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### The Mayfly Newsletter

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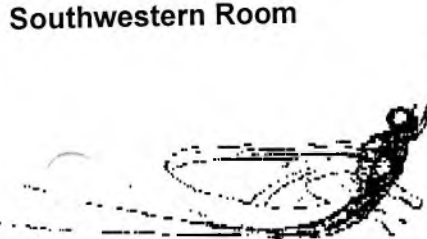
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# THE MAYFLY NEWSLETTER

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## DNA and Mayfly Taxonomy

### DNA Barcoding North American Mayflies - A Call for International Collaboration

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Because mayflies (Ephemeroptera) are common in many freshwater habitats, they are widely used in freshwater biomonitoring. However, difficulties in species-level identifications and the uncertainty of certain life stage associations present a major impediment. The recently launched DNA barcoding initiative can help to standardize identifications and enable confident association of larval and adult stages.

DNA barcoding is based on the observation that a short, standardized segment of the genome can enable species identification and discovery. There is now clear evidence that a 658-bp segment positioned near the 5' terminus of the mitochondrial cytochrome c oxidase subunit I (COI) gene is extraordinarily effective in discriminating members of the animal kingdom, allowing unambiguous identification of more than 98% of animal species in studies that have examined a wide range of taxonomic groups.

A large-scale program to identify North American freshwater macroinvertebrates utilizing COI sequences has been launched by the Canadian Centre for DNA Barcoding (CCDB, <http://www.dnabarcoding.ca>) at the University of Guelph. In order to develop this approach to the identification of aquatic insects, a COI barcode reference library must first be established from expertly identified specimens. Once a COI barcode is linked with a named species, query sequences from unidentified specimens can be compared with the reference barcode and suggested identifications are generated based on the result.

Among all freshwater macroinvertebrates, mayflies clearly deserve high priority for developing a barcode reference library because of their great diversity and abundance. Thus, researchers at the CCDB are launching an international

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### Large-scale DNA Sequencing in Taxonomy and Conservation: A Case Study with the Mayfly Family Heptageniidae (Ephemeroptera) in the Alps and Madagascar

Laurent Vuataz, Michel Sartori, Olivier Glazot and Jean-Luc Gattolliat, Musée cantonal de zoologie, Palais de Rumine, Place de la Riponne 6, CH-1014 Lausanne, Switzerland, Vuataz@unil.ch. In collaboration with Luca Fumagalli, Lausanne University, and Michael Monaghan, Natural History Museum, London, UK.

Advances in high-throughput DNA sequencing and analysis are transforming most aspects of organismal biology, but their potential for the study of biodiversity, taxonomy, and evolution has yet to be fully realized. Applied to whole communities and entire faunas, large-scale sequencing could provide rapid species inventories and a means to characterize biodiversity where an existing taxonomy is incomplete or absent. The need for such advances is critical - only 10-20% of Earth's species have been formally described and the rate of conventional species description would require a 1000-fold increase to meet the existing taxonomic needs of the global community (Seberg, 2004). This is particularly true for the functionally important but morphologically cryptic meio- and micro-fauna (Blaxter *et al.*, 2004). Freshwater ecosystems are the most threatened of the world's natural resources (Abell, 2002), and the knowledge of their fauna is particularly relevant for conservation purposes (Dudgeon *et al.*, 2006).

Here we develop novel, DNA-based methods to provide

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DNA barcoding project with the aim of barcoding all mayfly species of the Nearctic fauna. Because there are more than 600 species of mayflies in North America, no single researcher or institute can carry out this project. However, the task can be completed through an international collaboration involving mayfly systematists, freshwater ecologists, and biomonitoring specialists. All collaborators will benefit from the results of this project - barcode sequences will advance our understanding of mayfly biology by discovering cryptic species, by revealing cases of over splitting, by supplementing the description of new species, by associating life stages, by tracing dispersion histories, and by creating an easy system for the identification of any life stage of any species.

The tool of DNA barcoding shows great potential for use by those studying the systematics of many Ephemeroptera species groups. One example of the utility of barcoding is the verification of stage associations, especially those not made by careful rearing. Recent revisionary work on the family Ephemerellidae Klapalek provides an illustration. The species concept of *Ephemerella altana* Allen, a western Nearctic taxon, had been based on a larva belonging to the genus *Ephemerella* Walsh and an adult of *Serratella* Edmunds. Had barcoding technology been available at the time of *E. altana*'s discovery and description, it potentially could have shown that this association was erroneous. Furthermore, barcoding could have helped to resolve the species identities of the larva and adult. Based on traditional specimen comparisons, the larva is thought to be that of the transcontinental species, *Ephemerella excrucians* Walsh, and the adult to be that of the western species, *Serratella micheneri* (Traver). *Ephemerella excrucians* exhibits an amazing amount of morphological variability throughout its wide geographic range, which begs the question of whether the current species concept might contain various cryptic lineages that are unrecognizable by traditional, morphological means. Barcoding technology could be used to study various populations, including those from type locales, and could provide a guideline for decisions about species identities and boundaries.

We seek (1) specimens from throughout the Nearctic Region and (2) assistance in their identification from any researcher interested in joining this project as a collaborator.

#### **Collecting and preserving mayflies for DNA analysis**

The conventional ways of preserving mayfly specimens include preservation of larval and adult stages in 70% ethanol. However, DNA degrades rapidly in ethanol because of the acidification of ethanol through time. As a result ethanol preserved specimens should be analyzed within a year or two of capture. Collecting and preserving mayflies in high concentration ethanol (95%) does slow DNA degradation. Ideally, ethanol should be changed a few days after the initial collection. This is particularly critical for larval specimens because of the large amount of water in their bodies. Other factors, such as temperature and exposure to sunlight can affect the life of DNA as well. Specimens

should be kept in a refrigerator when possible. Unfortunately, mayfly specimens, especially adults, become very brittle in high concentration ethanol. A potential solution would be preparing a separate set of specimens for DNA analysis. Alternatively, legs can be pulled and kept in 95% ethanol for DNA purpose while the remainder of the specimens can be stored in the traditional way.

Male adults and larvae are preferred, but female adults can also be used, especially if they have been identified to species-level by association with identifiable stages. Our recent study on the Ephemeroptera of the Great Smoky Mountains National Park has shown that life-stage associations for most species were correct. In order to measure the levels of genetic divergence within species, we plan to analyze multiple (>5) individuals for each species. The widest range of intraspecific morphological differentiation from the widest geographic distribution should be included when choosing the particular individuals for DNA analysis.

#### **Museum materials**

While fresh materials are preferred for establishing DNA barcode library, museum collections may serve as an alternative and critical resource. Although museum materials generally have lower success rate in DNA sequencing, they typically provide much more complete species coverage than new collection efforts. Additionally, freshly collected specimens can be examined against the type specimens that are deposited in various museums over the world using DNA sequences. A series of studies have shown that a very short fragment of COI sequences (~130 bps on the 5' terminus) can provide surprisingly good resolution (>95%) for species-level identification in Lepidoptera as well as in Ephemeroptera, Plecoptera, and Trichoptera (EPT). Because short DNA fragments are much easier to amplify in old specimens, museum materials, including type specimens, can be associated with fresh materials using these "mini-barcodes."

#### **Identification of mayfly specimens**

Since the barcoding project was launched in mid-2007, mayfly specimens have been contributed from various sources (including amateur collectors and biomonitoring programs) to the CCDB. Researchers at the CCDB have also collected over 1,000 specimens at varied sites in the eastern half of North America ranging from Hudson Bay to the southern USA. Many of the species encountered still need to have their identifications confirmed by specialists. Certainly, with the help from Ephemeroptera specialists, the process of collecting reference barcodes for North American mayflies will be greatly accelerated.

#### **General protocols of submitting specimens to the project**

DNA barcodes are collected and associated with individual specimens. Thus the specimens that are used for DNA barcoding need to be preserved individually, each with a unique sample ID. In contrast to all other DNA depository databases, the Barcode of Life Data System (BOLD, <http://www.barcodinglife.org>) holds detailed information

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for all individual specimens that are used for barcoding, such as collection date, locality, taxonomy, sex, life stage, coordinates, elevation, specimen image, etc. All information is associated with the original voucher specimen via its unique sample ID. Additionally, DNA sequence and the original trace files (the raw electropherograms from which the DNA sequences were read) are linked to the specimen after a barcode is generated.

Mayfly specimens are routinely assembled into Matrix boxes. A Matrix box is a plastic container that each hold 96 tubes organized in a format that is compatible with PCR plates used in most molecular labs. Each of the 96 tubes holds one individual specimen, which is tagged with a unique sample ID. Along with the Matrix box, two Excel spreadsheets are provided so collaborators can record the position of each specimen and relevant information (collection information, taxonomy, etc.).

#### Lab protocols

Once Matrix boxes and data arrive at the CCDB, specimen information is transferred onto BOLD. Specimens are sub-sampled, i.e. tissue (in most cases, 1 leg) is removed from the specimen and placed into a well in a lysis plate. The remainder of the specimen is stored in the original tube and deposited at the CCDB or sent back to the collaborator after being photographed. Tissue samples subsequently go through a high-throughput lab procedure that includes DNA extraction, PCR, cycle sequencing, and sequencing. The project manager oversees the whole procedure. Depending on the success rate of a particular plate of samples, positive hit picking of PCR products or negative hit picking of failures can be done on automatic robots. The turn-around time for the lab work is normally 1-2 weeks. The CCDB has the capacity to process 500,000 specimens each year.

#### Sharing data via BOLD

All collaborators will be registered as project users on BOLD, providing them with direct access to the management console for their project, making it possible to monitor progress, and to run analysis using tools provided in BOLD before the results are available publicly. Additionally, Ephemeroptera systematists can edit specimen information, e.g., updating taxonomy directly on the webpage. BOLD serves not only as a data depository, but also a communication platform between barcoders and collaborators. For example, the taxonomic browser that is integrated in BOLD will show what species have been barcoded and what species are needed.

Ephemeroptera barcodes will eventually be published and become publicly accessible. At the meantime, barcodes will be submitted to GenBank and cross-linked on both websites.

#### Post-sequencing

Collecting barcodes for mayflies is not the only goal of the barcoding initiative. Almost certainly, DNA barcodes will indicate some very interesting questions that might have been overlooked by conventional approaches. For instance, cryptic species that share nearly identical morphology can be separated into distinct groups on the DNA tree.

Other nominal species may need to be synonymized. As a general rule, all cases of conflict between the DNA tree and morphological assignment deserve additional investigation. DNA barcoders will continue working with mayfly workers, ecologists, and many other researchers after DNA sequences are generated.

#### Structure of the DNA barcoding North American mayflies project

The barcoding projects can be organized based on taxonomy (e.g., North American Baetidae), geographic regions (e.g., Ephemeroptera of the Great Smoky Mountains National Park), or collections (either of a museum or a specific collaborator).

#### A call for international collaboration

International support for the DNA barcoding North American mayflies is requested. Anyone who is interested in joining the alliance should contact Xin Zhou, who is coordinating this effort.

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## Course on South American Mayflies

### Eduardo Dominguez

Claudio Froehlich, from the University of São Paulo (at Ribeirão Preto), Brazil, kindly invited me to give a one week course on "Systematics, Biology and Biogeography of South American Ephemeroptera" for graduate students. Thirteen students from different universities of Brazil participated in the course and had the chance to collect and identify gorgeous nymph and adult mayflies.

I was delighted to find out that several of them are doing



their master or doctoral work on different aspects of Ephemeroptera. The course included talks, labs and a field trip to the nearby mountains that was especially useful to practice techniques on collecting and rearing different groups.

We were able to determine most of the specimens to the species level with the book *Ephemeroptera of South America* (Dominguez, E., C. Molineri, M. L. Pescador, M. D. Hubbard & C. Nieto, 2006). As usual in this part of the world, something new appeared! Fortunately, there are many young, new mayfly students in South America that are working to improve our knowledge of the systematics and biology of our beautiful species.

# Mayfly Conference News

## International Conference Update

The XIIth International Conference on Ephemeroptera and the XVIth International Symposium on Plecoptera are rapidly approaching. So far more than 100 participants (including 20 accompanying persons) from 30 countries are already registered for the meeting. The deadline for the submission of abstracts has ended, but one may still register for the meeting. To do so, please visit the conference website at <http://www.jointmeeting08.naturkundemuseum-bw.de> for further instructions.

Please make sure to arrange your accommodation soon - the contingents we arranged with some hotels were only reserved until the end of February. The International Youth Hostel is full, but there are still rooms available at other hotels. Please check the website for an update on availability.

There will be a major update of the conference site in March. All registered participants, and those who have subscribed to the conference newsletter, will receive a respective notice by e-mail. Then you will be able to learn more about the specifications to prepare your poster or talk.

I look forward to meeting you in Stuttgart! Arnold Staniczek, Staatliches Museum für Naturkunde, Abt. Entomologie, Rosenstein 1, D-70191, Stuttgart, Germany, phone ++49 (0) 711 8936 239, fax ++49 (0) 711 8936 100, [stanieczek.smns@naturkundemuseum-bw.de](mailto:stanieczek.smns@naturkundemuseum-bw.de).

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## Auction to Support Travel Scholarships and Lifetime Achievement Awards

An auction will be held at the XIIth International Conference in Stuttgart to benefit the William L. Peters Travel Scholarship Fund. This fund provides financial support to help students, who are studying mayflies, to attend the international conference. During the Montana conference, two students were awarded travel scholarships and a total of US\$716 was raised at the auction for this fund.

This will be a silent auction, where all items to be auctioned will be displayed for several days during the meeting. A sheet of paper will be placed next to each item so that individuals may write down the amount they wish to bid for the item. At the end of the auction, the person who submitted the highest bid wins that item. Since this meeting is in Germany, bids will be in euros.

For now we are asking you to think about what you might like to donate to this auction. Certainly anything with a mayfly theme would be appropriate, such as books or other publications, photographs, artwork or jewelry. Collecting equipment, rare or unusual mayfly specimens, and tied flies for fly-fishing would be good options, too.

Donated items do not have to deal with mayflies, though. For example, consider donating interesting art or craftwork from your country or possibly souvenirs of Germany.

Also, the Permanent Committee will be presenting, for the first time, lifetime achievement awards to our colleagues who have made significant contributions to the study of Ephemeroptera. Funds raised from this auction will be used to purchase those awards. The Committee hopes that these awards will become a tradition at future conferences.

We are hoping to increase donations by offering two special prizes. One prize will be for the person who submits the highest bid. All individuals, who donate an item, have a chance to win a second prize.

If you will not be attending the meeting, but would like to donate items for the auction, please mail the items to Arnold Staniczek, Staatliches Museum für Naturkunde, Abt. Entomologie, Rosenstein 1, D-70191, Stuttgart, Germany, phone ++49 (0) 711 8936 239, fax ++49 (0) 711 8936 100, [stanieczek.smns@naturkundemuseum-bw.de](mailto:stanieczek.smns@naturkundemuseum-bw.de).

Donations of money may be mailed to Donna Giberson, Treasurer, Department of Biology, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada, C1A 4P3, [giberson@upe.ca](mailto:giberson@upe.ca).

In the meantime, if you have any suggestions or questions about this auction, please contact either Eduardo Domínguez, Facultad de Ciencias Naturales, Universidad Nacional de Tucumán, Miguel Lillo 251, 4.000 Tucumán, Argentina, fax 54(81)248025, [mayfly@unt.edu.ar](mailto:mayfly@unt.edu.ar) or Pete Grant (see publication box on p. 5).

## Want to Host the Next International Conference?

The next international joint conference will be held in Stuttgart, Germany, 8-14 June 2008. If you are considering the possibility of hosting the conference after Germany, please note the following.

Representatives from the International Conferences on Ephemeroptera and the International Society of Plecopterologists established a set of guidelines for submitting proposals to host the joint conferences. These guidelines are:

### Preliminary Proposals

Preliminary proposals to host a conference may be submitted six years prior to the year of the proposed conference, but a final vote on the conference site will not be made until three years prior to the actual conference date.

### Final Proposals

1. Proposals should be submitted at least one month prior to the conference during which the proposal will be officially presented.
2. A copy of this proposal should be sent to the chair of each committee - International Conferences for Ephemeroptera (Michel Sartori, michel.sartori@vd.ch) and the International Society of Plecopterologists (John Brittain, j.e.brittain@nhm.uio.no).
3. Proposals should be submitted by email. This facilitates distribution of the proposal to the members of the two committees.
4. Proposals should contain detailed information regarding plans to host the conference.

So, if you would like to host the conference after Germany, or if you have questions about hosting a conference or submitting a proposal, please contact either Michel and John. Either individual can assist you with your questions.

## Copies of Conference Proceedings

Copies of the Proceedings of the 6th Ephemeroptera and 10th Plecoptera Conferences (Granada, Spain) are available for US\$55 (price includes postage). To purchase a copy, contact Michael Hubbard, Laboratory of Aquatic Entomology, Florida A&M University, Tallahassee, FL 32307 USA, michael.hubbard@fam.u.edu.

Copies of the Proceedings of the 8th Ephemeroptera and 12th Plecoptera Conferences (Lausanne, Switzerland) are available for US\$55 (price includes postage). To purchase a copy, contact Michel Sartori, Musée cantonal de zoologie, Palais de Rumine, Place de la Riponne 6, CH-1014 Lausanne, Switzerland, michel.sartori@vd.ch.

It would be a good idea to contact Michael or Michel prior to purchasing a copy of the proceedings to check on availability and to determine currency used for purchasing. All proceeds from the sale of these publications will be placed in the travel scholarship fund.

## Members of the Permanent Committee

**Javier Alba-Tercedor**, Universidad de Granada, Facultad de Ciencias, Departamento de Biología Animal Ecología y Genética, 18071 Granada, Spain, jalba@ugr.es

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communication among ephemeropterists. Subscriptions to the *Newsletter* are free. To place your name on the mailing list or to contribute information for the next issue, contact Peter M. Grant, editor, *The Mayfly Newsletter*, Department of Biological Sciences, Southwestern Oklahoma State University, 100 Campus Drive, Weatherford, Oklahoma 73096-3098 USA, phone (580) 774-3294, FAX (580) 774-7140, email peter.grant@swosu.edu. This publication was authorized by the Dean of Arts and Sciences and was printed at a cost of \$492.50 for 500 copies.

a reliable, accurate, and stable taxonomy of species using mitochondrial and nuclear gene sequence data from ca. 1800 individuals of ca. 50 species of mayflies (Ephemeroptera, Heptageniidae). We then apply the data to a comprehensive study of their macroecology, population genetics, and speciation to identify key species and habitats for conservation. We study two different biogeographical regions, the European Alps and the rainforests of Madagascar, to compare faunas with different evolutionary histories. First, we use both novel and well-established evolutionary criteria to delimit putative species using DNA. Group delineation is an important first step, but determining whether these are species constitutes the critical step in classification. The ability of DNA data to recover the species will be tested by examining congruence with 29 described species of *Rhithrogena* and geographical grouping methods (*sensu* De Salle *et al.*, 2005). By delimiting putative species from the data and then corroborating these using other criteria (e.g. geography, morphology), we propose an alternative to 'barcoding' in which *a priori* (and potentially incorrect) entities are databased and compared to unknowns based on phenetic similarity.

Using the extensive database of species and sequence data, we will examine species richness and turnover, gene flow among populations, and speciation rates in both Alpine and tropical environments. We will use our results to test a recent theory that key watersheds are responsible for buffering the effects of climate change and therefore give rise to contemporary biodiversity (Wilmé *et al.*, 2006). This mechanistic model of speciation proposes that low-elevation watersheds act as zones of isolation during periods of climatic change and therefore harbour a greater number of endemic species. Aquatic insects presumably provide an appropriate model system for more rigorous tests of the theory, as they are more closely tied to the watershed boundaries by habitat and dispersal constraints.

We expect sequence variation to be partitioned into clearly recognisable groups or "clusters" of sequences detectable with a variety of parsimony-based statistical methods and newly developed likelihood analysis of branching (cladogenesis) rates. A lack of groups in the Alps and clearly defined groups in the tropics, or *vice versa*, would suggest incipient or recent speciation in one group that is not easily detected by our genetic methods. The magnitude of variation among Alpine and tropical taxa is likely to be different; greater divergence between tropical congeners may be the result of extinction within ancient lineages. Alternatively, a recent arrival to Madagascar and recent speciation could result in closely related species. Regardless, we predict that DNA sequences will accurately and consistently recover diagnosable species. It is highly possible that morphology and DNA differ in their group assignments and one of our proposed tasks is to quantify the magnitude and nature (e.g., over-splitting) of difference. As the evolutionary process will have left individual lineages at different levels of

separation, there may be occasions where clustering based on various markers disagree. A critical empirical result from the study will be the degree to which congruent signals will be obtained from the two gene markers and morphology.

When the data are examined for all species, we predict higher species turnover across watershed boundaries with low-elevation sources in Madagascar, but that local richness may be independent of this turnover. A failure to see such a pattern in continental watersheds (e.g., Alps) would imply that reduced dispersal across marine barriers is a critical factor or that speciation is responsible for tropical but not Alpine biodiversity. Alternatively, similar climatic constraints may be broadly responsible for distribution patterns in both biogeographical regions, with dispersal playing a relatively minor role.

We suggest that novel, DNA-based methods will provide both a reliable, accurate taxonomy of all species and a rapid means to quantify the genetic diversity and evolutionary history of an entire fauna. Phylogenetic and population-genetic analyses using the growing genetic data sets will drastically improve our understanding of the evolution, community structure, and conservation status of freshwater invertebrates in the very near future. Testing the role of key watersheds in generating biodiversity (based on mammal distribution data) is ideally suited to a large-scale study of aquatic insects. Finally, the growing DNA taxonomy established here will provide short mtDNA sequences ('barcodes') for the identification of all mayfly species.

## Online Entomology Publications

The number of entomology publications that are available online continues to increase.

Take a look at Google Book Search (<http://books.google.com/intl/en/googlebooks/about.html>) if you haven't already. A search for "entomology" netted 8639 hits, a search for "aquatic entomology" netted 618 hits, and a search for "Ephemeroptera or mayflies" netted 633 hits. All of these hits were for full view texts which show the entire contents of a publication. I found publications such as Rousseau's *Annales de Biologie Lacustre* (1918), Needham's *Aquatic Insects in New York State* (1903), and Sheringham's *Elements of Angling – A book for Beginners* (1908).

Mike Quinn, in the Texas Parks and Wildlife Department (<http://www.texasento.net/earlyentobks.htm>), is compiling a list of early entomological books that are hyper-linked to Google Book Search.

The American Museum of Natural History now has all of its issues of the *Bulletin of the American Museum of Natural History* and the *American Museum Novitates* online. These contain publications by Spieth, Needham, Cockerell and Rabb.

And, of course, Mike Hubbard, is maintaining the "Bibliography of the Ephemeroptera" (<http://www.famu.org/mayfly/mfbib.php>) on Ephemeroptera Galactica, which contains many PDF files.

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# 2006 Mayfly Bibliography

Editor's note: This bibliography was published as the Ephemeroptera portion of the 2006(2007) North American Benthological Society's (NABS) *Current and Selected Bibliography on Benthic Biology*.]

The following is a list of current publications on Ephemeroptera that have been published up to and during 2006 and have not appeared in previous NABS Bibliographies.

I would appreciate receiving a reprint or complete bibliographic reference of any article about mayflies, especially if it contains scientific names, so that it may be included in next year's bibliography. Also, I would like to be informed of any corrections or omissions in this or past bibliographies. Suggestions are always welcome.

Please send all correspondence to Peter M. Grant, Department of Biological Sciences, Southwestern Oklahoma State University, 100 Campus Drive, Weatherford, Oklahoma 73096-3098 USA, phone (580) 774-3294, fax (580) 774-7140, email [peter.grant@swosu.edu](mailto:peter.grant@swosu.edu).

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