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***Thelomma ocellatum*, a range extension to the Yukon Territory and case study in the use of molecular data to recognize asexually reproducing crustose lichens**

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Abstract: An unusual sterile, asexually reproducing crustose lichen was encountered during fieldwork in the Yukon Territory of Canada. Genus and family level placement of the taxon were precluded by a lack of both sexual characters and any non-sexual characters that would have suggested an unambiguous generic affiliation. Examination of mtSSU and nrITS sequence data of the taxon revealed it to be a member of the Caliciaceae, with a sister relationship to the genus *Tholurna*. Subsequent molecular phylogenetic analyses of mtSSU sequence data suggested conspecificity with *Thelomma ocellatum*, one of the few calicioid lichens that reproduce asexually via lichenized diaspores. Comparison of the material from the Yukon with reference specimens and descriptions of *T. ocellatum* confirmed the identification of these populations, which extend the known distribution of *T. ocellatum* considerably northward in North America.

Key words: Sterile crustose lichens, biogeography.

Introduction: During recent fieldwork to study the genus *Lepraria* s.l. in the Yukon Territory the author collected material of an unusual sterile crustose lichen growing on old, dry, weathered logs at two localities. The lichen resembled an overgrown form of *Buellia griseovirens* (Turner & Borrer *ex* Sm.) Almb. because of its white thallus with what appeared to be dark brownish-black soredia. Study of the specimens upon return to The New York Botanical Garden revealed they were morphologically and chemically discordant with *B. griseovirens*: in addition to the morphological differences noted in the field the material was found to lack both atranorin and norstictic acid, two substances considered diagnostic for *B. griseovirens* (Elix & Tønsberg 1999, Nordin 2000, Tønsberg 1992). Thus, despite the distinctive appearance of the material, the author was faced with two specimens that lacked any observable characters that would unequivocally allow them to be placed in a known genus or family of lichen forming fungi.

Normally the fate of such material would have been to be filed away with other undetermined problem specimens as a “known unknown” (cf. Spribille et al. 2010). However, the author had recently completed two studies aimed at using molecular phylogenetic analyses to place otherwise unassignable sterile crustose lichens into a higher level taxonomic framework (Hodkinson & Lendemer 2012; Lendemer & Hodkinson 2012). In the hope that similar protocols could be used to elucidate the identity of the specimens from the Yukon, nrITS and mtSSU sequences were generated for both specimens and analyzed. The results of these analyses, together with additional morphological investigations, are presented here.

Materials and Methods: *Microscopy and chemistry.* This study is based on material collected by the author and deposited in the herbarium of The New York Botanical Garden (NY). Reference material of *Thelomma ocellatum*

from that institution was also consulted (see Appendix 2). All specimens were studied following Allen et al. (2012) with both light and scanning electron microscopy techniques as well as illustration preparation following the methods of those authors. Thin Layer Chromatography (TLC) was performed in Solvent C following Culberson and Kristinsson (1970).

DNA extraction, PCR amplification, and sequencing. DNA extraction, PCR amplification, sequencing, and sequence assembly follow the methods of Hodkinson and Lendemer (2012) for both the mtSSU and nrITS regions.

Taxon sampling and phylogenetic methods. The protocol for assessing the phylogenetic placement of the taxon in question largely followed that outlined by Hodkinson and Lendemer (2012). That protocol was developed specifically to elucidate the higher level placement and affinities of sterile crustose lichens that cannot be placed in a higher level phylogenetic context using only non-molecular characters. Based on the results of the BLASTn searches of both mtSSU and nrITS sequences detailed above, the taxon was found to have affinities to the Buellioideae lineage of the Caliciaceae in the order Caliciales (Gaya et al. 2012).

The mtSSU gene was used to assess the higher level affinities of the taxon (Hodkinson and Lendemer 2012) and a dataset of representative mtSSU sequences of members of the lineages within the orders Caliciales and Teloschistales (*sensu* Gaya et al. 2012) was assembled as follows. First, the full multi-gene dataset of the Lecanoromycetes from Miadlikowska et al. (2006) was downloaded. Using Mesquite 2.74 the dataset was reduced to 1) contain only data from the mtSSU region, 2) only representatives of the Caliciales and Teloschistales, and 3) only representatives of these orders with mtSSU sequence data. The following sequences were then added to the pruned dataset: 1) the two mtSSU sequences generated for this study and 2)

any of the top twenty sequences obtained by BLASTn searches of the newly generated mtSSU sequences that represented taxa not already present in the dataset. The multiple sequence alignment tool available in Mesquite was then used to align the newly added sequences to those already present in the dataset. Subsequent to the automated alignment, the alignment was adjusted by hand and ambiguously aligned regions were defined as an exclusion set following Lendemer and Hodkinson (2010).

An initial maximum likelihood (ML) analysis was then conducted on the dataset to assess whether there was support for placement of the taxon in question in the Buellioideae lineage of the Caliciales as was suggested by the BLASTn searches. The input file for this analysis was produced by manually deleting the ambiguously aligned regions from the alignment and then exporting the alignment from Mesquite as a PHYLLIP file. A rapid ML search and bootstrap analysis with 500 replicates, using model GTRGAMMA was conducted. The results (see Appendix 1) recovered the sequences of the taxon in question as both sister to *Tholurna dissimilis*, and a member of the Caliciales with strong bootstrap support (ML-BP 96 and 100 respectively).

Based on the results of the analysis described above, the dataset was further pruned to contain only members of the Caliciales. The alignment and ambiguously aligned regions were then redefined to reflect the revised dataset. The dataset was then prepared for maximum parsimony (MP), ML, and Bayesian Inference (BI) analyses. The input files for all analyses conducted for this study have been deposited in the Dryad Data Repository as DOI doi:10.5061/dryad.6mp50.

MP analyses were conducted using PAUP* 4.0b10 on a version of the dataset with ambiguously aligned regions and constant sites excluded. The former were not included as

recoded characters. An initial search was made with 1000 random-addition-sequence (RAS) replicates and tree bisection reconnection (TBR) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were saved with branch lengths, and a strict consensus tree was computed for reference. In this analysis the best tree island was hit in 96% of the replicates, and 14 equally most parsimonious trees were recovered. Branch support for MP trees was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with five RAS per bootstrap replicate, with all other settings as above. The results of these analyses were visualized in PAUP and the trees exported as PICT files.

ML analyses were run using RaxML 7.2.6 on a version of the dataset wherein ambiguously aligned regions had been manually deleted using Mesquite and the alignment then exported as a PHYLLIP formatted file. A rapid ML search and bootstrap analysis with 500 replicates, using model GTRGAMMA was conducted. The results were visualized in FigTree 1.3.1.

For BI analyses MrModeltest (Nylander 2004) was used to select a proper model of nucleotide substitution. Using the Akaike Information Criterion (AIC; Akaike 1973), HKY+I+G was selected. BI was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) and a NEXUS formatted version of the alignment. A MrBayes block was produced through the online automated from found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html). The model parameters produced by MrModeltest were pasted directly into the MrBayes block. The Markov chain Monte Carlo parameters consisted of 10,000,000 generations, with four chains, and a tree sampled every 100 generations. The first 10,000 trees were discarded as burn-in and the results were summarized as a 50% majority rule consensus tree.

Results: The results of the molecular phylogenetic analyses of mtSSU sequence data of members of the Caliciales are presented in figure 1. These analyses recovered the sequences generated from the unknown taxon collected in the Yukon Territory as members of a strongly supported (MP/ML/BI: 100/100/1.0) clade together with the one reference sequence of *Thelomma ocellatum* available in GenBank. *Thelomma ocellatum* is one of the few calicioid fungi that reproduce via lichenized diaspores (Tibell 1976) and is characterized by its bullate areolate gray thallus that does not react with spot tests and produces abundant lichenized diaspores in the form of short globose isidia and coarse, dark brown-black soredia (Tibell 1976; see figures 2-3 herein). Although apothecia were not observed in the populations from the Yukon, the thalli in these populations match the published descriptions and comparative material (see Appendix 2) of *T. ocellatum* in all respects. As such the populations from the Yukon are considered to belong to *T. ocellatum* and the clade containing these sequences is hereafter referred to using this name.

Assignment of the Yukon material to *Thelomma ocellatum* represents a significant range extension for the taxon in North America (figure 4). Until now the species has generally been thought to occur in the mountains of western North America only from southern California, north to British Columbia (McCune & Rosentreter 1995). Recently, Schiefelbein (2011) summarized the worldwide distribution of *T. ocellatum* and included several reports from northern North America based on the distribution map published by Thomson (1997) (U. Schiefelbein pers. comm.). Since these records were not included in the distribution mapped by McCune and Rosentreter (1995) it was necessary to borrow the relevant vouchers from WIS to confirm their identification. Examination of these specimens (see Appendix 3) revealed that they represented *Cypheium inquilans* (Sm.) Trevis., a very different calicioid

lichen that lacks soralia. It is interesting to note that the sequences of *Thelomma ocellatum* were recovered with moderate to strong support (MP/ML/BI: 81/85/1.0) as members of a clade containing two other rare calicioid members of the Caliciaceae that occur in western North America, namely *Texosporium sancti-jacobi* (Tuck.) Nádv. and *Tholurna dissimilis* (Norman) Norman.

Discussion: Despite a significant increase in study in recent years, many crustose lichens that are typically sterile and reproduce via lichenized diaspores remain poorly understood, inadequately studied, or undescribed (Kantvilas & Lumbsch 2010; Hodkinson & Lendemer 2012). As has been summarized by Hodkinson and Lendemer (2012) these problems are due in large part to two factors: the widespread perception that sterile crustose lichens are difficult to identify and the fact that in the absence of sexual fruiting bodies it can be difficult or impossible to place a specimen or hypothetical taxon in a higher level taxonomic framework based only on secondary non-sexual characters. In the context of the present study it would have been impossible to identify the material from the Yukon as *Thelomma ocellatum* based on non-sexual characters alone unless one was already familiar with the existence, and more importantly, the gestalt of the taxon. Indeed, the combination of characters present in the Yukon material (e.g., lack of secondary chemistry, gray bullate thallus, dark isidia or soredia, non-micareoid coccoid photobiont) would hardly allow placement in a single genus, family, or order.

Paradoxically the same suite of characters results in a distinctive morphological and ecological gestalt that is instantly recognizable in the field. It is true that in hindsight the species-level identification of this material as *Thelomma ocellatum* would have been possible using non-molecular characters and certain specific keys for nearby geographic regions (e.g., McCune (2012);

though not Nash (2007) where it is keyed as being isidiate and having norstictic acid). However, considering the geographic separation between the Yukon populations and those previously reported by McCune and Rosentreter (1995) the identification would have been tentative in the absence of fertile material. This is particularly true because of the similarity between the thallus of *T. ocellatum* and some lignicolous species of *Caloplaca* described from the Great Plains and arid western interior of North America (e.g., Wetmore 2009).

In the present case the role of mtSSU sequence data were analyzed to inform a higher level placement for the material such that the existing taxonomic literature could be consulted in search of a species identification or affiliation. It is surprising that relatively few published studies have explicitly attempted to use molecular data to elucidate the phylogenetic affinities, and ultimately the identifications, of otherwise unassignable sterile crustose lichens. That this is the case is all the more remarkable because nearly all such studies have met with a high degree of success (e.g., Hodkinson & Lendemer 2012, Lendemer & Lumbsch 2008, Nelsen et al. 2012, Vondrák et al. 2010), although this is not universal (Kantvilas & Lumbsch 2010). Some may consider this study a minor note, reporting range extension for *Thelomma ocellatum* in North America. Echoing Vondrák et al. (2010) however, it is nonetheless a timely and much needed one that reiterates the remarkable potential for molecular data, used in tandem with reciprocal studies of chemical, ecological, and morphological data, to reshape the study of asexually reproducing crustose lichens. In addition, it is important in terms of allowing effective phylogenetic placement and taxonomic description, as well as facilitating the biogeographic documentation of an understudied component of many lichen biotas worldwide.

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Literature cited

- Akaike H. 1973. Information theory and an extension of the maximum likelihood principle. Pp. 267-281 in: Petrov, B. N. and F. Csaki, eds. *Proceedings of the 2nd International Symposium on Information Theory*. Akademiai Kiado, Budapest.
- Allen, J. L., B. P. Hodkinson and C. R. Björk. 2012. A major range expansion for *Platismatia wheeleri*. North American Fungi 7(10): 1–12. <http://dx.doi.org/10.2509/naf2012.007.010>
- Culberson, C. F. and H. Kristinsson. 1970. A standardized method for the identification of lichen products. Journal of Chromatography 46: 85–93. [http://dx.doi.org/10.1016/S0021-9673\(00\)83967-9](http://dx.doi.org/10.1016/S0021-9673(00)83967-9)
- Elix, J. A. and T. Tønsberg. 1999. Notes on the chemistry of some lichens from Norway. Graphis Scripta 10(1&2): 4–6.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. <http://dx.doi.org/10.2307/2408678>
- Gaya, E., F. Högnabba, A. Holguin, K. Molnar, S. Fernández-Brime, S. Stenroos, U. Arup, U. Söchting, P. van den Boom, R. Lücking, H. J. M. Sipman and F. Lutzoni. 2012. Implementing a

- cumulative supermatrix approach for a comprehensive phylogenetic study of the Teloschistales (Pezizomycotina, Ascomycota). *Molecular Phylogenetics and Evolution* 63: 374–387.
<http://dx.doi.org/10.1016/j.ympev.2012.01.012>
- Hodkinson, B. P. and J. C. Lendemer. 2012. Phylogeny and taxonomy of an enigmatic sterile lichen. *Systematic Botany* 37(4): 835–844.
<http://dx.doi.org/10.1600/036364412X656536>
- Huelsenbeck J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Kantvilas, G. and H. T. Lumbsch. 2010. A new species and new record of Australian *Scoliciosporum*. *Australasian Lichenology* 66: 16–23.
- Lendemer, J. C. and B. P. Hodkinson. 2012. *Chirleja buckii*, a new genus and species of lichenized-fungi from Tierra del Fuego, southern South America. *New Zealand Journal of Botany* 50(4): 449–506.
<http://dx.doi.org/10.1080/0028825X.2012.732093>
- Lendemer, J. C. and B. P. Hodkinson. 2010. A new perspective on *Punctelia subrudecta* (Parmeliaceae) in North America: previously rejected morphological characters corroborate molecular phylogenetic evidence and provide insight into an old problem. *Lichenologist* 42(4): 405–421.
<http://dx.doi.org/10.1017/S0024282910000101>
- Lendemer, J. C. and H. T. Lumbsch. 2008. *Protoparmelia capitata* sp. nov., and *P. isidiata* Diederich, Aptroot & Sérus., two species of *Protoparmelia* (Lecanorales, Ascomycota) from south-eastern North America. *Lichenologist* 40(4): 329–336.
<http://dx.doi.org/10.1017/S0024282908007810>
- McCune, B. 2012. Key to the Lichen Genera of the Pacific Northwest. Published by the author, Corvallis. 94pp.
- McCune, B. and R. Rosentreter. 1995. Distribution and ecology of *Thelomma ocellatum* in western North America. *Evansia* 12(3): 102–106.
- Miadlikowska, J., F. Kauff, V. Hofstetter, E. Fraker, M. Grube, J. Hafellner, V. Reeb, B. P. Hodkinson, M. Kukwa, R. Lücking, G. Hestmark, M. G. Ojalora, A. Rauhut, B. Büdel, C. Scheidegger, E. Timdal, S. Stenroos, I. M. Brodo, G. B. Perlmutter, D. Ertz, P. Diederich, J. C. Lendemer, P. F. May, C. Schoch, A. E. Arnold, C. Gueidan, E. Tripp, R. Yahr, C. Robertson and F. Lutzoni. 2006. New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* 98(6): 1088–1103.
<http://dx.doi.org/10.3852/mycologia.98.6.1088>
- Nash, T. H. 2007. Sterile crustose lichens. Pp. 408–411 in: Nash, T. H., C. Gries and F. Bungartz, eds. *Lichen Flora of the Greater Sonoran Desert Region. Volume 3*. Lichens Unlimited, Arizona State University, Tempe.
- Nelsen, M. P., R. Lücking, C. J. Andrew, E. Rivas Plata, J. L. Chaves, M. E. S. Cáceres and N. Ventura. 2012. Dismantling *Herpothallon*: *Herpothallon antillarum* (Arthoniomycetes: Arthoniaceae) is a member of the genus *Diorygma* (Lecanoromycetes: Graphidaceae). *Bryologist* 115: 313–321.
<http://dx.doi.org/10.1639/0007-2745-115.2.313>
- Nordin, A. 2000. Taxonomy and phylogeny of *Buellia* species with pluriseptate spores

(Lecanorales, Ascomycotina). *Symbolae Botanicae Upsalienses* 33(1): 1–117.

Nylander J. A. A 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala, Sweden.

Schiefelbein, U. 2011. Distribution and ecology of the lichen *Thelomma ocellatum* (Caliciaceae) in Pomerania and the world. *Plant Diversity and Evolution* 129(3-4): 241–251.

<http://dx.doi.org/10.1127/1869-6155/2011/0129-0046>

Spribille, T., S. Pérez-Ortega, T. Tønsberg and D. Schirokauer. 2010. Lichens and lichenicolous fungi of the Klondike Gold Rush National Historic Park, Alaska, in a global biodiversity context. *Bryologist* 113(3): 439–515.

<http://dx.doi.org/10.1639/0007-2745-113.3.439>

Thomson, J.W. 1997. *American Arctic Lichens. 2. The Microlichens*. The University of Wisconsin Press, Madison. 675 pp.

Tibell, L. 1976. The genus *Thelomma*. *Botaniska Notiser* 129: 221-249.

Tønsberg, T. 1992. The soorediate and isidiate, corticolous, crustose lichens in Norway. *Sommerfeltia* 14: 1–331.

Wetmore, C.M. 2009. New species of *Caloplaca* (Teloschistaceae) from North America. *The Bryologist* 112(2): 379-386.

<http://dx.doi.org/10.1639/0007-2745-112.2.379>

Vondrák J., O. Redchenko, D. Himelbrant, I. Stepanchikova and E. Kuznetsova. 2010. Some sterile *Caloplaca* crusts identified by molecular data from the Leningrad region (Russia). *Folia Cryptogamica Estonica* 47: 97–99.

Figure 1, Phylogeny of representatives of the Caliciales, including *Thelomma ocellatum* (clade shaded in gray), inferred from mtSSU sequence data and displayed as the most likely tree with Physciaceae selected as the outgroup. Support was evaluated using maximum parsimony (MP) and maximum likelihood (ML) bootstrap proportions (BP) and Bayesian posterior probabilities (B-PP). Support values are displayed at each node in the following format: MP-BP/ML-BP/B-PP. Thickened branches are those that were recovered with MP-BP and ML-BP support $\geq 70\%$ and B-PP $\geq 95\%$.

Figure 2, comparison of morphology of European population of *Thelomma ocellatum* (left, *Tibell 6313*) to one from the Yukon Territory (right, *Lendemer 28967*). A-B, gross morphology of the thallus (scales = 1.0 mm). C-D, detail of the thallus (scales = 0.5 mm). E-F, detail of the lichenized diaspores (scales = 0.25 mm).

Figure 3, scanning electron micrographs of *Thelomma ocellatum* (all from *Lendemer 28767*). A-B, morphology of the thallus. C-D, detail of the regions where lichenized diaspores develop. E-F, detail of the lichenized diaspores. Scale bars are as indicated.

Figure 4, geographic distribution of *Thelomma ocellatum* in North America as presently known (red dots = records from CNALH; yellow dots = new reports from this study).

Appendix 1

Results of preliminary ML analysis of mtSSU sequence data of the unknown lichen from the Yukon Territory and members of the Teloschistales and Physciales derived from the pruned dataset published by Miadlikowska et al. (2006). The results are displayed as the most likely tree, with branch support evaluated as ML-BP. Members of the Teloschistales were selected as the outgroup based on Miadlikowska et al. (2006). Support values $\geq 50\%$ are displayed and thickened branches are those that were recovered with ML-BP support $\geq 70\%$.

Appendix 2

Specimens of Thelomma ocellatum examined. – **Canada:** *Yukon Territory:* N side of Wheaton River, S of Annie Lake Rd., at km post 24, on log, 2011, *Lendemer 28767* (NY; Isolate NY-1311, GenBank #KF020558 (ITS), #KF020560 (mtSSU)); E side of Klondike Hwy. (Yukon 2), 3.5 km S of Carcross, on log, 2011, *J.C. Lendemer 28967* (NY; Isolate NY-1313, GenBank #KF020559 (ITS), #KF020561 (mtSSU)). **Sweden:** *Jämtland:* Offerdal par., 42 km NE of Åre, Ytterolden, on W facing wall of old logged barn, 1975, *L. Tibell 6313* (NY).

Appendix 3

Specimens from WIS annotated by J.W. Thomson as Thelomma ocellatum but which actually represent Cyphelium inquinans. – **Canada:** *Northwest Territories:* Mackenzie District, Reindeer Station, on an old stump, 1964, *J.W. Thomson & J.A. Larsen 14998* (WIS-L-0099226), *J.W. Thomson & J.A. Larsen 15616* (WIS-L-0099227), on a dead spruce stub, 1964, *J.W. Thomson & J.A. Larsen 15520* (WIS-L-0099228); Keewatin District, Roseblade Lake, on open spruce and tamarack, 1977, *J.D. Johnson 3833* (WIS-L-0099222); unnamed tributary of the Arctic Red River, entering from the east side, ca. 120 mi WNW of Norman Wells, on spruce stub, *C.D. Bird & J.W. Thomson 19861* (WIS-L-0099229).