*, and further, *Rhodophana* is characterized by the presence of hyphal clamp connections. Morphologically, this genus appears to be an intermediate between *Clitopilus* and *Rhodocybe*.

Clitopilopsis Maire, Bull. Soc. Hist. nat. Afr. N. 28: 113. 1937.

Description. Lamellae close to sub-distant and decurrent to subdecurrent. Basidiospores are pinkish in deposit, have a slightly thickened wall (≥ 0.5µm), are evenly cyanophilic and nearly smooth in all views, and are round or obscurely angular in polar view. Pseudocystidia and clamp connections are absent.

Type. Clitopilopsis hirneola (Fr.) Kühner, Bull. trimest. Soc. Mycol. Fr. 62:138. 1946.

Clitopilus (Fr. ex Rabenh.) P. Kumm. Führ. Pilzk.: 23, 96. 1871.

Description. Lamellae are long-decurrent, narrow, and close on centrally stipitate species, but adnate or adnexed on pleurotoid forms. Basidiospores are pinkish in deposit, binucleate, longitudinally ridged in face and profile views and angular in polar view. Pseudocystidia and clamp connections are absent.

Type. Clitopilus prunulus (Scop.: Fr.) P. Kumm., Führ. Pilzk.: 97. 1871.

Rhodocybe Maire, Bull. Soc. Mycol. France 40: 298. 1924 [1926].

Description. Lamellae are variously attached ranging from adnexed to adnate or sub-decurrent (but never crowded and long-decurrent in all fruiting bodies). Basidiospores are distinctly angular in polar view, have thin walls that are evenly

cyanophilic, are pinkish in deposit, and have pronounced undulate-pustulate ornamentations. Pseudocystidia present in some species, pleurocystidia and cheilocystidia are often present, and clamp connections are absent.

Type. Rhodocybe caelata (Fr.) Maire Bull. Soc. Mycol. France 40: 298. 1924 [1926].

Rhodophana Kühner Bull. Soc. Mycol. Fr. 87: 23. 1971.

Description. Lamellar attachment ranges from adnexed to adnate (but never decurrent). Basidiospores are distinctly angular in polar view, have thin walls that are evenly cyanophilic, are pinkish in deposit, and have discrete undulate-pustulate ornamentations. Clamp connections are present. Pseudocystidia are absent.

Type. Rhodophana nitellina (Fr.) Kühn. Bull. Soc. Mycol. Fr. 87: 23. 1971.

Clitocella fallax (Quelet) K. L. Kluting, T. J. Baroni & S. E. Bergemann, comb. nov.

Basionym, *Omphalina fallax* Quelet, Compt. Rend. Ass. Franc. Av. Sci. 24: 617. 1895 [1896].

Clitocella mundula (Lasch) K. L. Kluting, T. J. Baroni & S. E. Bergemann, comb. nov.

Basionym. *Agaricus mundulus* Lasch, Linnaee 4: 527. 1829.

Clitocella popinalis (Fr.) K. L. Kluting, T. J. Baroni & S. E. Bergemann, comb. nov.

Basionym. *Agaricus popinalis* Fr., Systema Mycologicum 1: 194. 1821.

Rhodophana melleopallens (Orton) K. L. Kluting, T. J. Baroni & S. E. Bergemann

Basionym. *Rhodocybe melleopallens* Orton, Trans. Brit. Mycol. Soc. 43: 380. 1960.

Rhodophana stangliana (Bresinsky & Pfaff) K. L. Kluting, T. J. Baroni & S. E. Bergemann

Basionym. *Squamanita stangliana* Bresinksy & Pfaff, Z. Pilzk. 34: 169. 1968.

CHAPTER FIVE: DISCUSSION

This phylogenetic hypothesis for the Rhodocybe-Clitopilus clade recognizes five resolved internal clades based on a three-gene analysis. Co-David et al. (2009) provided a phylogenetic analysis to evaluate generic boundaries within the Entolomataceae and found no evidence for clear segregation of species placed *Clitopilus* and *Rhodocybe*. In contrast, more recent studies provide support for multiple well-resolved clades within the Rhodocybe-Clitopilus clade (Baroni and Matheny 2011, Baroni et al. 2011). The analysis presented here is in close agreement with the analysis of Baroni and Matheny (2011) with two exceptions. First, these results provide support for two sub-clades differentiated by stipe attachment within the Clitopilus clade. Additionally, the results of this study indicate that the taxa formerly classified as *Rhodocybe fallax, R. mundula* and *R. popinalis* constitute a segregate sister lineage to the Clitopilus clade. The results of this analysis support the recognition of five genera within the Rhodocybe-Clitopilus clade.

A new genus, *Clitocella*, is proposed to accommodate *Rhodocybe mundula*, *R. popinalis*, and *R. fallax*, a unique lineage with long-decurrent lamellae and basidiospores that have unorganized and obscurely defined pustules on all surfaces. *Clitocella* species macroscopically resemble centrally stipitate *Clitopilus* species with regard to the clitocyboid stature (centrally stipitate with crowded, narrow, long-decurrent lamellae) but possess basidiospores that are covered in pustules as opposed to the longitudinally ridged basidiospores found in *Clitopilus*. The pustules and basidiospore angularity in polar view of *Clitocella* are not nearly as conspicuous as those observed in *Rhodocybe* and

Rhodophana species. *Clitocella* appears to be a morphological intermediate to *Clitopilus* and *Rhodocybe*.

Additionally, the recognition of *Clitopilopsis*, *Rhodocybe*, and *Rhodophana* to accommodate the three remaining internal lineages is proposed since our analyses agree with those of Baroni and Matheny (2011) in providing support for the Clitopilopsis, Rhodocybe s. str. and Rhodophana clades. The Rhodocybe s. str. clade includes the type species for the *Rhodocybe*, *R. caelata*, and therefor should retain the generic epithet *Rhodocybe.* The Clitopilopsis clade resolved in the analysis presented here and in the analysis of Baroni and Matheny (2011) is comprised of multiple isolates of *Clitopilopsis* (*Rhodocybe*) *hirneola,* the type species for the genus. *Clitopilopsis* is distinct because it has thickened, obscurely ornamented basidiospores that appear slightly roughened to nearly smooth. At present, at least two species are associated with the genus *Clitopilopsis*. At least one more species, *Rhodocybe heterospora* (Murr.) T. J. Baroni, shares this unique basidiospore morphology but was not represented in this study since the species is only know from a single type collection. The currently accepted name for *C. hirneola*, according to indexfungorum.org and mycobank.org, is *Rhodocybe hirneola* (Fr.) P. D. Orton (1960). Placement in *Rhodocybe* reflects the recognition of pustulate basidiospore walls (Baroni 1981). Only recently has this taxon been referred to as *Clitopilopsis hirneola* in phylogenetic analyses (Moncalvo et al. 2002, Baroni and Matheny 2011). Based on the presence of a unique basidiospore morphology and the number of samples tested in phylogenetic analyses, there is now ample evidence to formally resurrect the genus *Clitopilopsis*.

The second resurrected genus in this proposed classification is *Rhodophana*. Kühner (1947) described the genus based on the type species *Rhodophana nitellina* (Fr.) Kühn. to accommodate all *Rhodocybe* species with uninucleate basidiospores and abundant clamp connections. The formal description lacked the necessary components to be accepted, however, and thus received the designation of nomen nudum. *Rhodophana* was not formally validated until Kühner (in Kühner & Lamoure 1971) provided a Latin diagnosis, where he recognized it as a subgenus of *Rhodocybe* (*Rhodocybe* subgenus *Rhodophana*) instead of recognizing it as a unique genus (*Rhodophana*) (Pegler and Young 1975). Regardless of whether *Rhodophana* was intended as a generic or as a subgeneric taxon, the Latin diagnosis provided by Kühner has been accepted as the description of a novel generic concept (Indexfungorum.org, Mycobank.org). Baroni (1981) and Singer (1986) agreed that the presence of abundant clamp connections was an important character and both recognized *Rhodocybe* section *Rhodophana* in their infrageneric classifications for *Rhodocybe*. The phylogenetic data presented in this study support a clade comprising species that possess abundant clamp connections as well as basidiospores with undulate-pustulate ornamentation. The use of *Rhodophana* is proposed to accommodate these species and is formally resurrected here. It is also important to note that Kühner (in Kühner & Lamoure 1971) reported the presence uninucleate basidiospores in *Rhodocybe nitellina* and binucleate basidiospores in *R. popinalis* and *R. caelata*. Based on this observation, he designated the presence of uninucleate basidiospores as a defining feature for *Rhodophana*. The number of nuclei per basidiospore is a character that was not examined in this study, although this character should be evaluated in future studies.

The revised generic classification proposed here conflicts with the classification proposed by Co-David et al. (2009). Co-David et al. (2009) amended the circumscription of *Clitopilus* to include all of the species within the Rhodocybe-Clitopilus clade since they did not find evidence for the clear segregation of *Rhodocybe* and *Clitopilus* species based on their phylogenetic analysis. Although this delimitation recognizes a monophyletic group, it disregards the diagnostic character of spore ornamentation used for separating taxa that were placed into *Clitopilus* and *Rhodocybe* by recognizing one large genus with basidiospores that vary widely morphologically. In addition to morphological evidence for segregate genera, five well-resolved internal clades are supported by the data presented here. The results of the current analysis support the monophyly of *Clitopilus* and the four distinct clades for species traditionally placed in *Rhodocybe* based on spore ornamentation and each of these clades is supported by morphological evidence. Recognizing *Clitopilus*, *Clitocella*, *Clitopilopsis*, *Rhodocybe* and *Rhodophana* as five distinct and monophyletic genera as opposed to only a single genus more precisely depicts the morphological and evolutionary diversity within the Rhodocybe-Clitopilus clade.

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