Barcoding the base of Lepidoptera: exploring global diversity and evolution of the Micropterigidae

David Lees, Rodolphe Rougerie, Christof Zeller-Lukashort, Georges Gibbs

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2 – 4 June 2010
Braga, PORTUGAL
BOOK OF ABSTRACTS

Welcome to Braga and University of Minho

Dear colleagues,

The European Consortium for the Barcode of Life (ECBOL) and the Centre for Molecular and Environmental Biology (CBMA) welcome you to the 2nd ECBOL conference, under the focal theme of the **2010 United Nations' International Year of Biodiversity** (IYB). As a fortunate coincidence, the IYB will also see the formal start up of a major DNA barcoding programme (International Barcode of Life – iBOL), which will create a library of DNA barcodes for 500K eukaryotic species and 5 million specimens by 2015.

ECBOL2 will hopefully provide the opportunity for strengthening the implementation of DNA barcoding in Europe and to further explore interactions between European partners and the barcoding community across the world.

Concurrently, it will be also a timely occasion to contribute to further raise awareness to the crisis of biodiversity loss, and to discuss the role of the Barcode of Life to help countering this global problem.

On behalf of all the people involved in the organization of this meeting, I wish you a pleasant and productive stay in our beautiful city and country.

Filipe Costa

Scientific Committee

Filipe Costa	CBMA, University of Minho, Portugal
Gary Carvalho	Bangor University, UK
Jan Pawlowski	University of Geneva, Switzerland
Peter Bonants	Wageningen University, Netherlands
Peter Hollingsworth	Royal Botanic Garden, Edinburgh, UK
Mehrdad Hajibabaei	University of Guelph, Canada
Ursula Eberhardt	CBS-Fungal Biodiversity Center, Netherlands
Wieslaw Bogdanowicz	Polish Academy of Sciences, Poland

Organizing Committee

Filipe Costa	CBMA, University of Minho, Portugal
David Schindel	CBOL, Smithsonian Institution, USA
Pedro Crous	CBS-Fungal Biodiversity Centre, Netherlands
Sarah Samadi	National Museum of Natural History, France

Local Organizing Committee

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Design by Sara Pimenta (www.sarapimenta.com), Cândida Lucas and Miguel Pinheiro (CBMA)

Tuesday, 1 June

19.30	**Welcome reception** Museu Nogueira da Silva (Braga Center) - **Registration desk will be available**

international BARCODE OF LIFE

Sponsors

Wednesday, 2 June

8.00 - 9.00	**Registration and posters' affixation**
9.00 - 12.30	**S1. Opening session / Chair Peter Hollingsworth**
9.00 - 9.15	**Welcome note Filipe Costa**
9.15 - 10.00	S1O1. Stuart Pimm - How many more species are there? And where do they live?
10.00 - 10.20	S1O2 - David Schindel - Consortium for the Barcode of Life (CBOL).
10.20 - 10.45	S1O3 - Pedro Crous - What is happening in the European Consortium for the Barcode of Life (ECBOL).
10.45 - 11.15	Coffee break
11.15 - 11.45	S1O4. Bob Hanner - The international Barcode of Life (iBOL) project.
11.45 - 12.15	S1O5. Peter Bonants - QBOL. Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health.
12.30 - 14.00	Lunch
14.00 - 16.15	**S2. Barcoding campaigns and biotas session / Chair Sarah Samadi**
14.00 - 14.30	S2O1. Mehrdad Hajibabaei - Next generation biodiversity analysis.
14.30 - 14.45	S2O2. Nicolas Puillandre - The MarBOL project in the MNHN, Paris. The crustacean and mollusc collections sequenced.
14.45 - 15.00	S2O3. Alessia Cariani - DNA barcoding of the Mediterranean chondrichthyans (ELASMOMED).
15.00 - 15.15	S2O4. Agnès Dettai - CEAMARC: Barcoding East Antarctic Deuterostomia.
15.15 - 15.30	S2O5. Rodolphe Rougerie - The iBOL Lepidoptera campaign: towards barcode records for 80K species by 2015.
15.30 - 15.45	S2O6. David Lees - Barcoding the base of Lepidoptera. Exploring global diversity and evolution of the Micropterigidae.
15.45 - 16.00	S2O7. Erik Van Nieukerken - DNA barcoding of the leaf - mining moth subgenus *Ectoedemia* s. str. (Lepidoptera: Nepticulidae). Are cryptic species recognised?
16.15 – 16.45	Coffee break

16.15 - 16.45	Coffee break
16.45 – 17.45	**S3. Plant barcoding session / Chair Peter Hollingsworth**
16.45 - 17.15	S3O1. Peter Hollingsworth - Choosing and using a plant barcode.
17.15 - 17.30	S3O2. Sofia Caetano - Do plants' life cycles influence assignment success in DNA barcoding? An answer based on seven genera of trees, shrubs and herbs.
17.30 - 17.45	HS3O3. Harald Meimberg - Multiple origins as potencial error source for barcoding in polyploids. Patterns of chloroplast haplotypes shared between polyploid and diploid representatives of the genus *Aegilops* (Poaceae).

Thursday, 3 June

9.00 – 10.30	**S4. Fungi barcoding session / Chair Ursula Eberhardt**
9.00 - 9.15	S4O1. Johannes Groenewald - Barcoding fungi of quarantine importance to Europe – WP2 of QBOL.
9.15 - 9.30	S4O2. Pedro Crous - DNA barcoding the genus Calonectria.
9.30 – 9.45	S4O3. Seena Sahadevan - DNA barcoding of fungi: a case study using ITS sequences for identifying aquatic hyphomycete species.
9.45 - 10.00	S4O4. Jozsef Geml - The use of DNA-barcodes in biodiversity assessments of ectomycorrhizal fungi in arctic and boreal ecosystems.
10.00 – 10.15	S4O5. Nelson Lima – Portuguese isolates of *Aspergillus* section *flavi* unravelled by the calmodulin gene
10.30 - 11.00	Coffee Break
11.00 - 12.30	**S5. Protist barcoding / Chair Jan Pawlowski**
11.00 - 11.15	S5O1. Mónica Moniz - Barcoding Diatoms with ITS – evaluation and case study.
11.15 - 11.30	S5O2. El Mahdi Bendif - A morphogenetic assessment of micro-evolution in extant *Noelaerhabdacean coccolithophorids*.
11.30 - 11.45	S5O3. Edward Mitchell - COI phylogenies reveal unsuspected, pseudo-cyptic diversity in testate amoebae (Rhizaria: Euglyphida & Amoebozoa: Arcellinida).
11.45 - 12.00	S5O4. Jan Rueness - DNA barcoding of selected freshwater and marine red algae (Rhodophyta).
12.00 - 12.15	S5O5. Jonas Zimmermann - Diatom barcoding: Water monitoring using molecular tools.
12.30 - 14.00	Lunch
14.00 - 16.00	**S6. Data management and analyses session / Chair Mehrdad Hajibabaei**
14.00 - 14.15	S6O1. Robert Vaughan - Services for the BARCODE community at the European Nucleotide Archive.
14.15 - 14.30	S6O2. Vincent Robert - QBOL and ECBOL data management and analyses system.
14.30 - 14.45	S6O3. Karl Larsson - The UNITE database for molecular identification of fungi.
14.45 - 15.15	S6O4. Discussion Panel with session speakers open to audience. Methods for data analyses and management for DNA barcoding data.
15.30 - 17.00	Poster sessions with coffee break
17.15 - 20.00	**Visit to the city of Guimarães - bus leaves at 17.30h.**
20.00	**Dinner at the HOTEL DE GUIMARÃES**

Friday, 4 June	
9.00 - 10.30	**S7. Applied barcoding Part I / Chair Mehrdad Hajibabaei**
9.00 - 9.30	S7O1. Simon Creer - Environmental barcoding and second-generation sequencing. A meiofaunal perspective.
9.30 - 9.45	S7O2. Martin Meijer - DNA Barcoding of the mycobiota in indoor environments.
9.45 - 10.00	S7O3. Jan Pawlowski - DNA barcoding and deep sequencing of eukaryotes diversity.
10.00 - 10.15	S7O4. Donald Baird - Biomonitoring 2.0: fusing next-generation sequencing and phenomics to illuminate ecological assessment.
10.30 - 11.00	Coffee break
11.00 - 12.30	**S7. Applied barcoding session Part II / Chair Peter Bonants**
11.00 - 11.15	S7O5. Joanna Zaluga - Phylogenetic study and identification of plant pathogenic clavibacters.
11.15 - 11.30	S7O6. Bart van de Vossenberg - QBOL: DNA barcoding from the perspective of diagnostic laboratories.
11.30 - 11.45	S7O7. Eva Rolo - Database of insect species with forensic interest in Portugal: molecular perspective.
11.45 - 12.00	S7O8. Neela Enke - Manual on field recording techniques and protocols: organizing specimen and tissue preservation in the field for subsequent molecular analyses.
12.00 - 12.15	S7O9. Tadeusz Malewski - Identification of forensically important blowfly species (Diptera: Calliphoridae) by high-resolution melting PCR analysis.
12.30 - 14.00	Lunch
14.00 - 17.30	**S8. Progress in animal barcoding; barcoding poorly known animal taxa / Chair Gary Carvalho**
14.00 - 14.30	S8O1. Bob Ward – Fish barcoding and its contributions to fisheries science.
14.30 - 14.45	S8O2. Frederic Sinniger - DNA barcoding in deep-sea zoanthids, unexpected diversity in Gold Coral-related zoanthids.
14.45 - 15.00	S8O3. Adriana Radulovici - DNA barcoding and phylogeographic patterns in marine amphipods from North Atlantic and Arctic waters.
15.00 - 15.15	S8O4. Julien April - DNA barcoding of North American freshwater fishes.
15.15 - 15.30	S8O5. Michael Raupach - DNA barcoding and supplementary nuclear marker for species identification. Two arthropod case studies (Coleoptera: Carabidae; Isopoda: Asellota).
15.45 - 16.15	Coffee break
16.15 - 16.30	S8O6. Charlotte Schoelinck - Parasite biodiversity in coral reef fishes: delimitation and description of new species of *Pseudorhabdosynochus* (Monogenea: Diplectanidae) from the gills of groupers (Serranidae: Epinephelinae) off New Caledonia.
16.30 - 16.45	S8O7. Andrea Galimberti - A DNA barcoding approach to discriminate Italian bats.
16.45 - 17.00	S8O8. Astrid Cruaud - Barcoding-based inferences of speciation mode. The case study of figwasps (Chalcidoidea, Sycophaginae).
17.00 - 17.15	S8O9. Thibaud Decaens - DNA barcodes for soil animal taxonomy.
17.30 - 18.00	**Discussion panel** **Moderator David Schindel** - international research networks and related aspects - wrap up of ECBOL2
18.00	**Conference closure – Filipe Costa**

Oral Presentations

S1O1.
How many more species are there? And where do they live?

Pimm S.

Nicholas School of the Environment, Duke University, Durham, USA.
Presenting author's email: stuartpimm@me.com

Species are going extinct one hundred times faster than the geological background rate. Worse, that rate is poised to increase to one thousand times faster in the near future. I will first review the "laws of biodiversity" — the global patterns of where species occur, the size of their geographical ranges, and their abundances. These patterns are derived for well-known taxa — birds and mammals, principally. This begs the question of how well we understand other groups where the taxonomic catalogue is much less complete. The most obvious first question is how incomplete is the catalogue? For flowering plants, modelling the process of species description suggests than another 10-20% of species remain undiscovered. There are, however, some serious discrepancies between the opinions of taxonomists for some plant families that likely reflect the complex patterns of species description. Where are these missing species? Broadly, the places where as-yet unknown species are likely concentrated are disproportionately where human actions are destroying habitats. More specific predictions require better documentation of the taxonomic process. Using these results and experimental knowledge of how long rare species will survive in fragmented habitats, we can produce effective conservation measures to reduce extinction.

S1O2.
Consortium for the Barcode of Life (CBOL)

Schindel D.E., CBOL Executive Secretary

Smithsonian Institution, Washington, DC.
Presenting author's email: schindeld@si.edu

CBOL has been working to promote DNA barcoding as a global standard for species identification since May 2004. It has 200 Member Organizations in 50 countries who participate in a range of CBOL activities including Working Groups, workshops, outreach meetings in developing countries, short courses and other training activities, and an international conference that is held every two years. The outcomes of CBOL's activities have been to raise awareness, organize and launch barcoding projects, increase the capacity of Member Organizations to generate barcode data, set community standards for barcoding, and engage users (especially government agencies) in barcoding activities. ECBOL and the Network of European Leading Labs (NELL) are directly involved in these activities.
CBOL is supported by the Sloan Foundation and is now in its fourth two-year grant period. The goals for 2010-2012 are to support the successful launch of iBOL and its Working Groups, promote technology development in barcoding, and accelerate production of barcode data in three critical areas: agricultural pests, disease vectors, and endangered species.

S1O3.

What is happening in the European Consortium for the Barcode of Life (ECBOL)?

Crous P.W.

CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.
Presenting author's email: p.crous@cbs.knaw.nl

Calibrating Europe's Biodiversity using DNA Barcodes (ECBOL) is a European consortium that is part of EDIT (European Distributed Institute of Taxonomy), an EU Sixth Framework Programme. The goal of ECBOL is to liaise closely with CBOL to establish a coordinated European approach to DNA barcoding. Presently ECBOL has more than nine European countries that actively participate in these activities, and are also contributing to the International Barcode of Life (IBOL) initiative. ECBOL pursues its goal by several means. A Network of European Leading Labs (NELL) for DNA barcoding has recently been established across Europe. These labs will barcode specimens from existing European natural history collections and specimens acquired by ATBIs (all taxa biodiversity initiatives) or targeted taxonomic sampling, such as in QBOL (Quarantine Barcode of Life). A centralised bioinformatics hub (E-BOLD) is planned to make information present in national databases (i.e. collection databases, taxonomic resources, sequence repositories) available through a single, integrated interface, and link to hubs in Canada and China. Furthermore, DNA barcoding related applications will be developed in dialogue with stakeholder needs. Members of EDIT have also agreed to continue supporting ECBOL activities for the coming 5-year period, also providing financial support for the coordination of barcoding activities.

S1O4.
The International Barcode of Life Project

Robert Hanner

Canadian Barcode of Life Network, Biodiversity Institute of Ontario & Department of Integrative Biology, University of Guelph, Canada.
Presenting author's email: rhanner@uoguelph.ca

The International Barcode of Life Project (iBOL) has one overarching goal - to assemble the sequence library and the technology necessary to identify organisms rapidly and inexpensively. This goal is underpinned by the observation that sequence diversity in short, standardized gene regions (DNA barcodes) enables both the identification of known species and the discovery of new ones. By building an identification system based on digital DNA strings rather than on analogue traits, DNA barcoding promises a massive improvement in our capacity to monitor and manage biodiversity with profound societal and economic impacts. iBOL will construct the richly parameterized barcode library needed as the foundation for a DNA-based identification system; 5M specimens representing 500K species will be analyzed within 5 years. iBOL will also deliver technologies enabling both massive biodiversity screens and point-of-contact identifications. Its work will be advanced through an international research consortium of biodiversity scientists, genomicists, technologists and ethicists. Data sharing, the structure of iBOL and its launch will be discussed.

S1O5.

QBOL: Development of a new diagnostic tool using DNA Barcoding to identify quarantine organisms in support of plant health.

Bonants P.

BU Biointeractions & Plant Health, Plant Research International, Wageningen, Netherlands.
Presenting author's email: peter.bonants@wur.nl

Development of accurate identification tools for plant pathogens and pests is vital to support European Plant Health Policies. For this project Council Directive 2000/29/EC is important, listing some 300 organisms for which protective measures against introduction into and their spread within the Community needs to be taken. Those threats are now greater than ever because of the increases in the volumes, commodity types and origins of trade, the introduction of new crops, the continued expansion of the EU and the impact of climate change. Currently identifying pathogens (in particular new emerging diseases) requires a staff with specialised skills in all disciplines (mycology, bacteriology, etc.); which is only possible within big centralised laboratory facilities. Taxonomy, phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction. Modern molecular identification/detection techniques may tackle the decline in skills since they often require much less specialist skills to perform, are more amenable for routine purposes and can be used for a whole range of different target organisms. Recently DNA barcoding has arisen as a robust and standardised approach to species identification. QBOL wants now to make DNA barcoding available for plant health diagnostics and to focus on strengthening the link between traditional and molecular taxonomy as a sustainable diagnostic resource. Within QBOL collections harbouring plantpathogenic Q-organisms will be made available. Informative genes from selected species on the EU Directive and EPPO lists will be DNA barcoded from vouchered specimens. The sequences, together with taxonomic features, will be included in a new internet-based database system. A validation procedure on developed protocols and the database will be undertaken across worldwide partners to ensure robustness of procedures for use in a distributed network of laboratories across Europe. More information can be found at www.qbol.org.

S2O1.
Next-generation biodiversity analysis

Mehrdad H.

Biodiversity Institute of Ontario & Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
Presenting author's email: mhajibab@uoguelph.ca

Understanding biodiversity is fundamental to much biological research and key to our health, environmental management and economic systems. However, the science of biology has been faced with a stiff challenge in documenting, characterizing and understanding biodiversity across the domains of life. DNA sequencing technologies have made it possible to gather genomics information to study biodiversity, ranging from the deepest trunks to the finest tips of the Tree of Life, and how these organisms interact with their various environments. What is the species composition of a particular ecosystem? How does biodiversity change over time, space, and in relation to environmental changes? How closely related are different species that share a particular environment? These questions are fundamental to understanding of our environment, how it is changing over time, and how best to reap its benefits in a sustainable manner. In long-term we would like to be able to use genomics in monitoring environmental change, ecosystem relationships and dynamics of biodiversity birth and death not only in model systems, but in any environmental setting. Advancements in next-generation technologies have created a paradigm shift in understanding and using biodiversity robustly and cost-effectively.

S2O2.
The MarBOL project in the MNHN, Paris: the crustacean and mollusc collections sequenced.

Puillandre N., Boisselier M., Brisset J., Buge B., Corbari L., Lambourdière J., Lorion J., Terryn Y., Tillier S., Utge J., Bouchet P. and Samadi S.

Systématique & Evolution, MNHN, Paris, France.
Presenting author's email: puillandre@mnhn.fr

The MarBOL project started in the Muséum National d' Histoire Naturelle (MNHN) of Paris in 2008, targeting two major groups of marine invertebrates: crustaceans and molluscs. Around 40,000 specimens preserved in alcohol were collected during the last 35 years in the Indo-Pacific Ocean by the Tropical Deep-Sea Benthos program. The first task was to develop a database to manage this large amount of data, linked to the system already used for "traditional" collections in the MNHN. We developed a ″specimen″ database (i.e. taxonomic ID, collecting event…) that meets the requirements of a barcoding approach, i.e. keep the link between each specimen and all the associated data. We also developed a ″molecular″ database (tissue, DNA, sequences…) to manage the newly created collections of tissues and DNA in the MNHN. So far, ~ 15,000 specimens have been databased, and ~7,500 of them have been sequenced for the COI gene. Preliminary analyses of the crustacean sequences show an overall congruency between species delimitation in the literature and clusters of COI sequences, even if in several cases a single species name hides several DNA clusters. Contrary to the crustacean dataset, where most of the specimens were already identified at the species level by taxonomists, many molluscs are only identified at the genus or even familial level, generally because most of the sequenced specimens certainly correspond to new species. In these cases, the barcoding approach becomes the preliminary step for an integrative taxonomy approach, where species are delimited using other characters in addition to the COI gene. In this context, we analysed several groups of crustaceans and molluscs and identified and described several new species.

S2O3.
DNA barcoding of the Mediterranean chondrichthyans (ELASMOMED)

Cariani A.[1], Nero A.[1], Arculeo M.[2], Bertucci V.[1], Cannas R.[3], Cau A.[3], Charilaou C.[4], Dimech M.[5], Fiorentino F.[6], Follesa M.[3], Galafassi D.[1], Garofalo G.[6], Golani D.[7], Gravino F.[5], Hemida F.[8], Knittweis L.[5], Lo Brutto S.[2], Mancusi C.[9], Manfredi C.[1], Morey G.[1], Mulas A.[3], Najib E.[1], Piccinetti C.[1], Serena F.[9], Sion L.[1,2], Stagioni M.[1], Steinke D.[1,3], Vrogc N.[1,4] and Tinti F.[1]

[1]Dept. Experimental Evolutionary Biology, University of Bologna, Bologna, Italy [2]Dept. Animal Biology, University of Palermo, Palermo, Italy [3]Dept. Animal Ecology and Biology, University of Cagliari, Cagliari, Italy [4]Department of Fisheries and Marine Research, Ministry of Agriculture, Natural Resources and Environment, Nicosia, Cyprus [5]Capture Fisheries, MRRA, Fort San Lucjan, Marsaxlokk, Malta [6]CNR-IAMC, Mazara del Vallo (TP), Italy [7]Department of Evolution, Systematics and Ecology, The Hebrew University of Jerusalem, Jerusalem, Israel [8]Ecole Nationale Supérieure des Sciences de la Mer et de Aménagement du Littoral, Algiers, Algeria [9]ARPAT, Livorno, Italy [10]Fisheries Department, Balearic Islands Government, Palma de Mallorca, Spain [11]Centre Régional de Institut National Recherche Halieutique, Nador, Morocco [12]Dept. Environmental and Animal Biology, University of Bari, University of Bari, Italy [13]Canadian Centre for DNA Barcoding, Guelph, Canada.
Presenting author's email: alessia.cariani@unibo.it

Within ELASMOMED, a regional initiative of the Fish-BOL campaign aiming at the DNA barcoding of Mediterranean chondrichthyans (sharks, skates, rays and chimeras), we have sampled and barcoded ca. 1000 cartilaginous fishes collected from several locations in the Western and Eastern Mediterranean and adjacent Eastern Atlantic. Individuals were obtained from international and national scientific trawl surveys (e.g. MEDITS) or at the fish markets (e.g. Poissonerie de Algiers) and provisionally assigned to species based on the available morphological identification keys and guidelines. Finclip or muscle tissue specimens were collected following Fish-BOL protocol and processed for DNA barcoding at the Canadian Centre for DNA Barcoding (CCDB) of Guelph or at the own molecular labs at the Universities of Bologna, Cagliari and Palermo. Barcodes were deposited in the BOLD under the ELASMOMED Project (http://www.boldsystems.org/views/projectlist.php?&). We obtained 835 COI-5P sequences of high quality (< 1% of Ns) belonging to 58 cartilaginous fish species (Mediterranean: 16 shark species out of the 42 reported in the basin; 19/36 skates and rays; 1/1 chimera; Eastern Atlantic: 27 skates and rays). According to the DNA barcode variation, we get evidence for 1) the high taxonomic resolution and reliability of the barcode identification, 2) the occurrence of species misidentification or missing identification of few sharks and numerous skate individuals based on the external rough morphology during routine data collection of the fishery survey programmes; 3) the allopatric cryptic speciation in Atlantic and Mediterranean skates, and 4) the geographical structuring in skates among Atlantic, Western and Eastern Mediterranean population samples. These outcomes highlighted and reinforced general issues which are relevant for understanding the evolution and the population biology of chondrichthyans in the Mediterranean as well as for planning the conservation and management of endangered or declining species (Dulvy & Reynolds 2009; Ferretti et al. 2008; Griffiths et al. 2010).

References:
(1) Dulvy NK *et al.* (2009) *Nature* **462**:417-417.
(2) Griffiths AM *et al.* (2010) *Proceedings of the Royal Society B: Biological Sciences* **DOI 10.1098/rspb.2009.2111**.
(3) Ferretti F *et al.* (2008) *Conservation Biology* **22**:952-964.

S2O4.
CEAMARC: Barcoding East Antarctic Deuterostomia

Dettai A.[(1)], Froger A.[(1)], Hemery L.[(2)], Denys G.[(2)], Gallut C.[(1)], Monniot F.[(2)], Cruaud C.[(3)], Lecointre G.[(1)], Ozouf-Costaz C.[(1)], Eleaume M.[(2)] and Ameziane N.[(2)]

[(1)]Systematique et Evolution, MNHN, Paris, France [(2)]Milieux et Peuplements Aquatiques, MNHN, Paris, France [(3)]Génoscope, Centre National de Séquençage, Evry, France, presenting author's email: adettai@mnhn.fr

Pictures and videos of the benthic communities of the Antarctic shelf show that Deuterostomia, especially ascidians, echinoderms and teleosts, are dominant in many of the habitats. However, the morphological identification of most species is not easy. Ascidian identification is particularly difficult and requires skilled experts, but even for well-known echinoderms and teleosts, younger specimens are difficult to assign to the adult form, as allometric trends tend to obscure diagnostic characters in juvenile specimens. Molecular identification is therefore very promising, but requires validation of its efficiency and error rate before being routinely used for identification in these crucial groups for Southern Ocean biodiversity studies. The CEAMARC cruises have allowed the sampling of benthic species from depths yet unexplored in the eastern part of the Antarctic continent. In collaboration with experts from a majority of taxa, the specimens have been or are being identified morphologically. The COI barcodes and the morphological identifications are then compared to one another, as well as to additional nuclear markers. With few exceptions, teleost barcoding has been validated as an ID technique in our 540 specimens and 68 species dataset. Exceptions generally correspond to taxonomic problems, and are being investigated with additional data. Several hundreds of COI and ribosomal sequences have been analysed for crinoids, and a phylogeographic study has also been performed. For ascidians, despite initial technical difficulties, COI and 18S sequences have been obtained and compared with morphological identifications for almost all the collected species. There are currently no sequences in the BOLD for ascidians from the Southern Ocean. In addition, the first comprehensive sample of Asteroidea from East Antarctica is also under study, and already includes 473 specimens. These results represent a major contribution to the creation of a reference dataset, and initiate the exploration of the limits and strengths of barcoding for these groups in the Dumont d'Urville Sea. This is especially important since the sampling area is undergoing major transformations following the calving and drifting away of a massive iceberg from the Mertz Glacier in mid-February 2010 (http://www.esa.int/esaCP/SEM4D59KF6G_France_1.html).

References:
(1) Dettai A *et al.* (accepted) *Deep Sea Research II*.

S2O5.
The iBOL Lepidoptera campaign: towards barcode records for 80K species by 2015

Rougerie R.[(1)]*, Hausmann A.*[(2)] *and Hebert P.D.N.*[(1)]

[(1)]Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada [(2)]Entomology Section, Bavarian State Collection of Zoology, Munich, Germany.
Presenting author's email: rrougeri@uoguelph.ca

Lepidoptera have served as a model group to test the effectiveness and utility of DNA barcoding. Today, these insects are the most heavily sampled organisms, numerically (>380K barcodes in BOLD), taxonomically (>40K species sampled), and geographically (samples from 189 different countries). The campaign developed within iBOL is progressing at a rapid pace and we expect to surpass the target of gaining barcode coverage for half of all known Lepidoptera species (165K species) by 2015.In this presentation, we dissect the fundamentals of the campaign, emphasizing the strategic and methodological elements that underpin its success. We first emphasize the strong participation by the community of lepidopterists worldwide as the most critical factor. Their participation has been encouraged by both the success of barcode recovery and the efficiency of DNA barcodes as species-specific markers in this group. Participation has been further strengthened by the immediate taxonomic implications of the results, and by the development of new applications based on the DNA barcode reference library. Finally, we describe how some major sub-campaigns and projects are organized and coordinated, introducing the recently launched iBOL Lepidoptera website.

S2O6.

Barcoding the base of Lepidoptera: exploring global diversity and evolution of the Micropterigidae

Lees D.C.[1], Rougerie R.[2], Zeller-Lukashort C.[3] and Gibbs G.W.[3]

[1]Dept. Entomology, Natural History Museum, London, UK [2]Biodiversity Institute of Ontario, Canadian Centre for DNA Barcoding, Guelph, Canada [3]Forsthubfeld 14, 5303, Thalgau, Austria [4]Dept. Biology, Wellington University, Wellington, New Zealand
Presenting author's email: dclees@gmail.com

Interim results are presented summarizing the global DNA barcoding campaign recently initiated for the Micropterigidae. This family is thought to be the sister group to all other extant Lepidoptera (Kristensen et al., 2007). Despite its potential significance for understanding the diversity and early evolution of the Lepidoptera, to date there is no COI data for this family available on Genbank. Preliminary phylogenetic analysis based on COI barcodes reveals the surprising potential informativeness of this marker for inferring deep relationships within the family. For example, it supports a sister relationship of the Eurasian and primarily W. Palaearctic genus *Micropterix* with other northern hemisphere micropterigids. As expected, DNA barcodes are also highly efficient for species-level discrimination and grouping. There are in total approximately 139 currently recognised species and subspecies of Micropterigidae, out of an estimated collected diversity that probably exceeds 230 species. Currently over 36% of described species and 21% of total inventoried species diversity has been barcoded, including over 26% of described species within *Micropterix*. Overall, the barcoding campaign is nearly half complete, considering an additional 63 undescribed taxa worldwide that have been sequenced, including five new *Micropterix*, while some countries such as New Zealand are nearing completion. We have prioritised poorly known diverse faunas such as New Caledonia and Madagascar to boost descriptive efforts. With several genera awaiting description, we may ultimately reach a larger number of undescribed than described species in this family. New results from tropical localities where micropterigids have been systematically searched for, suggest that the micropterigid fauna of many entire countries still awaits the start of an inventory. It will thus be difficult to reach a truly comprehensive barcode inventory of this family without expansion of this campaign with the help of lepidopterists in Europe and worldwide.

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S2O7.

DNA barcoding of the leaf-mining moth subgenus *Ectoedemia* s. str. (Lepidoptera: Nepticulidae): are cryptic species recognised?

Doorenweerd C., van Nieukerken E.J., Stokvis F. and Groenenberg D.S.J.

Terrestrial Zoology, Netherlands Centre for Biodiversity Naturalis, Leiden, Netherlands.
Presenting author's email: nieukerken@naturalis.nl

We sequenced 665bp of the Cytochrome C Oxidase I (COI) barcoding marker for 258 specimens belonging to the leaf-mining subgenus *Ectoedemia* (*Ectoedemia*) in the basal Lepidopteran family Nepticulidae. Next to that we tested a 482bp section of the nuclear Elongation Factor 1-alpha (EF1-alpha) for 240 specimens. We did this to circumvent some of the pitfalls of working with mitochondrial DNA and to see if using a second barcoding marker might help to clarify the status of cryptic species. The dataset includes 46 out of 48 European *Ectoedemia* s. str. species and several species from Africa, North America and Asia. The morphological characters needed for identifying species in this family are often found only in the male genitalia, dissecting these is labourious. Both COI and EF1-alpha proved reliable as an alternative to conventional species identification for the majority of species and the combination can aid in species validation. The results of potential cryptic species are discussed in detail. In the common species *Ectoedemia albifasciella* we discovered two haplotypes of 2.17% divergence in COI in sympatric populations. These haplotypes are not found with EF1-alpha, and not supported by other characters.

S2O8.

Barcoding Fauna Bavarica – Capturing Central European Animal Diversity

Hausmann A.[1], Schmidt S.[1], Balke M.[1], Haszprunar G.[1] and Hebert P.[2]

[1]Entomology, Zoologische Staatssammlung, Munich, Germany [2]Biodiversity Institute of Ontario, Guelph, Canada.
Presenting author's email: Coleoptera-ZSM@zsm.mwn.de

The Barcoding Fauna Bavarica (BFB) is an All Species Barcoding campaign ran by the Zoologische Staatssammlung in Munich and the Canadian Centre for DNA Barcoding. Core funding comes from the Bavarian Ministry for Science, Research and the Arts and from Genome Canada through the Ontario Genomics Institute. The initial funding period is from 2009–2013.Bavaria has the highest biodiversity of all German states, with at least 35000 animal species reported, representing a significant portion of the central European species diversity. Ecoregions include high altitude biomes, foothill areas and forested lowlands. The Zoologische Staatssammlung (ZSM) is one of the largest German natural history research institutions. It holds the world's largest collection of Lepidoptera and Germany's largest Hymenoptera collection. Since mid-2009, the BFB project has contributed DNA barcode records from 5772 specimens representing 2598 species and is therefore, after less than one year, one of the most comprehensive sources for local DNA barcode data. The focus groups for the initial phase were Lepidoptera (1750 species barcoded), bees (283 species), ants (32 species) and aquatic insects (265 species). Work on these focal groups will continue during 2010, with the goal to complete 80% of the Bavarian focal group species by the end of the year. New focal groups are Diptera and Coleoptera, targeting 1000 species in 2010.Most tissue samples come from specimens in the ZSM collection, and where this was not feasible from freshly collected and identified specimens. This rapid progress reflects the strong involvement of taxonomists throughout the process, which is one of our key missions. We have implemented a system which co-ordinates vouchers stored in our main collection, with tissues as well as DNA samples in our DNA bank. See our website at: www.faunabavarica.de and in our Barcoding Fauna Bavarica site on www.facebook.com

S3O1.
Choosing and using a plant barcode

Hollingsworth P.

Royal Botanic Garden Edinburgh, Inverleith Row, Edinburgh, EH3 5LR.
Presenting author's email: p.hollingsworth@rbge.org.uk

DNA barcoding aims to establish a central community resource for large scale use of DNA sequences for organismal identification and taxonomic clarification. The approach was pioneered in animals using a portion of the cytochrome oxidase (CO1) mitochondrial gene and has since resulted in a large number of studies applying the technique to study animal biodiversity. In plants, establishing a standardized DNA barcoding system has been more challenging. In this talk, I will review the process of selecting and refining a plant barcode, and describe some applications of DNA barcoding in plants. I will also summarise some of the major emerging projects, and outline some of the outstanding research requirements required to take plant barcoding forward.

S3O2.
Do plants' life cycles influence assignment success in DNA barcoding? An answer based on seven genera of trees, shrubs and herbs

Caetano S., Christe C., Aeschimann D. and Naciri Y.

Conservatoire et jardin botaniques, Unige, Geneva, Switzerland.
Presenting author's email: sofia.caetano@ville-ge.ch

Rates of molecular evolution are consistently lower in trees and shrubs displaying quite long generation times, when compared to herbaceous plants, which usually have annual generation times. When using a DNA barcoding approach, a higher assignment success to the species level is thus expected in annuals when compared to woody perennial plants. Our study specifically addresses this question and analyzes seven groups of closely related species that have different life cycles. We selected *Acer* and *Salix* as trees representative, *Lonicera*, *Gentiana* and *Adenostyles* for shrubs and perennials, and *Veronica* and *Geranium* for annuals. Within each genus, three to five closely related species have been chosen. A sampling scheme based on an average of 16 populations per species and three individuals per population over geographical ranges as large as possible was applied. Four chloroplast sequences were used as barcodes (*trn*H-*psb*A; *rpo*B; *rpo*C1; *mat*K) following the proposal of the Royal Botanical Garden of Kew at the time the project started (http://www.kew.org/barcoding/update.html). Such population genetics approach, allowed us to estimate the influence of the intra- *versus* interspecific variability on correct species assignments in the different genera. According to expectations, haplotypes found for annual genera displayed a huge number of mutations, when compared to those characterizing woody plants. Within annuals, divergent haplotypes were still shared among species, which indicates that assignment success to the species level is not as straightforward as expected. Full species assignments were only obtained within the perennial *Lonicera* genus. Rather good species recognition (~75%) was also observed within *Acer* and *Veronica*. Conversely, the four barcode loci failed to correctly assign species in the remaining genera (*Salix*, *Gentiana*, *Adenostyles* and *Geranium*). Our results show that assignment success to the species level does not directly depend upon life cycle. This success rather seems highly related to the evolutionary history of each species' group, whether because speciation occurred in very recent times, or because hybridization is still ongoing. The sampling strategy used in the present study furthermore allows delineating some sampling rules to be applied in plant DNA Barcode databasing effort in order to account for as much intraspecific variation as possible.

S3O3.

Multiple origins as potential error source for barcoding in polyploids: patterns of chloroplast haplotypes shared between polyploid and diploid representatives of the genus *Aegilops* (Poaceae)

Meimberg H.[1] *and McKay J.K.*[2]

[1]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, University of Porto, 4485-601 Vairão, Portugal [2]Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, 80523, USA.
Presenting author's email: meimberg@mail.icav.up.pt

Polyploidization is a major speciation processes in the plant kingdom and it is likely that the majority of angiosperms have polyploid histories. Polyploids contain the merged genomes of both parental progenitors and the chloroplast genome of one progenitor. Differences in chromosome number result very likely in reproductive isolation of the new polyploid and can lead to instantaneous speciation. Polyploids can originate recurrently by repeated hybridization, which is probably the rule rather than the exception and had been shown for many cases. Each independent origin constitutes a unidirectional gene flow event resulting in the introduction of a new chloroplast (cpDNA) haplotype into the gene pool of the polyploid. In case bidirectional multiple origins this can result in both cpDNA types from the progenitors to be found in the allopolyploids. We investigated the amount of cpDNA haplotypes shared between the different species of the genus *Aegilops* (Poaceae) in the context of an ecological study. We comparatively sequenced about 4000 bp in 6 loci including the trnK intron for multiple individuals per species. Most of the 11 polyploid species showed at least one haplotype shared with one of the progenitors, five species equal or very similar haplotypes with both progenitors. Several polypoids with the same progenitors shared one or more haplotypes. Even though the species are in general morphologically and genetically well characterized, this added up to 50% of their haplotypes being shared with another species for most of them. Depending on the divergence time and whether the gene flow is still ongoing, this can lead to high rate of misidentification using cpDNA sequences as barcodes. Especially in polyploid complexes where different species can have the same progenitors this could also result in haplotypes shared between polyploid species without direct hybridisation. This requires special attention in terms of initial sampling size and the amount of sequence information used for barcoding.

S4O1.
Barcoding fungi of quarantine importance to Europe – WP2 of QBOL

Groenewald J.Z.[(1)], *Quaedvlieg W.*[(1)], *Bonants P.J.M.*[(2)], *Boonham N.*[(3)] *and Crous P.W.*[(1)]

[(1)]Evolutionary Phytopathology Programme, CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands [(2)]Business Unit Biointeractions & Plant Health, Plant Research International BV, Wageningen, Netherlands [(3)]The Food and Environment Research Agency, York, UK.
Presenting author's email: e.groenewald@cbs.knaw.nl

The QBOL project consists of a consortium of 20 partners (www.qbol.org) and is financed by the 7th Framework Programme of the European Union to develop a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health. Quarantine organisms were selected from the EU Directive and EPPO lists based on the availability of specimens and/or taxonomic expertise and prioritised lists were created. The following fungi of quarantine importance and their relatives were selected for Work Package 2 (Fungi): *Ceratocystis fagacearum*, *Ceratocystis fimbriata* f. sp. *platani*, *Ceratocystis virescens*, *Cercospora angolensis* (=*Phaeoramularia angolensis* = *Pseudocercospora angolensis*), *Cercoseptoria pini-densiflorae* (=*Pseudocercospora pini-densiflorae*; teleomorph: *Mycosphaerella gibsonii*), *Melampsora farlowii*, *Melampsora medusae*, *Monilinia fructicola*, *Mycosphaerella dearnessii* (anamorph: *Septoria acicola*), *Mycosphaerella larici-leptolepis*, *Mycosphaerella populorum* (=*Davidiella populorum*; anamorph: *Septoria musiva*), *Puccinia pittieriana*, *Septoria lycopersici* var. *malagutii* (=*Septoria malagutii*) and *Thecaphora solani*. The internally transcribed spacer (ITS) regions of the nrDNA operon are initially used to confirm the taxonomic identity of all strains used in the project and to evaluate the resolution of this commonly used region. Other house-keeping genes, e.g. actin, beta-tubulin, cytochrome oxidase I and translation elongation factor 1-alpha, are screened additionally for species with no or poor ITS resolution. Genomic DNA will be maintained in a DNA bank and the resulting sequences and taxonomic features will be included in an online database. Although ITS provides a good indication at genus level, initial results show that it lacks resolution for several of the selected genera. We envisage a protocol for the end-user where ITS will be used as starting point and a flow chart will guide the end-user to the next step, if necessary, for a positive identification. I shall address the current status of the work package with regards to the resolution of the gene regions for the different species and their relatives based on the data available at that stage.

S4O2.
DNA barcoding the genus *Calonectria*

Lombard L.[(1)], Crous P.W.[(2)], Wingfield B.D.[(1)] and Wingfield M.J.[(1)]

[(1)]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa [(2)]CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands
Presenting author's email: p.crous@cbs.knaw.nl

Species of the genus *Calonectria* (*Hypocreales*) are important plant pathogens, several of which have a worldwide distribution. Taxonomic studies on these fungi have chiefly relied on DNA sequence comparisons of the β-tubulin (BT) gene region, because the ITS gene has proven inordinately conserved to facilitate species discrimination. There are about 68 species of *Calonectria* associated with root, stem and leaf diseases of numerous plant hosts. Attempts to find a suitable barcoding region have resulted in a multigene database employing seven gene regions including actin (ACT), BT, calmodulin (CAL), histone H3 (HIS3), ITS, translation elongation 1-alpha (TEF-1α) and the 28S large subunit RNA gene, for all species. Although the BT gene region provided valuable insights into relationships among species of *Calonectria*, analyses of individual coding gene regions and single nucleotide polymorphisms showed that CAL sequence data provided the best resolution in distinguishing between them. Sequence data for the TEF-1α, HIS3, BT and ACT gene regions were less useful. Unfortunately, there is no standard gene region that can be used for barcoding the *Hypocreales*, and future research is required to determine if CAL can successfully be applied to other genera in the Order.

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S4O3.
DNA barcoding of fungi: a case study using ITS sequences for identifying aquatic hyphomycete species

Seena S., Pascoal C., Marvanová L. and Cássio F.

CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal.
Presenting author's email: seena.sahadevan@gmail.com

Aquatic hyphomycetes were first reported by Ingold in 1942 and currently about 300 species with a worldwide distribution are known. This polyphyletic group of fungi plays a crucial role in organic matter turnover in streams, acting as intermediaries between plant litter and invertebrate shredders and, thereby, contributing to the functioning of freshwater ecosystems. The common approach in stream ecology is to identify aquatic hyphomycetes based on morphology of conidia collected in water or obtained from leaves colonized in nature through aeration methods in laboratory. Identification based on these criteria is often limited by our ability to induce conidium production from colonized plant debris and to establish pure cultures. The conidia is known to have evolved convergently hence, similar conidia may be produced by different conidiogenous processes and ignoring this may lead to false identification. In the All Fungi Barcoding meeting held in 2007 the nuclear ribosomal Internal Transcribed Spacer (ITS) region was proposed as the most appropriate candidate for barcoding of true fungi. In an attempt to examine the suitability of whole ITS region or a subregion (ITS1 or ITS2) to identify aquatic hyphomycetes, the ITS region was sequenced and compared in 94 isolates belonging to 19 species. The fungal isolates used in this study were collected from Portuguese streams with different environmental conditions between 1999 and 2007, and include cosmopolitan and rare species of aquatic hyphomycetes. The ITS sequences were also compared with those published at the National Center for Biotechnology Information. The ITS1, ITS2 and ITS1-5.8S-ITS2 sequences of the Portuguese isolates of aquatic hyphomycetes and those from the GenBank exhibited taxonomic cohesiveness, all the isolates grouped with their respective species except for Tricladium species. The three ITS sequences allowed the discrimination of the 19 species of aquatic hyphomycetes. Cohesiveness was not observed between isolates with respect to location, condition of stream or date of collection. Evolutionary divergences (ITS1-5.8S-ITS2 sequences; Kimura 2-parameter distance) between con-specific isolates were shallow and a deep divergence between species was generally observed. The phylogenetic trees based on ITS1 and ITS2 sequences had lower statistical support for many internal nodes, so we propose mainly the usage of entire ITS1-5.8S-ITS2 as barcodes for identifying species of aquatic hyphomycetes.

S4O4.
The use of DNA-barcodes in biodiversity assessments of ectomycorrhizal fungi in arctic and boreal ecosystems

Geml J.[(1)], Timling I.[(2)], Nusbaum H.C.[(3)] and Taylor D.L.[(2)]

[(1)]National Herbarium of the Netherlands, Netherlands Centre for Biodiversity Naturalis / Leiden University, Leiden, Netherlands [(2)]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, USA [(3)]Sequence and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, USA.
Presenting author's email: geml@nhn.leidenuniv.nl

Although critical for the functioning of ecosystems, fungi are poorly known in high-latitude regions. Among northern fungi, ectomycorrhizal (ECM) species have particular importance, because 1) they include the most diverse and most abundant fungal genera in the Arctic and 2) form symbioses with all arctic and boreal woody plant species. Despite their importance, the true extent of their diversity and taxonomy in the high latitudes remain largely unknown. Here, we provide examples of DNA-based biodiversity assessments of ECM fungi in various arctic and boreal regions. We analyzed ITS and LSU rDNA sequences generated in high-throughput fashion from curated sporocarps and soil clone libraries from multiple vegetation types, bioclimatic subzones, and geographic regions as part of our kingdom-wide molecular diversity assessments for arctic and boreal fungi. Soil clone sequences were grouped into operational taxonomic units (OTUs) at 97% ITS sequence similarity level. Representatives of these were included in phylogenetic analyses along with sequences generated from sporocarps deposited at the University of Washington Herbarium (WTU) and at the Natural History Museum, University of Oslo (O). We observed that sporocarp and soil DNA sampling gave complementary views on biodiversity, with a relatively high number of taxa detected only by one of the two methods. Some phylogroups were identified to known species, while others were previously unsequenced, and may or may not represent novel taxa. Our results provide the first large scale phylogenetic diversity assessment of ECM fungi in the Arctic, and reveal several unidentified clades that have not been documented previously.

S4O5.

Portuguese isolates of *Aspergillus* section *Flavi* unraveled by the calmodulin gene

Rodrigues P.[1,2], Torres A.[1], Nunes F.[1], Leirós C.[2], Santos C.[2], Venâncio A.[2] and Lima N.[2]

[1]CIMO - Centro de Investigação de Montanha, Escola Superior Agrária de Bragança, Bragança, Portugal, [2]IBB - Institute for Biotechnology and Bioengineering, Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal
Presenting author's email: nelson@iec.uminho.pt

Aspergillus is a large genus, with a complex and ever evolving taxonomy. Section *Flavi* is one of the most significant sections in the genus. Taxonomy and species identification is subject of great interest for scientists aiming to clarify the species concept and limits within the section. Furthermore, this section comprises both toxigenic and non-toxigenic species/strains, with great interest to biotechnology and food industry. Various genes, namely the rRNA (ITS region), calmodulin and ß-tubulin genes, have been widely reported as good markers for *Aspergillus* species identification, because they are rapid and cost-effective. In the present study, we evaluated the discriminatory power of the ITS region and the calmodulin gene to distinguish closely related taxa within *Aspergillus* section *Flavi*. For this purpose, 26 isolates of *Aspergillus* section *Flavi* obtained from Portuguese almonds were characterized at various levels: i) phenotypic, regarding various aspects of morphology and physiology; ii) spectral, using MALDI-TOF ICMS to obtain protein fingerprinting; and iii) genotypic, by sequence analysis of a 730 bp segment of the calmodulin gene and a 908 bp segment of the ITS region. For the various methods, dendrograms were created and results were compared. Both genotypic and spectral analyses divided the isolates in 3 groups corresponding to closely related taxa of *A. flavus*, *A. parasiticus* and *A. tamarii*. Except for the ITS region, all sets of analysis positioned 5 of the 26 isolates in two unidentified clades close to *A. parasiticus,* and divided the *A. flavus* group in two distinct clades. The phylogenetic analysis of the calmodulin sequences resulted in very similar dendrograms when using various methods of analysis (Neighbor-Joining, Maximum Likelihood, Bayesian Inference), and altering the analytical parameters did not result in significant changes. Furthermore, the genetic dendrograms were strongly supported by the phenotypic and spectral analyses. These results confirm the calmodulin gene as a robust and reliable genomic marker for this group of fungi. The unsolved isolate identifications are currently under further analysis.

S5O1.
Barcoding Diatoms with ITS – evaluation and case study

Moniz M.B.J.[(1)], Amorim A.[(2)], Kaczmarska I.[(3)] and Alves de Matos A.P.[(4)]

[(1)]MRI/National University Ireland, Galway, Galway, Ireland [(2)]Instituto de Oceanografia/Universidade de Lisboa, Lisbon, Portugal [(3)]Mount Allison University, Sackville, Canada [(4)]Anatomic Pathology, Curry Cabral Hospital, Lisbon, Portugal.
Presenting author's email: monica.j.moniz@gmail.com

DNA-barcoding is based on the premise that the divergence of a small DNA fragment coincides with biological separation of species. If true, it offers an additional tool for worldwide consistent species identification. In this context, we evaluated 618 sequences representing 114 diatom species belonging to the two most species-rich classes of diatoms (Mediophyceae and Bacillariophyceae). We proposed a DNA-barcode fragment (300 to 400 bp), starting at the 5' end of 5.8S and ending in the conserved motif of helix III of ITS2 secondary structure, which is successful in identifying biological and well defined diatom morpho-species and effective in separating 88% of species examined (1). We compared this marker with two others as diatom barcodes: the small ribosomal subunit (SSU) and a 5' end fragment of cytochrome c oxidase subunit 1 (coxI). We found SSU had high success rate in amplification and sequencing and was readily aligned but required a long fragment to show enough divergence for species separation. CoxI only needed a small fragment to separate species and was also readily alignable, but it showed very low amplification and sequencing success rates. 5.8S+ITS2 was easily amplifiable and sequenced and was the most variable marker of the three. We concluded that the fragment 5.8S+ITS2 was the best candidate as a diatom DNA barcode (2). Using the proposed barcode, we have focused on the role of barcoding and cultures in a first morphological and molecular assessment of *Pseudo-nitzschia* species from Lisbon bay. Ten strains of *Pseudo-nitzschia* were isolated from net samples (20μm) collected in Lisbon Bay. Five species were identified based on SEM and TEM: *P. australis*, *P. fraudulenta*, *P. multiseries*, *P. multistriata* and *P. subpacifica*. The identification was confirmed molecularly, comparing the DNA region 5.8S+ITS2 of these strains with reference data bases. For all tested species a 100% sequence identity was recorded. Although most strain sequences in these data bases were from the NE and NW Atlantic, this was also observed when strains from other Oceans or sea basins were compared thus confirming this DNA region is an effective marker for the studied species even when the reference sequences were obtained from geographically distant sites. This study permitted the comparison and validation of barcoding, morphological identification techniques and a first characterization of morphological, molecular diversity of *Pseudo-nitzschia* species in Lisbon Bay (3).

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S5O2.
A morpho-genetic assessment of micro-evolution in extant noelaerhabdacean coccolithophores

Bendif E.[1], Probert I.[1], Hagino-Tomioka K.[2], Romac S.[1], Young J.[3] and de Vargas C.[1]

[1]UMR7144 - Adaptation et diversité en milieu marin, CNRS/UPMC, Roscoff, France [2]UMR7144 - Adaptation et diversité en milieu marin, CNRS/UPMC, Roscoff, France [3]Division of Biological Sciences, Hokkaido University, Sapporo, Japan
Presenting author's email: bendif@sb-roscoff.fr

The coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica* are widely distributed and well known phytoplankton representatives. Due to their ecological importance in marine carbon fluxes, we conducted a multi-strain phylogenetic reconstruction inferred from the mitochondrial COI gene in order to assess the delineation of genetic species within this group. SSU rDNA sequences of *E. huxleyi* and *G. oceanica* are identical and LSU rDNA sequences differ by only 1 base pair. No significant ultrastructural differences have been reported between the two species and they share the same coccolith crystal structure, differing only in the degree of calcification and the presence of an additional calcite bridge in *G. oceanica*. Different *E. huxleyi* morphotypes have been described based on calcification level. The analysis revealed cryptic speciation within both morphospecies while highlighting the close relationship between them. Diversity of haplotypes appears to be higher in *G. oceanica*, maybe due to its earlier appearance during the Pleistocene around 1.5 Ma ago. *E. huxleyi*, which is a young species (first appearance 0.25 Ma ago), has less genetic variation with two distinct clades corresponding to two ecological niches. *E. huxleyi* seems to have derived from a sub-lineage within *G. oceanica*, with part of the *E. huxleyi* diversity sharing the same haplotype as *G. oceanica*.

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S5O3.

COI phylogenies reveal unsuspected, pseudo-cyptic diversity in testate amoebae (Rhizaria: Euglyphida & Amoebozoa: Arcellinida)

Mitchell E.A.D., Heger T.J., Kosakyan A. and Lara E.

Laboratory of Soil Biology, University of Neuchâtel, Neuchâtel, Switzerland.
Presenting author's email: edward.mitchell@unine.ch

COI has been proposed as a universal barcoding gene for animal species and some other groups of eukaryotes. However while this gene allows to identify species in some taxonomic groups such as animals, it fails to do so for others such as plants. Few barcoding studies exist to date for the unicellular groups of Eukaryotes that constitute most of the diversity at higher levels. Here we present results from the two main groups of shelled (or testate) amoebae in the Euglyphida and Arcellinida. Using three model genera, *Cyphoderia* and *Assulina* in the Euglyphida and *Nebela* (s.l.) in the Amoebozoa, we show that COI allows to identify known species, and that it is also useful in revealing hitherto unsuspected, cryptic and pseudocryptic diversity. These results suggest that the diversity of testate amoebae is currently strongly underestimated. This has important potential implications for the study of biogeography of free-living protists.

S5O4.
DNA barcoding of selected freshwater and marine red algae (Rhodophyta)

Rueness J.

Department of Biology, University of Oslo, Oslo, Norway.
Presenting author's email: jan.rueness@bio.uio.no

The red algae (Rhodophyta) are one of the oldest groups of eukaryotes, comprising around 6000 species world-wide, nearly all are marine with only about 150 freshwater species. Species identifications using morphological features alone can be very difficult due to phenotypic plasticity, many examples of heteromorphic life histories, lack of sexual reproduction in many populations etc. Various molecular markers have been employed over the past decades that have enabled huge advances in red algal taxonomy and phylogeny. The use of DNA barcoding in red algal species identification from sequences of the mitochondrial marker cytochrome oxidase subunit I (cox1) was successfully introduced by Saunders (2005), who developed novel primers. Numbers of red algal cox1 sequences are rapidly accumulating, but far more species and geographical isolates are required. I present the first European data for the freshwater species *Batrachospermum helminthosum*, a species that has been investigated throughout its range in North America. The marine algae studied include the invasive *Gracilaria vermiculophylla* that in recent years has spread on both sides of Atlantic waters (Rueness 2005) as well as on the west coast of USA and Canada. DNA barcoding of specimens from Europe and East Asia acquired in this and other studies show remarkably little variation. *Ptilota serrata* and *Ptilota gunneri* have been analyzed from the Norwegian coast and from Spitzbergen and compared with sequence data from Canada. The results show that *P. gunneri* that has not been considered part of the flora on the western Atlantic coasts, in fact is represented in the BOLD database as *Ptilota serratus* sp.1. The only *Ptilota serrata* access in GenBank represents a third species. Other species analyzed include two other introduced species on the Norwegian coast: *Dasysiphonia japonica* and *Antithamnion nipponicum*. *Pantoneura fabricii* and *Euthora cristata* were isloated and analyzed from Spitzbergen, in addition a few others species from the Norwegian west coast. For most of the taxa mentioned, other molecular markers such as the cox2-3 spacer, Rubisco spacer and rbcL were acquired in addition to cox1 sequences.

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S5O5.
Diatom barcoding: water monitoring using molecular tools

Zimmermann J., Jahn R. and Gemeinholzer B.

Botanic Garden and Botanical Museum Dahlem, Freie Universität Berlin, Berlin, Germany.
Presenting author's email: j.zimmermann@bgbm.org

Within the microscopic algae the diatoms represent the sole group being present in all types of water bodies and can therefore be excellently used for water monitoring. Because environmental conditions influence the species diversity in a given water body, diatoms are suitable indicators to detect water quality. Furthermore, continuous screening of algal biodiversity can provide information about the diversity development in present ecosystems. Thus, diatoms are an ideal model group to establish DNA barcoding methods to develop an easy to use, quick, efficient, and standardised organism identification tool to serve routine water quality assessments, as conventional morphological identification demands specialised in-depth knowledge. For this approach we created an artificial mixed sample of known as well as morphologically validated taxa for molecular treatments. DNA was extracted and deposited in the DNA Bank of the BGBM (Droege et al. 2008) where DNA aliquots and sampling information is available through the DNA Bank Network (Gemeinholzer et al. 2009). We have established a fast standardised molecular identification tool via DNA barcoding: We identified a short segment (about 420 bp) of the ribosomal SSU (18S) which is generally applicable for identifying diatom taxa and provided a standardised barcoding protocol including standard primers for this group of microalgae. The retrieval success after sequencing and cross-referencing against the database is always 100 % of the taxa contained in the mixed sample. It is not possible to get a quantification of the fraction of each taxon in the mixed sample from the pure molecular identification approach without further effort. However, this might be achieved through 454-sequencing. Pre-tests turn out to be rather promising. In the end, its application on natural samples and the feasibility for a faster and more convenient statement about the quality as well as the quantity of diatom diversity and taxon composition should be tested.

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S6O1.
Services for the BARCODE community at the European Nucleotide Archive

Vaughan R.J., Bower L., Cheng Y., Cleland I.S., Leinonen R. and Cochrane G.R.

European Nucleotide Archive, EMBL-EBI, Cambridge, UK
Presenting author's email: vaughan@ebi.ac.uk

The ENA (European Nucleotide Archive) has recently developed a range of new services of particular relevance to the barcoding community. These range from a new, optimised submission system with specific features and validation for barcode data, through the application of the barcoding standards to data and the addition of a community controlled keyword to entries which meet the standard, to our new search and data presentation tools. We also provide similar tools for the MIENS (Minimal Information about an Environmental Sequence) standard, meeting the needs of the microbiology barcoding community. To improve the efficiency and accuracy of submission to the ENA we have introduced a new template based submission system; for barcode data all of the required fields are presented and explained, and our validation systems check that all of the submitted data follows the standard. The template nature of the system makes it possible to very quickly submit anything from a single sequence to thousands of sequences, all using the same interface. Through the INSDC (International Nucleotide Sequence Database Consortium) we have agreed a controlled BARCODE keyword with our partners (Genbank and DDBJ) that is applied to records which meet the standard defined by the barcoding consortia. This keyword is added to entries which meet the criteria, and can be removed at the request of the barcoding community if entries are subsequently judged not to meet the standard required. Our new search system provides fast and easy access to data for both term and homology queries, and our new browser provides facilities for viewing and downloading data easily, regardless of the size of the dataset. Queries on organism name and taxon are supported, and we hope to expand our services in this area in the future. The ENA is committed to working with communities to develop standards, to drive the adoption of these standards, and to help ensure that the data collected meets those standards, and is available to the scientific community in appropriate formats.

S6O2.
QBOL and ECBOL data management and analyzes system

Robert V., Szoke S., Jabas B., Vu D., Chouchen O. and Stegehuis G.

Bioinformatics, CBS-KNAW fungal biodiversity centre, Utrecht, Netherlands
Presenting author's email: v.robert@cbs.knaw.nl

For the Quarantine Barcoding of Life (QBOL) as well as the FES (Dutch government) projects, we have developed a complete scientific data management system based on the BioloMICS software. Morphological, physiological, chemistry, ecological, molecular, geographical, administration, bibliography and taxonomy, as well as many others, can each be stored, handled and analyzed in the most appropriate ways. The system includes all relevant biological data related to Bacteria, Fungi, Insects, Nematodes, Phytoplasmas, Plants and Viruses associated with the quarantine problematic. The system not only allows data to be saved but also to be analyzed (polyphasic identification, classification, statistics, automated curation, etc) but also to be published online. The system is completely dynamic in the sense that new characters or fields, tables and records can be added to the system within minutes without the intervention of software developers. The same system will soon be used for the ECBOL website and will allow any Internet user to freely deposit and manage strains and sequences data. Submissions to BOLD or Genbank is made easy. Public release of the data will be decided by the depositor/owner of the data without any limitation in time.

S6O3.
The UNITE database for molecular identification of fungi

Larsson K.H.(1), Kõljalg U.(3), Abarenkov K.(3) and Nilsson H.(2)

(1)Natural History Museum, University of Oslo, Oslo, Norway (2)Plant and environmental sciences, University of Gothenburg, Göteborg, Sweden (3)Department of Botany, University of Tartu, Tartu, Estonia
Presenting author's email: k.h.larsson@nhm.uio.no

The large amount of environmental sequence data produced by next generation sequencing facilities will transform the way we think about ecology and taxonomy and blur the distinction between these disciplines. Databases and bioinformatic tools are currently the main bottleneck for a full-scale exploitation of the new possibilities. The UNITE database for molecular identification of fungi (http://unite.ut.ee) is presented. UNITE was initiated in 2001 as a collaboration within a NordForsk funded network of Nordic-Baltic fungal ecologists and taxonomists. The database was originally intended for identification of Nordic ectomycorrhizal fungi but lately opened for all fungal groups from any part of the world. UNITE primarily stores rDNA ITS sequences from expert identified, vouchered fruiting bodies. However, UNITE also stores unidentified environmental samples together with a copies of the ITS sequences available in INSD (NCBI, EMBL, DDBJ) and offers the user a choice to include them when running queries. Identification can be done either using simple BLAST searches or through phylogenetic analysis. In the latter case the result is presented as a phylogenetic tree. Recent developments of UNITE include possibility for multi-sequence queries and annotation of INSD sequences. We are also preparing UNITE for dealing with next generation sequencing data and a 454 pipeline is in place but not yet publicly available. UNITE is now accompanied by a web-based project management system called PlutoF where users can store field data, document the sequencing lab procedures, manage sequences, and make analyses. PlutoF intends to make it possible for taxonomists, ecologists and biogeographers to use a common platform for data storage, handling and analyses and ultimately facilitate an integration of their disciplines. A user can have an unlimited number of projects but still make analyses across any project data available to him.

S7O1.
Environmental barcoding and second generation sequencing: A meiofaunal perspective.

Creer S.[1], Fonseca V.G.[1], Lallias D.[1], Sung W.[2], Thomas W.K.[2], Carvalho G.R.[1], Power D.M.[3], Blaxter M.L.[4], Neill S.[5], Hiddink J.G.[5], Johnson H.F.[1], Packer M.[6], Hall N.[7], Ferrero T.[6], Barnes N.[6] and Lambshead P.J.D.[8]

[1]School of Biological Sciences, Bangor University, Bangor, UK [2]Hubbard Center for Genome Studies, University of New Hampshire, Durham, USA [3]Centre of Marine Sciences, CCMAR-CIMAR Associate Laboratory, Faro University, Faro, Portugal [4]Institute of Evolutionary Biology, Edinburgh University, Edinburgh, UK [5]School of Ocean Sciences, Bangor University, Bangor, UK [6]Department of Zoology, The Natural History Museum, London, UK [7]School of Biosciences, Liverpool University, Liverpool, UK [8]School of Ocean and Earth Science, National Oceanography Centre, Southampton, UK
Presenting author's email: s.creer@bangor.ac.uk

Taxon assessment is the key to understanding the relationship between biodiversity and ecosystem processes, but the identification of microbial eukaryotes is impeded by a number of factors. The small size of taxa, different life history stages, morphological convergence, poorly defined species concepts and intraspecific variation create logistical and taxonomic problems. However, the most important restricting factor confounding the ecological research of any microbial community is the mismatch between diversity and the number of taxonomists that are able to simultaneously identify and catalogue inter-phylum diversity. Accordingly, a molecular operational taxonomic unit (MOTU)-based approach has been advocated for en mass biodiversity assessment, but it has been hitherto restricted by the lack of throughput afforded by chain termination sequencing. Contemporary pyrosequencing offers a solution to this problem in the form of environmental metagenetic analyses (ie. the large-scale analysis of taxon richness via the analysis of homologous genes), but this represents a novel field of biodiversity assessment. Here, we cover some of the pros and cons of 454 Roche environmental metagenetic sequencing analyses via reference to example datasets derived from novel bioinformatic analyses of over 1 million nuclear small subunit 18S (nSSU) sequence reads of the meiofaunal biosphere. Soft-bottom benthic meiofauna are a ubiquitous, highly abundant guild (between 500 and 45µm) that play a crucial role in ecosystem functioning and services. Comprised of between 50% (shallow water) and 90% (deep water) nematodes, meiofaunal assemblages contribute significantly to bentho-pelagic coupling in the form of nutrient cycling, water column processes, pollutant distribution, secondary production and stability of sediments. The data provide quantitative, objective and revealing insights into the relative magnitude, composition and identity of the meiobenthic biosphere in marine and estuarine environments.

S7O2.
DNA barcoding of the mycobiota in indoor environments

Meijer M. and Samson R.A.

Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands
Presenting author's email: m.meijer@cbs.knaw.nl

The mycobiota occurring in indoor environments have attracted an increasing interest because of the health problems and biodegradation of the residences. About 200 culturable mould species are common in buildings in North America and Europe. However little data are available of the indoor mycobiota occurring in the subtropics and tropics. Surveys of the indoor moulds are often done by culturing, but the uncultured profile of indoor moulds is poorly understood. Recent studies show that uncultured fungi occur in great abundance, but their significance and frequencies remain unknown. In a collaborative project we assembled a set of dust samples from all continents except the Antarctic, and subjected them to standard protocols for isolation and morphological identification, high throughput dilution to extinction, and to 454 pyrosequencing. The internal transcribed spacer of the ribosomal operon (ITS) was used as a standard barcoding marker in all the work, but we are also mining available fungal genomes searching for barcode markers with higher resolution. About 2200 cultures have been isolated, from various geographical regions, identified and sequenced using conventional techniques. The high-throughput method was adapted for house dust and implemented on the global sample, and the first set of 600 cultures is sequenced. First pyrosequencing results demonstrate a taxonomically diverse and geographically patterned mycobiota, with a higher diversity in temperate zones in comparison to the tropics; a subset of 36 taxa (Dothideomycetes) has a cosmopolitan distribution, with regional fungal profiles exhibiting spatial autocorrelation at national and hemispheric scales. During the sequence experiments with the culturable mycobiota we experienced that the taxonomy of many “common” fungal genera is not elucidated and that much taxonomic research is needed to clarify the species/genus concept in these fungi.

S7O3.
DNA barcoding and deep sequencing of eukaryotes diversity

Pawlowski J., Lecroq B. and Lejzerowicz F.

Zoology and Animal Biology, University of Geneva, Geneva, Switzerland
Presenting author's email: Jan.Pawlowski@unige.ch

High-throughput sequencing opened new avenues for exploring diversity of micro-organisms and small-sized animals and protists. The richness of these inconspicuous organisms revealed by environmental DNA surveys in water, soil, and sediment samples by far exceed that obtained by traditional microscopic observations. However, the identification of short sequence tags is problematic. Here, we will present the results of our studies of deep-sea eukaryotes diversity, using 454 and Solexa massively parallel sequencing technologies. We will discuss the pitfalls of deep sequencing of environmental DNA focusing on selection of appropriate markers, development of environmental databases and systems of taxonomic identification.

S7O4.

Biomonitoring 2.0: fusing next-generation sequencing and phenomics to illuminate ecological assessment.

Baird D.J.[(1)]*, Shokralla S.*[(2)] *and Hajibabaei M.*[(2)]

[(1)]Environment Canada @ Canadian Rivers Institute, University of New Brunswick, Fredericton, NB, Canada
[(2)]Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, Canada
Presenting author's email: djbaird@unb.ca

Biomonitoring science is now established in most developed countries as an effective approach to assess human impacts on the environment through the development of index or model-based criteria by which to detect significant change in ecosystem structure. The current state of the science in biomonitoring permits detection of impact signals against a noisy background of environmental variation, yet in multiple stressor situations, diagnosis of cause and related stressor ranking remains difficult. Two emerging and rapidly linking areas in biomonitoring science offer some hope in this area: traits-based approaches and high-capacity genomics. By offering the possibility to link morphological, physiological and ecological character variation (the phenome) mechanistically to stress tolerance, trait-based approaches show great promise, yet the level of taxonomic resolution required to fully develop this approach (genus / species) is currently unachievable by traditional taxonomic approaches. However, the advent of high throughput genomics technologies offers the exciting prospect of rapid, high-capacity, high-resolution taxonomic observation at acceptable costs. Combining these two emerging areas offers the prospect of biomonitoring science advancing to a new level, yielding affordable diagnostic tools coupled with an unparalleled immediacy of assessment outcome.

S7O5.

Phylogenetic study and identification of plant pathogenic clavibacters

Zaluga J.[1], Heylen K.[1], Maes M.[3] and De Vos P.[1,2]

[1]Department of Biochemistry and Microbiology, Gent University, Gent, Belgium [2]BCCM/LMG Bacteria Collection, Gent, Belgium [3]Institute for Agricultural and Fisheries Research-ILVO, Merelbeke, Belgium
Presenting author's email: Joanna.Zaluga@ugent.be

The genus *Clavibacter* includes 5 subspecies, most of them belong to quarantine or q-alert organisms and cause a variety of plant diseases and serious crop losses. To protect plants rapid and reliable identification is required. The development of accurate DNA-based diagnostic tools is essential for quick and effective identification and in consequence for preventing the distribution of the diseases in pathogen-free areas. In the frame project QBOL (Quarantine Barcoding of Life) we focus on the fine taxonomic resolution of *Clavibacter* and close relatives using a polyphasic approach, with the development of barcodes as the final goal. To reevaluate taxonomic/phylogenetic position in the genus *Clavibacter*, a total number of 140 strains of 5 *Clavibacter michiganensis* subspecies (*C. michiganensis* subsp. *michiganensis* (Cmm), *C. michiganensis* subsp. *sepedonicus* (Cms), *C. michiganensis* subsp. *nebraskensis* (Cmn), *C. michiganensis* subsp. *insidiosus* (Cmi), *C. michiganensis* subsp. *tesselarius* (Cmt)) and some outgroups were included for sequence analysis of the 2 housekeeping genes gyrB and rpoB, and of the 16S rRNA gene. A new method introduced into bacterial taxonomy, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) was evaluated for chemotaxonomic typing. Cell extracts from strains were measured to establish proteins pick profiles. Only one out of 3 published gyrB primer sets gave a good amplification of a 500-bp fragment in all subspecies and outgroups. Gene sequence analysis in BioNumerics 5.1 and Mega 4.1 software shows that the gyrB gene fragment might be a good taxonomic marker. 16S rRNA gene analysis was used to obtain first subspecies grouping but its taxonomic resolution was too low. In case of the published rpoB primer set no amplification was observed in any tested groups of strains. The search for other suitable taxonomic markers continues. The two available *Clavibacter* genome sequences, namely from a Cmm and a Cms strain, together with the genome of the outgroup *Leifsonia xyli* subsp. *xyli* were already searched for shared single copy genes through whole genome comparison but resulted in a high number of potentially suitable genes for further research. From this list, genes will be selected for primer design and further research. In our ongoing MALDI-TOF study Cms strains were clearly distinguished from Cmm and other subspecies, so this technique seems promising for preliminary subspecies clustering.

S7O6.
QBOL – DNA barcoding from the perspective of diagnostic laboratories

van de Vossenberg B.

National Reference Centre - Molecular Biology, National Plant Protection Organisation, Wageningen, Netherlands
Presenting author's email: b.t.l.h.van.de.vossenberg@minlnv.nl

World-wide, many barcoding initiatives have been launched. These initiatives are often knowledge-driven and usually focus on barcoding specimens and filling databases from a taxonomic viewpoint. The use of barcodes as identification tool in diagnostic laboratories (in the phytosanitary field) is relatively new. Before introducing identification using DNA barcodes as a reliable diagnostic tool, barcoding protocols and the barcodes present in the database (Q-bank) need to be validated. Within the EU project QBOL, DNA barcoding protocols for the quick and accurate identification of regulated (quarantine) arthropods, bacteria, fungi, nematodes, phytoplasmas and viruses are under development. Developed protocols will be used to generate sequence data of quarantine organism and their taxonomic context. Sequence data is added to the Q-bank database as developed in the FES program financed by the Dutch government. The development of such identification tools for plant pathogens and pests is vital to support European Plant Health legislation. Protective measures against the introduction of quarantine organisms into, and their spread within, the community have a high impact on international trade, agriculture and horticulture. It is important that tools used for the detection and identification of quarantine organisms are robust and reliable. By means of validation, the usability and reliability of the developed DNA barcoding protocols and database will be determined. In total 20 QBOL partners world-wide will participate in the validation study. The validation consists of two ring tests and a survey among possible end-users of Q-bank. One ring test is organised to validate the usability of the DNA barcoding protocols developed in the other QBOL work packages. The DNA barcoding protocols include instructions for DNA extraction, amplification, sequencing and database-searching. The other ring test is organised to validate Q-bank. The survey is organised to inventory the needs and expectations among possible end-users of the QBOL DNA barcoding protocols and Q-bank. More information can be found at www.qbol.org

S7O7.
Database of insect species with forensic interest in Portugal: molecular perspective

Rolo E.[1,2], *Rebelo M.T.*[1,2] *and Dias D.*[1,2]

[1]Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal [2]Centro de Estudos do Ambiente e do Mar, Portugal.
Presenting author's email: evarolo@gmail.com

The creation of a molecular database has proven to be an important step for the identification of species. DNA barcoding, i.e., the ability to apply molecular analysis to discriminate the group which each specimen belongs to (mainly at the species level) through barcode sequence, seems to be useful. A standard region such as the mitochondrial gene cytochrome *c* oxidase (COI) has been considered mandatory and most effective (1). In Forensic Entomology, a molecular database can help identifying the necrophagous insects that colonize human corpses. This information can be crossed with the prior knowledge regarding the developmental stages of each species and allows the determination of relevant aspects with medicolegal purposes, including the *post mortem* interval (PMI). Consequently, this work consists in the building of a National Molecular Database integrating reference barcode sequences of insect species found in forensic context (in Portugal). In this way, we sequenced samples of several fly species (Insecta: Diptera), *Calliphora vicina* (Robineau-Desvoidy, 1830), *Calliphora vomitoria* (Linnaeus, 1758), and *Musca domestica* (Linnaeus, 1758). The amplification was performed in COI region using universal primers (2). The results suggest that these three species can be differentiated with a distance of ≈ 4% in the same genus and between 10,9% and 12,3% to different genus (results obtained with p-distance model test). Thus, our next step is to increase sampling of these species and extend the analysis to other groups, such as Coleoptera, in order to create a complete and robust National Molecular Database, available to forensic researchers.

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S7O8.

Manual on Field Recording Techniques and Protocols: Organizing specimen and tissue preservation in the field for subsequent molecular analyses

Enke N.

Botanic Garden Berlin, Freie Universitaet, Berlin, Germany
Presenting author's email: n.enke@bgbm.org

During the last decade DNA-based methods have revolutionized almost all areas of biological research. While DNA isolation techniques are continuously being improved, the impact and importance of adequate pre-DNA-isolation treatment are still largely underestimated. In the present review, we present some guidelines on how to organize specimen and tissue preservation in the field for optimized subsequent molecular analyses. Recommendations are given on how to set up a collection plan and sampling strategy, how to gather information on the environment, habitat and taxa to be collected, and how to deal with legal issues. Furthermore, we review currently used field tissue storage methods and their efficiency for different types of samples and organisms, taking into account the available resources and the intended use of the sampled material. We also make suggestions about logistics, precautions, and safety as well as on how to carry out field work and how to prevent contamination. When collecting specimens (vouchers) and parts of specimens (DNA, tissue) both the short-term and long-term preservation of the samples and their subsequent storage in natural history collections must be guaranteed. Checklists of documentation essentials and equipment for collection trips are appended (AbcTaxa, Vol. 8, 2010).

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S7O9.

Identification of forensically important blowfly species (Diptera: Calliphoridae) by high-resolution melting PCR analysis

Malewski T., Draber-Mońko A., Pomorski J., Los M. and Bogdanowicz W.

Polish Academy of Sciences, Wilcza 64, 00-679, Museum and Institute of Zoology, Warszawa, Poland
Presenting author's email: tmalewski@miiz.waw.pl

We describe here the successful coupling of real-time polymerase chain reaction (PCR) and high-resolution melting (HRM) analysis to rapidly identify 15 forensically important species of blowfly from the family Calliphoridae (Diptera), which occur in Poland. Two short regions (119 and 70 base pairs, respectively) of cytochrome oxidase gene subunit I with sufficient sequence diversity were selected. In the case of lacking taxa (e.g., reference species) these amplicons can be synthesized using sequences deposited in gene banks. The technique utilizes low template DNA concentration and is highly reproducible. The melting profile was not altered up to a 10,000-fold difference in DNA template concentration (ranging from 5 pg to 50 ng). The several HRM runs performed on different specimens from Poland belonging to the same species and on different days resulted in only minor variations in the amplification curves and in melting temperatures of the peaks. Intraspecific variation in a larger scale was tested using synthesized oligonucleotides from cosmopolitan *Lucilia illustris* originating from Poland, France, Great Britain, India, and USA. As HRM PCR analysis is sensitive to even single base changes, all geographic variants of this species were identified. This technique is also cost effective and simple, and it may even be used by non-geneticists. A working protocol was ultimately constructed for the purpose of rapid and accurate species identification in most countries in Europe regardless of which stage or which part of a blowfly was collected.

S8O1.
Fish Barcoding and Its Contributions to Fishery Science

Ward R.

CSIRO Marine and Atmospheric ResearchHobart, Tasmania 7001, Australia
Presenting author's email: Bob.Ward@csiro.au

Fishes were among the first groups of animals to be subjected to DNA barcoding, using the COI gene. A global campaign (FISH-BOL) was initiated in 2005 and so far has barcoded nearly 8000 of the world's 30,000 fish species. While much analysis remains to be done, initial data show that barcoding enables the discrimination of about 98% of fish species. Fisheries science now has a tool that permits the accurate and rapid identification of all life history stages, from egg to adult, including much processed material. I will present an overview of some of the achievements to date of fish barcoding, and will give examples of its application to tuna, shark fin, egg and processed product identification. Barcoding has also proved its effectiveness in highlighting likely new species, and in validating the distinctiveness of some newly described species. Examples of such will also be given. While much still remains to be done, barcoding's effectiveness as a new species diagnostic tool for fishes cannot be questioned.

S8O2.
DNA barcoding in deep-sea zoanthids, unexpected diversity in Gold Coral-related zoanthids.

Sinniger F. and Baco A.

Oceanography, Florida State University, Tallahassee, USA
Presenting author's email: fredsinniger@hotmail.com

Zoanthids (Cnidaria, Hexacorallia) are distributed in all marine environments from the deep sea to the intertidal and from the Antarctic to Arctic. Identification of zoanthid species based on histological and morphological characters has always been difficult and controversial. Because of these difficulties, this group of anthozoans is regularly ignored in diversity and ecological surveys. Molecular methods have proved helpful in investigating shallow water zoanthid diversity. However, most diversity is expected to be found in deep-sea environments. Thus, information from deep-sea taxa is essential to understand the biology and evolution of this group. This study used a DNA barcoding approach to clarify the taxonomic diversity of the Zoantharia from Hawaiian and New Zealand seamounts. We compared the taxonomic resolution and variation of 4 genetic markers in relation to the taxonomy and evolutionary hypotheses recently developed for zoanthids. Our results suggest a need to reconsider some traditionally used morphological characters (e.g. the secretion of an axis in the genus Savalia). Instead, substrate specificity appears to be tightly linked to the evolution of this group. The investigation of deep seamount zoanthid biodiversity throughout the Pacific revealed an important unknown diversity of octocoral-associated zoanthids which further support this hypothesis.

S8O3.

DNA barcoding and phylogeographic patterns in marine amphipods from North Atlantic and Arctic waters

Radulovici A.E.[(1)], Dufresne F.[(1)], Sainte-Marie B.[(2)], Steinke D.[(3)] and Costa F.O.[(4)]

[(1)]Department of Biology, University of Quebec at Rimouski, Rimouski, Canada [(2)]Department of Biology, University of Quebec at Rimouski, Rimouski, Canada [(3)]Fisheries and Oceans Canada, Maurice Lamontagne Institute, Mont-Joli, Canada [(4)]Fisheries and Oceans Canada, Maurice Lamontagne Institute, Mont-Joli, Canada
Presenting author's email: adriana.radulovici@gmail.com

DNA barcoding was proposed as a standard tool for species identification and discovery (by flagging divergent intraspecific clusters). In marine organisms, a DNA-based approach would have multiple fundamental and practical applications including reliable diagnosis across all life stages, identification of processed seafood substitutions or detection of invasive species. In the same time, it provides permanent species tags unchanged during taxonomic revisions. DNA barcodes are rapidly accumulating and, as such, their implications for various types of research are growing. For instance, a large database could be useful for inferring comparative phylogeographic patterns in related species inhabiting the same geographical area. Here we present results on phylogeographic patterns in *Gammarus* spp. from North Atlantic (Western and Eastern) and Arctic waters. More than 600 COI sequences showed that morphological species are usually resolved as cohesive barcode clusters and with at least 10 × greater divergence between than within species. At the intraspecific level, data showed a range of patterns for *Gammarus* spp. examined, from little or no genetic variation at large geographic scale (*G. duebeni*, *G. wilkitzkii*) to deep divergence between intraspecific clusters (*G. oceanicus*, *G. setosus*). A phylogeographic break was found between the Arctic and the temperate Atlantic zone for *G. oceanicus*, while at smaller scales (Atlantic Canada) this species showed some level of genetic structure. This study highlights the usefulness of DNA barcoding beyond species identification and stresses the importance of including geographic aspects during sampling to reveal the true extent of intraspecific variation and speciation.

S8O4.
DNA barcoding of North American freshwater fishes

April J.[1], Hanner R.[2], Mayden R.[3] and Bernatchez L.[1]

[1]Biologie, Universite Laval, Quebec, Canada [2]Integrative Biology, Guelph University, Guelph, Canada [3]Biology, Saint Louis University, Saint Louis, USA
Presenting author's email: julien.april.1@ulaval.ca

It is now clear that DNA barcoding is a useful and effective identification tool for a broad array of organisms. However, there are still few projects that achieve extensive coverage at large taxonomic and geographic scale. This is especially true for economically important species whose identification can be challenging. Such projects are important because they allow rigorous testing of the method and have the potential to be particularly useful for future users. In this context, we assembled the most exhaustive barcodes collection to date for freshwater fishes. Over 6000 specimens from Canada and the United-States were analyzed, representing 745 species (83% of known species). Specifically, we (1) investigated the accuracy of barcodes to identify freshwater fishes (which are thought to hybridize frequently and often exhibit deep intra-specific divergence values), (2) assessed the efficiency of barcodes to delineate closely related species within the most specious genera (3) searched for barcodes differentiation between paired-species of lampreys and (4) used extensive sampling coverage to evaluate the performance of barcodes to distinguish intra-specific lineages. Analyses confirmed that DNA barcoding is an accurate tool for species identification. Furthermore, results from this large scale project have important implications in conservation, taxonomy, forensics and in our understanding of fish evolution and biodiversity.

S8O5.

DNA barcoding and supplementary nuclear marker for species identification: two arthropod case studies (Coleoptera: Carabidae; Isopoda: Asellota)

Raupach M.J.[1], Astrin J.J.[2], Hannig K.[3], Peters M.K.[2], Stoeckle M.Y.[4] and Waegele J.W.[2]

[1]German Centre for Marine Biodiversity Research, Senckenberg Research Institute, Wilhelmshaven, Germany [2]Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany [3]Waltrop, Germany [4]The Rockefeller University, New York, USA,
Presenting author's email: m.raupach.zfmk@uni-bonn.de

The identification of vast numbers of unknown organisms using DNA sequences becomes more and more important in ecological and biodiversity studies. In this context, a fragment of the mitochondrial cytochrome c oxidase I (COI) gene has been proposed as standard DNA barcoding marker for the identification of organisms. However, limitations of the COI barcoding approach can arise from its single-locus identification system, the effect of introgression events, incomplete lineage sorting, numts, heteroplasmy and maternal inheritance of intracellular endosymbionts. Consequently, the analysis of an additional nuclear marker system could be advantageous. We tested the effectiveness of the COI barcoding region and a variety of nuclear ribosomal expansion segments in discriminating ground beetles of Central Europe and marine isopods of the Southern Ocean. Our results confirm the high potential of COI barcodes for the identification of even closely related carabid and deep-sea isopod species. We also demonstrate that the analysed nuclear ribosomal expansion segments constitute a valuable and efficient supplement for classical DNA barcoding to avoid potential pitfalls when only mitochondrial data are being used.

S8O6.

Parasite biodiversity in coral reef fishes: delimitation and description of new species of *Pseudorhabdosynochus* (Monogenea: Diplectanidae) from the gills of groupers (Serranidae: Epinephelinae) off New Caledonia

Schoelinck C., Samadi S. and Justine J.

UMR7138, UPMC, Paris, France
Presenting author's email: schoelinck@mnhn.fr

Studies on groupers (Perciformes: Serranidae: Epinephelinae) have shown that parasite biodiversity in coral reefs could reach more than ten times the numbers of fish species. Consequently, the extinction of fish species from endangered coral reefs could result in the co-extinction of ten times more parasite species. Although coral reef fishes are relatively well known, parasite biodiversity has rarely been evaluated. Species description of diplectanids (Platyhelminthes: Monogenea), a group of parasites located on the gills of groupers, is currently based only on morphological characters. We applied an integrative approach based on morphological and molecular analysis to assess species limits in this family. A barcode approach has never been used before to analyse diplectanid biodiversity and no COI sequences are reported in GenBank and BOLD. In this study, 300 specimens of parasites, collected from gills of groupers of the New Caledonian lagoon, were analysed. Each parasite was identified morphologically at the species level and the COI gene was sequenced. Preliminary molecular analyses of 300 diplectanid COI sequences using Bayesian approach were congruent with the hypotheses of species delimitation proposed by morphological approach. In one case, two lineages were found within what was recognized as a single morphospecies, *Pseudorhabdosynochus youngi* Justine, Dupoux & Cribb, 2009, from *Epinephelus fasciatus* Forsskål. The phylogenetic analysis also suggested that the different parasite species that co-occur on a single host are never sister-groups. We are now working to sequence a second gene to confirm our hypotheses, especially for the species *P. youngi*, which needs to be re-evaluated at the morphological level.

S8O7.
A DNA barcoding approach to discriminate italian bats

Galimberti A.[(1)]*, Spada M.*[(2)]*, Mucedda M.*[(3)]*, Martinoli A.*[(2)]*, Russo D.*[(4)]*, Agnelli P.*[(5)]*, Crottini A.*[(1)] *and Casiraghi M.*[(1)]

[(1)]Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milan, Italy [(2)]Dipartimento dell'Ambiente, Salute e Sicurezza, Università degli Studi dell'Insubria, Varese, Italy [(3)]Centro per lo Studio e la Protezione dei Pipistrelli in SardegnaSassari, Italy [(4)]Dipartimento Ar.Bo.Pa.Ve. Laboratorio di Ecologia Applicata, Università degli Studi di Napoli Federico II, Napoli [(5)]Sezione di Zoologia, Museo di Storia Naturale dell, Firenze, Italy.
Presenting author's email: a.galimberti3@campus.unimib.it

Chiroptera are characterized by wide distribution and high diversity in the number of taxa and ecological features. Despite mammals are one of the most studied animal group, recent molecular studies highlighted the presence of numerous cryptic species; and many of them are within bats. Bats are used as environmental bioindicators and it is renown that congeneric species can have different ecological requirements, therefore accurate monitoring of bat distribution and abundance in a country is essential. In this study we applied a DNA barcoding approach to shed more light on the biodiversity of Italian microchiropteran bats. Italy is one of the most important countries for bat diversity in Europe, due to the presence of at least 33 species, one of which is endemic (*Plecotus sardus*), out of the almost 39 most frequently recovered in Europe. However, knowledge on the ecology and distribution of several species are still unknown, due to the difficulties in species identification. Our dataset includes 170 coxI sequences from 31 Italian bat species collected across Italy (Sardinia included) and recognized by experienced researchers in the field. We applied an integrated approach testing for the coherence between traditional morphological identification (mostly based on taxonomic keys) and DNA barcoding in bat species identification. In particular, we developed an identification system based on the calculation of an Optimum threshold (OT) of molecular divergence. Our results clearly show a strong coherence between DNA-based and morphological identifications for almost all the examined species and revealed interesting patterns of intraspecific variability (in some instances related to geographical localization) within some taxa belonging to the family Vespertilionidae. Finally, we successfully tested our identification method identifying undetermined individuals belonging to the *Plecotus* and *Myotis* genus. Although further investigations are needed (i.e. it will be important to include bats of Sicily and minor Italian islands), the present study validates the effectiveness of DNA barcoding as a powerful species discriminator in microchiropteran bats.

S8O8.

Barcoding-based inferences of speciation mode: The case study of figwasps (Chalcidoidea, Sycophaginae)

Cruaud A., Rasplus J., Zahab R., Genson G. and Bazin E.

CBGP, INRA, Montferrier sur lez, France
Presenting author's email: cruaud@supagro.inra.fr

In the tropics, each *Ficus* species hosts small communities of 2-6 species of Sycophagine non-pollinating figwasps. These communities are structured by the development of the syconia, the thickness of the fig wall, the timing of oviposition and the feeding habits of the wasps (gallers versus cleptoparasites). Four ecological groups (guilds) of species can be recognized: 1) the large gall-inducers: large wasps with short ovipositors, 2) the small gall-inducers: smaller wasps, with medium to long ovipositor (longer than body length), 3) the ostiolar gall-inducers: species that enter the figs through the ostiole to oviposit, just like the pollinator species and 4) the cleptoparasites: smaller wasps with longest ovipositor. From these observations several question arose: What are the relative importance of speciation on the host compared to host shifts? Are co-occuring species more distantly related than expected by chance? Is niche conservatism (clustering of species with same length ovipositor) the rule or do we observe trait convergence? We use a multigenic barcoding to 1) develop a web-based tool to identify this unknown and still undescribed biodiversity 2) to estimate mitochondrial introgression and sister-taxa relationships 3) to infer speciation mode of Sycophagine fig wasps. We barcoded two mitochondrial and one nuclear markers (COI, Cytb and EF respectively, 2.7 kb) for 150 species (3 times the number of described species) on a least two individuals each. These wasps belong to all Sycophaginae genera. To unravel the impact of geography on Sycophagine speciation most *Ficus* species were sampled over their distribution range. We used several methods of species delimitation/assignation to test their accuracy in regards to the morphology and ecology. Our dataset reveals no introgression and COI appears a good marker for species assignation and identification of Sycophaginae figwasps. The main result of our study is that most of the Sycophagine communities are polyphyletic assemblages. This pattern strongly contrasts with pollinator communities. Closely related species of Sycophaginae frequently belong to the same guilds and have shifted to different hosts. However, speciation on the host fig species also exists and in that case sister taxa exhibit differences in ovipositor length and consequently in resource exploitation.

S8O9.
DNA barcodes for soil animal taxonomy

Rougerie R., Decaëns T., Deharveng L., Porco D., James S.W., Chang C., Richard B., Potapov M., Suhardjono Y. and Hebert P.D.N.

Lab ECODIV, University of Rouen, Mont Saint Aignan, France
Presenting author's email: thibaud.decaens@univ-rouen.fr

The biodiversity of soil communities remains very poorly known and understood. Soil biological sciences are strongly affected by the taxonomic crisis, and most groups of animals in that biota suffer from a strong taxonomic impediment. In that context, we investigate how DNA barcoding is a novel method using a microgenomic tag for species identification and discrimination that permits better evaluation of the taxonomy of soil biota. We analyze 1152 barcode sequences for two major groups of animals, collembolans and earthworms, presenting for the first time broad taxonomic and geographic sampling in both groups. Besides strongly reflecting the taxonomic impediment for both groups, with a large number of species-level divergent lineages remaining unnamed so far, our results also highlight a high (15%) level of cryptic diversity within known species of both earthworms and collembolans. These results are supportive of recent local studies using a similar approach. Within an impeded taxonomic system for soil animals, DNA assisted identification tools can facilitate and improve biodiversity exploration and description. DNA barcoding campaigns are rapidly developing in soil animals and we urge that the community of soil biologists embrace these methods.

Poster Presentations

S2P1.
Where the barcoding vouchers are? And where should they be?

Bastos-Silveira C.[1,2], Cartaxana A.[1], Carvalho D.[1] and Alves M.[1,3]

[1]Museu Nacional de História Natural, Universidade de Lisboa, Lisboa, Portugal, [2]Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal, [3]Centre for Environmental Biology (CBA), Universidade de Lisboa, Lisboa, Portugal
Presenting author's email: mjalves@fc.ul.pt

One of the core tenets of DNA Barcoding Initiative is that sequences should be linked to voucher specimens, which serves as a basis of study and are retained as references. Vouchers specimens should be kept in long-term, secure and publicly accessible scientific collections in museums and herbaria. However, these good practices have not been widely implemented by the scientific community involved in the DNA Barcoding Initiative. Specimens that provide the DNA, from which the barcodes are determined, are often dispersed in individual collections and not easily accessible. Therefore, we strongly suggest that storage of voucher specimens in scientific collections at museums and herbaria should become an obligatory request for the CBOL projects. We believe it will benefit the scientific community, enhance public engagement and strength ongoing efforts to know and preserve Earth's biodiversity. The Museu Nacional de História Natural – Portugal is involved in two CBOL initiatives, and presently is partner of many Portuguese barcoding projects as the final repository of voucher specimens. These partnerships have proved to be fruitful while promoting the interaction among experts from several disciplines such as taxonomy, systematics, molecular biology, and curation, fulfilling both the missions of Natural History Museums and of the DNA Barcoding Initiative.

S2P2.
Barcoding marine nematodes: an improved set of nematode 18S primers to overcome eukaryotic co-interference

Bhadury P. and Austen M.C.

Dept. of Biological Sciences, Indian Institute of Science Education and Research-Kolkata, Mohanpur, India
Presenting author's email: pbhadury@gmail.com

Nematodes form an important component of many benthic marine ecosystems and DNA barcoding approaches could provide an insight into their community composition. We amplified nematode 18S rDNA sequences from environmental DNA extracted from intertidal sediment collected from U.S.A and India to test whether the published marine nematode 18S rDNA sequences from GenBank database can effectively assign unknown nematode sequences into genus or species level. Most of the clones showed identities with published nematode 18S sequences but few could be assigned even to genus level. In addition, other eukaryotic sequences were found to be co-amplified with nematode primers. We found that the majority of the nematode 18S primers will co-amplify other eukaryotes if environmental DNA is targeted. We designed new nematode 18S primers and evaluated them using environmental DNA extracted from US and Indian sediments. The clone sequences showed identity with published nematode 18S sequences and no eukaryotic co-amplicons were detected. Seven clones showed 100% identity to published *Daptonema* and *Metachromadora* 18S sequences. The current molecular database for nematodes are dominated by sequences from Europe and need to be extensively populated with new nematode sequences collected from different locations including the tropics to better investigate marine nematode diversity based on DNA barcoding.

S2P3.

Revising the biodiversity of macrobenthos from the Portuguese coast using DNA barcodes

Borges L.M.S., Ferreira S.M., Landi M., Gomes P.A. and Costa F.O.

Department of Biology, University of Minho, Braga, Portugal
Presenting author's email: luisa.borges@bio.uminho.pt

The species composition of benthic communities is a key attribute for understanding the function of marine ecosystems and an essential factor for environmental monitoring programmes. Benthic surveys are very time consuming and require extensive and detailed taxonomic knowledge on a wide spectrum of phyla. As a consequence many of the organisms collected during those surveys are not identified to the species level. Taxonomic revisions may prove difficult as there is no established procedure to keep specimens easily accessible to the scientific community. Thus, erroneous identifications are perpetuated in the literature leading to continuous misjudgement of many species ranges and abundances. By means of building a reference library of DNA barcodes of macrobenthos from the Portuguese coast, we aim to improve the accuracy of the identifications and to create a system that enables revision of the identifications and reliable regional scale comparisons. In the initial phase of our study the target groups were the Mollusca – mainly Gastropoda and Bivalvia – and the Crustacea. Here we present and discuss the most relevant taxonomic ambiguities emerging from BOLD searches for sequences matching our reference library of cytochrome c oxidase 1 (COI) DNA barcodes. For instance, we were able to assign some of our specimens of *Gammarus* sp to the species *Echinogammarus marinus*. Work on these groups will continue during 2011 and 2012 with the aim of completing as much as possible the DNA barcode library that has already been initiated, and will include other phyla, namely Annelida, another important group in benthic communities

S2P4.
BioFresh: biodiversity of freshwater ecosystems: status, trends, pressures, and conservation priorities

Freyhof J.

Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany
Presenting author's email: freyhof@igb-berlin.de

BioFresh is a new large, integrative EU-funded project. Within BioFresh, 19 international partners aim to build a freshwater biodiversity information platform to bring together, and make publicly available, the vast amount of information on freshwater biodiversity currently scattered among a wide range of databases. BioFresh is constantly searching for freshwater biodiversity databases that can be associated with the BioFresh data portal. We also search for molecular/barcode databases. BioFresh will provide spatially-explicit information on the status and trends of freshwater biodiversity and its ecosystem services. Using the data to search for past and present impacts of multiple stressors, the project will significantly improve our ability to predict future responses of freshwater biodiversity and its services to climate and socioeconomic pressures. These responses will be investigated at global, continental and local scale with a focus on European biodiversity.
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S2P5.

Phylogeographic relationships of northern and southern temperate Soleidae and Pleuronectidae (Pleuronectiformes) based on COI, 16S and Cyt b data

Marques J.F.[(1)]*, Paterson A.M.*[(2)]*, Costa F.O.*[(3)] *and Cabral H.N.*[(1,4)]

[(1)]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [(2)]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [(3)]Department of Ecology, Agriculture and Life Sciences Faculty, Lincoln University, Christchurch, New Zealand [(4)]Department of Ecology, Agriculture and Life Sciences Faculty, Lincoln University, Christchurch, New Zealand
Presenting author's email: jimarques@fc.ul.pt

The phylogenetic relationships within and between the Soleidae and Pleuronectidae have been frequently evaluated due to the ecological importance and commercial value of these two flatfish families. Although several phylogenies based on morphological and molecular data have been produced, a well-supported molecular phylogeny at the species level remains to be resolved. Here we present a molecular phylogeny of the Soleidae and Pleuronectidae from the coastal waters of Portugal (north-eastern Atlantic) and New Zealand (south-western Pacific), including representatives of all genera inhabiting these areas. Genetic data were collected for 32 taxa using three mitochondrial genes that total 2321 nucleotides. Bayesian inference, parsimony and maximum likelihood methods produced phylogenetic trees with strong support for *Pelotretis flavilatus* (Pleuronectidae) as the earliest diverged current taxon and for an early divergence of the Pacific from the Atlantic Pleuronectidae included in the study. Within the monophyletic Soleidae, the several phylogenetic reconstruction methods evidenced *Synaptura lusitanica* as the earliest diverged current taxon and the polyphyletic origin of the genera *Dicologlossa* and *Microchirus*. Although analyses of data from the three genes resolved some clades in agreement with previous phylogenetic studies, tree topology of relationships between families and genera within differed from previous hypotheses. These phylogenetic data were used to clarify long-standing questions regarding the taxonomic status of the Soleidae species *Dicologlossa hexophthalma*, *Solea kleinii, Solea lascaris* and *Monochirus hispidus,* to support raising Rhombosoleinae to family level (Rhombosoleidae), to estimate dates of diversification of genera and to examine global biogeographic patterns.

S2P6.
The Norwegian Barcode of Life Network - Barcoding Activity in Norway

Marthinsen G.

Natural History Museum, University of Oslo, Norway
Presenting author's email: g.m.marthinsen@nhm.uio.no

The Norwegian Barcode of Life (NorBOL) is a network of the major biodiversity institutions in Norway, chaired by Natural History Museum at University of Oslo, and a recognized partner in the iBOL project plan. NorBOL aims for the role as a regional node of iBOL, with a special responsibility for coordinating barcoding efforts in the Arctic. The vision of NorBOL is to build a complete reference library of DNA barcodes for all eukaryotic species occurring in Norway and in the Arctic region within the framework of the iBOL project. The most recently established large barcoding project in Norway is The Norwegian Taxonomy Initiative (NTI) run by the Norwegian Biodiversity Information Centre. This project is established by the Norwegian government to strengthen the knowledge about biological diversity in Norway. Within NTI, eleven ongoing projects aim to describe and map poorly known species and species groups, comprising lichens, fungi, algae, insects and marine invertebrates. Specimens with preserved DNA from the projects will be delivered to NorBOL institutions for barcoding. Examples of other ongoing barcoding activities in Norway are collection of plant DNA from Scandinavia, the Arctic region, Africa and various alpine regions of the world, and projects on plant wasps, parasitoid wasps, bees, lepidopterans, ants, polychaetes and nudibranchs. So far, results from Norwegian barcode projects have been published for e.g. lump suckers, birds, biting midges, long-horned beetles and chironomids, along with results from projects on candidate markers for plant barcoding, reconstruction of past Arctic vegetation and climate, and analyses of diet of small herbivores.

S3P1.

DNA barcoding for delimitation of species boundaries in *Dalbergia* spp. from Western Ghats in India

Bhagwat R.M.[(1)], Dholakia B.B.[(1)], Balasundaran M.[(2)], Kadoo N.Y.[(1)] and Gupta V.S.[(1)]

[(1)]PMB Unit, Division of Biochemical Sciences, National Chemical Laboratory, Pune 411008, India [(2)]Forest Genetics and Biotechnology Divison, Kerala Forest Research Institute, Peechi 680653, Thrissur, India
Presenting author's email: rasikamb@yahoo.co.in

The Western Ghats (WG) in India are well known for their rich and unique assemblage of flora and fauna, and are amongst the 25 biodiversity hotspots identified in the world. *Dalbergia* [from the family of pea (Fabaceae)] is an important member of the WG flora; valued for decorative and often fragrant wood (rosewood, African blackwood, sisu) and is rich in aromatic oils. There is taxonomic confusion with respect to several *Dalbergia* species as these often have more than one species names. Hence, the size of the *Dalbergia* genus remains disputed. Although DNA barcoding is well established in animals, a universally accepted barcode is still lacking in plants. Hence, the main objective of this study is to develop a unique barcode for quick, accurate and reliable species identification using the *Dalbergia* genus as a model system. Leaf samples from 15 accessions each, belonging to six validated *Dalbergia* species (*melanoxylon*, *candenatensis*, *rubiginosa*, *latifolia*, *volubilis* and *paniculata*) were collected from different locations in WG and DNA extractions have been carried out from these as well as characterized herbaria samples. A total of 37 primer pairs specific to several chloroplast genes (*mat*K, *rpo*C, *rpo*B, *rbc*L, *acc*D, *ndh*J, *ycf*5 and *trn*H-*psb*A) as well as the nuclear genes were evaluated on the samples and 16 of these have been standardized for the six *Dalbergia* species. We are currently targeting the DNA sequences corresponding to *mat*K, *rpo*C, *rpo*B, *rbc*L, *trn*H-*psb*A and nuclear ITS. Based on the preliminary sequence data, the resolution of the species differentiation using the *rpo*C, *rpo*B and *mat*K genes individually was 66.66%. Further work is in progress to achieve 100% species resolution and develop a successful barcode using other important genes either individually or in combination.

S3P2.
Searching for tools to discriminate eucalypts

Carocha V., Ribeiro C., Araújo J. and Marques C.

Análise Genómica, RAIZ - Instituto de Investigação da Floresta e Papel, Oeiras, Portugal
Presenting author's email: carocha@itqb.unl.pt

Eucalyptus tree species, commonly referred to as eucalypts, are native to Australia and to a few adjacent islands. Although great variety can be found, eucalypts consist in long-lived, evergreen species from the angiosperm family *Myrtaceae*. The eucalypt group (broad sense speaking) includes seven genera, three of which are closely related: *Eucalyptus*, *Corymbia* and *Angophora*. In the latest eucalypt taxonomic revision, over 700 species belonging to 13 main evolutionary lineages were described (1). Some of these species exhibit remarkable characteristics, combining fast growing ability, valuable wood properties, wide adaptability to soils and climates, a blending that makes these trees excellent candidates for plantation forestry (2). RAIZ is a Portuguese private non-profit research institute funded by the Pulp & Paper Portucel, Soporcel Group. RAIZ aims to increase the productivity of the *Eucalyptus* forest in Portugal, implement sustainable forest management practices and reduce wood costs. In the context of its population management activities, RAIZ is interested in establishing a barcode identification system for the most interesting eucalypt species and investigate the potential of sequence information to discriminate individuals of the same species. This would result in the establishment of very efficient fingerprinting protocols that could be used to identify species from wood samples and to certify individuals in field tests, seed orchards and clonal gardens and also be valuable for protecting the intellectual property of elite clonal material. With the objective of testing the power of DNA barcode technology to discriminate eucalypts, 12 eucalypt species were selected. Leaves from up to 30 individuals per species were collected throughout the country, to ensure adequate sampling of available biological material. Following the recommendation from the CBOL Plant Working Group for a standard plant barcode system (3), the *2-locus* barcode set: *matK* + *rbcL* and four other regions were sequenced (*rpoC1*, *trnH-psbA*, *psbK- psbI* and *ITS1-ITS4*). *Locus* amplification, sequence quality and total fragment size were evaluated. The minimum number of sequence variations has been revealed for each species, aiming at the definition of reference sequences. These will be analyzed to check the potential of these markers to discriminate pairs of species. Finally, the results will be used to check the match between molecular discrimination and taxonomic information.

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S3P3.
DNA Barcode of *Quercus* species (Fagaceae) in Portuguese Forests

Costa J.S.[1], *Romeiras M.M.*[2], *Duarte M.C.*[2], *Batista D.*[3] *and Paulo O.S.*[1]

[1]Centro de Biologia Ambiental (CBA), Computational Biology and Population Genomics Group (http://cobig2.fc.ul.pt/), Faculdade de Ciências da Universidade de Lisboa (FCUL), Lisboa, Portugal, [2]Instituto de Investigação Científica Tropical (IICT), Jardim Botânico Tropical, Lisboa, Portugal, [3]Instituto de Investigação Científica Tropical (IICT), Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Quinta do Marquês, Oeiras, Portugal
Presenting author's email: mail@jscosta.com

DNA Barcoding of life using standardized *rbc*L and *mat*K sequences was recently proposed as a plant species identification system. In this study we tested the efficacy of these loci as DNA barcodes in one of the most ecological and economically important and predominant tree species complex in the Portuguese forests, by comparing *rbc*L and *mat*K sequences of some *Quercus* species occurring in Portugal, namely *Quercus coccifera* (carrasco); *Quercus ilex* (azinheira); *Quercus faginea* (carvalho-cerquinho); *Quercus canariensis* (carvalho-das-canárias); *Quercus pyrenaica* (carvalho-negral); *Quercus lusitanica* (carvalhiça); *Quercus robur* (carvalho comum), and *Quercus suber* (sobreiro). Sequence analysis of five individuals per taxa was used to assess for the capacity to discriminate this species and disentangle the intra and interspecific genetic variation. Based on these preliminary results the application of barcoding to these species is discussed. This study is the first attempt to specifically focus on the universality and atributes of candidate barcodes across *Quercus*.

S3P4.
Testing plant DNA barcode regions for species discrimination in *Silene* sect. *Siphonomorpha* Otth.

Cotrim H.M., Santos P.A. and Martins-Loução M.A.

Museu Nacional de História Natural, Jardim Botânico. Centre for Environmental Biology (CBA), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
Presenting author's email: hmcotrim@fc.ul.pt

In the last few years a great number of genomic regions with phylogenetic potential have been studied driven by Plant DNA barcode research. DNA barcode involves sequencing of a standard region of DNA as tool for species identification. CBOL Plant Working Group aims to identify a standard DNA barcode for land plants. A 2-locus combination approach was recently recommended, however species discrimination for this loci combination was only successful in 72% of cases (1). A future challenge for DNA barcoding in plants is to increase the proportion of cases in which unique species identifications are achieved. The study of closely related species and evaluation of the performance of proposed plant DNA barcodes in phylogenetic reconstruction has great significance. To improve species concepts, a more sophisticated approach to barcoding is needed, which would ideally include sequences from multiple independent markers, a multi-locus barcode, and specific inference tools that could be used to explore species limits and identify genetic gaps (2). This second type of barcode would improve the information base upon which the cruder plastid and mitochondrial DNA barcodes depend. Thus it is advantageous to identify such plant groups were unique species identification is not achieved by standard 2- loci barcode. Here we apply the recently proposed DNA barcode for land plants *mat*K and *rbc*L plus the supplementary gene *trn*H-*psb*A to *Silene* sect. *Siphonomorpha* species. This group of c. 35 species has an unresolved phylogeny for ITS and *trn*L-F, subsequent improvements in resolution were achieved by neutral molecular markers AFLPs (3). With this work we aim to select phylogenic informative regions for *Silene* sect. *Siphonomorpha* and contribute for the establishment of a species-level case study in the framework of present research of a DNA barcode for land plants.

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S3P5.
Identification of poisonous plants by DNA barcoding approach

Bruni I., De Mattia F., Galimberti A., Cattaneo F., Casiraghi M. and Labra M.

Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milan, Italy
Presenting author's email: a.galimberti3@campus.unimib.it

The human diet includes a lot of domesticated plant species (for instance *Hordeum vulgare* L., *Zea mays* L., *Oryza sativa* L., etc.) derived from an evolutionary continuum of people–plants interactions. However, several spontaneous plants are potentially toxic for human beings. The accidental ingestion of toxic plant portions (i.e. seed, fruit, root, etc.) can cause severe poisoning or even death. The clinical diagnosis of intoxicated patients is typically based on the morphological analysis of plant fragments in the stomach contents. This method is very tedious to perform, requires a considerable amount of training and usually a variable proportion of plant fragments remains unidentifiable. In addition, the plant species identifications can be difficult without residuals showing distinctive taxonomic elements. Recently, a new technique for plant identification based on the analysis of a short, standardized DNA region known as "DNA barcoding" (www.barcoding.si.edu) has been proposed. The objective of this research is to test DNA barcoding approach as a new universal tool to identify toxic plants univocally and rapidly. Five DNA barcode regions were evaluated: three cpDNA sequences (*trn*H-*psb*A, *rpo*B and *mat*K) and two nuclear regions (At103 and sqd1). The performance of these markers was evaluated in three plant groups: (1) a large collection of angiosperms containing different toxic substances, (2) congeneric species showing different degrees of toxicity and (3) congeneric edible and poisonous plants. Based on assessments of PCR, sequence quality and resolution power in species discrimination, the combination of plastidial and nuclear markers to identify toxic plants were commended. Concerning plastidial markers, *mat*K and *trn*H-*psb*A showed consistent genetic variability. However, in agreement with CBOL Plant Working Group, we selected *mat*K as the best marker, because *trn*H-*psb*A showed some problems in sequences sizes and alignments. As a final and relevant observation, we also propose the combination of *mat*K with a nuclear marker such as At103 to distinguish toxic hybrids from parental species. In conclusion, our data support the claim that DNA barcoding is a powerful tool for poisonous plant identifications. The next step of this research is the establishment of a dedicated poisonous plants database where all species are described either by morphological and molecular (DNA barcoding) approaches for a rapid identification of toxic plant by poisonous centres.

S3P6.
Lithuanian Flora Barcoding Initiative: LT-PlantBOL

Pasakinskiene I.[(1)]*, Skridaila A.*[(1)]*, Tupciauskaite J.*[(2)]*, Zilinskaite S.*[(1)] *and Hollingsworth P.*[(3)]

[(1)]Botanical Garden of Vilnius University, Vilnius University, Vilnius, Lithuania [(2)]Faculty of Natural Sciences, Vilnius University, Vilnius, Lithuania [(3)]Genetics and Conservation Section, Royal Botanic Garden, Edinburgh, UK
Presenting author's email: izolda.pasakinskiene@gf.vu.lt

Plant barcoding is a very new approach, which integrates classic taxonomy and molecular genetics. Only very recently, in August 2009 the members of the CBOL's Plant Working Group published an open access paper in PNAS recommending a standard plant barcode based on chloroplast DNA sequences of the partial regions of the *rbc*L and *mat*K genes (CBOL Plant Working Group, 2009). To answer the question how many species of Lithuanian Flora have DNA barcodes we have performed a search of a sample set of 200 plant species against the data at the Barcode of Life Data System (BOLD) repository. This search has revealed that species from Lithuanian Flora representing Central European Flora are very poorly presented in BOLD, >70% of species were found to be missing. In October 2009, the Botanical Garden of Vilnius University (BGVU) joined the Consortium for the Barcode of Life (CBOL). The BGVU conducts research programmes covering a broad rage of topics on plant taxonomy, genetics and physiology. BGVU site in Kairenai covers an area of 191.5 hectares in a terrain characterized by a hilly landscape formed by the last glaciations and further shaped by erosion. Large part of this site is present as natural or semi-natural flora habitats, such as remote slopes, low hills, valleys, ravines, springy wetlands, streams and ponds. Considering the variation of flora biotopes, we have selected the following three habitats for the model plant barcoding studies: (i) broadleaved forest, (ii) coniferous forest, and (iii) water pond and an adjacent meadow. Sampling of plants will cover a broad range of taxa representing Magnoliophyta, Pinophyta, Polypodiophyta, Equisetophyta and Bryophyta. In total, the dataset of ca. 300 species is expected. By carrying out the Lithuanian PlantBOL project, the implementation of plant DNA barcoding approach, as a modern means for management of Lithuanian plant genetic resources, will be achieved. LT-PlantBOL Initiative aligns within the area of investigation activities of the international TreeBOL and GrassBOL groups.

S3P7.
Testing a DNA barcode application on the Portuguese wild relatives of *Beta* species

Romeiras M.M.(1), Vieira A.(2), Veloso M.(3), Moura M.(4), Batista D.(5), Pina-Martins F.(6), Paulo O.S.(7) and Duarte M.C.(8)

(1)Tropical Botanical Garden (JBT), Tropical Research Institute, Lisbon (2)Computational Biology & Population Genomics Group, CBA, Faculdade de Ciências, Lisbon (3)Instituto Nacional de Investigação Agrária (INIA), INRB, Oeiras (4)Department of Biology, University of the Azores, Ponta Delgada (5)Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Tropical Research Institute, Oeiras (6)Computational Biology & Population Genomics Group, CBA, Faculdade de Ciências, Lisbon (7)Computational Biology & Population Genomics Group, CBA, Faculdade de Ciências, Lisbon (8)Tropical Botanical Garden (JBT), Tropical Research Institute, Lisbon, Portugal
Presenting author's email: mromeiras@yahoo.co.uk

The conservation, utilization and management of the biodiversity require that plant species can be delimited and identified. The genus *Beta* (Family Chenopodiaceae) show high levels of diversity and present some similarities to *Patellaria* genus. In Portugal, the genus *Beta* is represented by the following taxa: *B. vulgaris* ssp. *maritima* (Portugal mainland, Azores, Madeira); *B. macrocarpa* that occurs in Portugal mainland; *B. patula* that is endemic in the Madeira and the Desertas islands; and *B. vulgaris* ssp. *vulgaris* that is naturalized in Portugal mainland. The *Patellaria* genus (included in *Beta* by some taxonomists) is represented by *P. procumbens* (=*B. procumbens*) endemic to the Macaronesian archipelagos of Madeira, Canary and Cape Verde Islands; *Patellaria patellaris* (=*B. patellaris*) in Portugal mainland and in the Macaronesian archipelagos of Madeira, Canary and Cape Verde Islands. The aim of this study is to test the utility of DNA barcodes in determining species boundaries among Portuguese *Beta* species. The utility of the DNA barcodes, in the establishment of species boundaries will be discussed as well as how the cpDNA regions (*mat*K and *rbc*L) has accumulated or not enough base-pair differences needed to tell these entities apart, and so if their DNA barcodes can be helpful for the characterization of species diversity within *Beta* genus. Moreover the DNA barcode will provide molecular data for further studies on the distribution and ecology of each species, in order to contribute to the conservation of wild *Beta* populations in Portugal.

S3P8.
Barcoding of Bromeliaceae

Kanz B.[(1)], Schneider J.[(1)], Zizka G.[(1)] and Printzen C.[(1)]

[(1)]Botany and Molecular Evolution, Senckenberg Research Institute, Frankfurt am Main, Germany [(2)]Spezielle Botanik und Funktionelle Biodiverstiät, University Leipzig, Leipzig, Germany [(3)]Botany and Molecular Evolution, Senckenberg Research Institute, Frankfurt am Main, Germany [(4)]Botany and Molecular Evolution, Senckenberg Research Institute, Frankfurt am Main, Germany
Presenting author's email: jschneid@uni-leipzig.de

The neotropical family Bromeliaceae comprises c. 2600 species, many of which have decorative inflorescenses and are traded as ornamentals. Most known, however, are the fruits of the pineapple (*Ananas comosus*). The phylogenetic relationships of the family and its subfamilies have received considerable attentions in recent years. Nevertheless, delimitation and identification of species remain difficult in many groups. In an ongoing project we are exploring the potential of the recognized barcoding markers *mat*K and *rbc*L as well as other genetic markers. The long-term goal will be the barcoding of the majority of the species within Bromeliaceae.

S3P9.
DNA barcoding of trees: contrasting results from taxon based investigations in Italian *Quercus*, *Pinus* and *Acer*.

Simeone M.C.[(1)], Piredda R.[(1)], Armenise L.[(1)], Bellarosa R.[(1)], Attimonelli M.[(2)] and Schirone B.[(1)]

[(1)]FOR-BOL, Dipartimento Ambiente e Foreste (D.A.F.), Università della Tuscia, 01100 Viterbo, Italy [(2)]FOR-BOL, Dipartimento di Biochimica e Biologia Molecolare "E. Quagliariello", Università di Bari, 70125 Bari, Italy
Presenting author's email: mcsimeone@unitus.it

Trees play important roles in the conservation of numerous land ecosystems, in the wood trade, and in the definition of biogeographic processes; nevertheless, peculiar biological, evolutionary and taxonomical features are likely to constitute an intriguing challenge to barcoders. We examined whether 4 markers (*trn*h-*psb*a, *rbc*L, *rpoc*1, *mat*K) proposed by the Consortium for the Barcode Of Life (CBOL) matched species taxonomy in a preliminary tree biodiversity survey of Italian forested land. Our objective was to provide a test of future in situ applications of DNA barcodes by evaluating the efficacy of species discrimination under the criteria of uniformity of methods and natural co-occurrence of the species in the main forest ecosystems. Sixty species were included in a floristic study. We obtained 75% total discrimination success, with *trn*H-*psb*A + *rbc*L as the most efficient marker combination. Taxon-based investigations were performed on *Quercus* (thirty individuals, 12 species), *Acer* (14 individuals, 7 species) and *Pinus* 20 indivuals, 9 species). *Quercus* revealed as refractory to barcoding. Such a striking result was confirmed in several European provenances of the same species set. In contrast, 100% discrimination success was achieved across pines, whereas *Acer* displayed intermediate results. Intra-specific variation overlaps were absent in *Pinus*, present in closely related *Acer* species and complete in *Quercus*, where discrimination of haplotypes matched only subgeneric levels, and seemed to better reflect some regional patterns rather than species boundaries. Tree taxonomy and related forestry fields (forest ecology, protection, silviculture, product marketing) would largely benefit from standard, low-cost, consistent methods to assess species identity. Limitations appear to be mostly due to hybridization/introgression phenomena and share of ancestral polymorphism, which would prevent the correct match between DNA variation at the plastid level and species identity. Additional limitations are likely to be represented by biogeographical factors. Until more efficient markers will be developed, we recommend that improved and diversified sampling (multiple locations of sympatric and co-occurring congenerics) be embraced as a timely and important goal for precise assessment of haplotype specificity to allow productive application of barcoding in practice.

S3P10.
DNA barcoding contributes to species identification in *Orthotrichum* mosses

von Cräutlein M., Korpelainen H., Pietiläinen M. and Rikkinen J.

Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland
Presenting author's email: maria.voncrautlein@helsinki.fi

The species of the moss genus *Orthotrichum* (Orthotrichaceae) are typically identified based on the characters of the spore capsule. However, spore capsules are not always produced and found in harsh environments, e.g., at the margin of the area of distribution, where conditions for spore production are too cold or dry. Therefore, the identification of many *Orthotrichum* species may be impossible without additional tools of identification, and that is why we have initiated a DNA barcoding project on *Orthotrichum* species occurring in Finland. Many of these species are classified as threatened in Finland, because their natural habitats have been destroyed due to current forest management practises. Our aim is to employ DNA barcodes for species identification specifically in *Orthotrichum* but also in other difficult plant groups. The standard plant barcoding areas, *rbc*L and *mat*K, were tested first. Previous studies have revealed that the *rbc*L region, amplified with universal primers, commonly provides enough resolution to distinguish species in most bryophytes, while the amplification of matK with universal primers is generally unsuccessful. Our attempts to use *mat*K in *Orthotrichum* failed, while *rbc*L barcoding was highly successful. Additionally, the nuclear ITS region was sequenced to provide additional resolution. Firstly, we produced DNA barcodes (*rbc*L combined with ITS information) for all 19 *Orthotrichum* species known to occur in Finland and, secondly, we tested the usability of the developed barcodes for species identification in sterile *Orthotrichum* patches in a natural environment.

S4P1.

Cytochrome b sequence diversity for distinction of genera, sections and species in fungi

Moreira P.[1,2], Pereira F.[1], Amorim A.[1,2] and Araújo R.[1]

[1]IPATIMUP, University of Porto, Porto [2]Faculty of Sciences, University of Porto, Porto, Portugal
Presenting author's email: ricjparaujo@yahoo.com

Aspergillus fumigatus is one of the most prevalent airborne saprophytic fungi responsible for life-threatening infections. The species belongs to the (recently described) *Aspergillus* section *Fumigati*, a complex of morphologically related species. Since long, mitochondrial DNA (mtDNA) has been useful for molecular identification of species owing to its uniparental inheritance and relative high mutation rate, particularly in mammals. Many sequences of cytochrome b (Cytb) and cytochrome oxidase subunit 1 (Cox1) genes are now available for phylogenetic inferences in taxonomic groups of fungi. The aims of this study were: (1) to determine the level of sequence divergence in intergenic and coding mtDNA sequences of *A. fumigatus*, and (2) to infer the phylogeny of *A. fumigatus* using a larger section of the mitochondrial genome. Therefore, we have sequenced part of dehydrogenase subunit 1 and 2 (Nad1 and Nad2) genes, the complete Cytb and two intergenic regions (in a total of 2,945 bp) in three strains of *A. fumigatus*. New data from *A. fumigatus* mtDNA were compared with the available sequence information from other *Aspergillus* species, namely *A. oryzae*, *A. niger*, *A. tubingensis* and *A. terreus*. The mtDNA region of 2,945 bp was very similar among *A. fumigatus* strains (n=3), with only one polymorphic site observed in the Nad2 regions, two polymorphic sites in Cytb, and one in the intergenic region between Cytb and Nad1. The phylogeny of combined mtDNA regions (2,945 bp) supported previous observations obtained with partial Cytb sequences (402 bp), with *A. niger* and *A. tubingensis* well separated from other *Aspergillus* species (both species belong to Section *Nigri*). The nucleotide differences between *Aspergillus* species ranged from 0 to 64 % in the coding regions (particularly in Cytb) and from 5 to 87 % in the intergenic regions. In a near future, a single nucleotide polymorphism based screening tool may be developed in order to discriminate *A. fumigatus* strains, avoiding expensive sequencing analyses. Cytb gene was suitable for clear distinction of genus, section and species taxonomic levels in fungi.

S4P2.
A *Saccharomyces cerevisiae* strain collection from winemaking environments

Drumonde J.[1,2], *Vieira E.*[1,3], *Gambon B.*[4], *Valero E.*[5], *Gomes A.C.*[6], *Sousa S.*[6], *Lima M.T.*[2], *Araújo I.*[3], *Santos M.*[6,7], *Dequin S.*[4], *Casal M.*[1] *and Schuller D.*[1]

[1]Centre of Environmental and Molecular Biology (CBMA), University of Minho, Braga, Portugal, [2]Research Centre for Agricultural Technology, University of Azores, Angra do Heroísmo, Portugal, [3]Vinalia - Biotechnology Solutions for Viticulture and Enology, *Spin-off* of the University of Minho, Braga, Portugal, [4]INRA Montpellier, SupAgro, Montpellier, France [5]Dep. of Molecular Biology and Biochemical Engineering, Pablo de Olavide University, Seville, Spain [6]BIOCANT - Biotechnology Innovation Center Cantanhede, Portugal, [7]CESAM and Biology Department, Aveiro University, Aveiro, Portugal
Presenting author's email: drumondeneves@gmail.com

The model organism *Saccharomyces cerevisiae* stands today at the forefront of molecular biology and functional analysis in genetics and genomics. However, understanding of the ecological, evolutionary and population genetic features that shaped the biology of this species is underscored by a wealth of knowledge on molecular and cellular biology obtained from a very limited number of laboratory strains. In this reasoning, we constituted one of the largest bio-databanks of *S. cerevisiae* that were obtained from winemaking environments in Portugal and France.
During the harvest time of 2001 to 2009, 604 grape samples were collected in appellations of origin in Portugal (Vinho Verde, Dão, Douro, Bairrada, Estremadura, Palmela, Ribatejo, Açores) and France (Languedoc). The grape samples belonged to the varieties Alvarinho, Aragonez, Arinto, Avesso, Baga, Bical, Castelão, Carignan, Loureiro, Maria Gomes, Terrantez, Touriga Nacional and Verdelho. Yeast populations, in particular *S. cerevisiae*, were isolated after spontaneous fermentation of the extracted grape juice. From the final stage of 258 fermentations, 7740 yeast isolates were obtained, belonging mainly (5496 isolates) to the species *S. cerevisiae*. An initial genetic screen, based on mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP), electrophoretic karyotyping or interdelta sequence analysis, was followed by microsatellite analysis of strains with unique genetic profiles. Isolates were assigned to 752 different strains, based on their microsatellite allelic distribution. The resulting web-based autochthonous strain collection is one of the largest *S. cerevisiae* bio-databanks and is a resource for sustainable biodiversity preservation, equitable sharing of genetic data and winemaking strain selection.

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S4P3.

Usage of ITS barcodes as unambiguous discriminators of strains of *Articulospora tetracladia*

Seena S., Duarte S., Pascoal C. and Cássio F.

Centre of Environmental and Molecular Biology (CBMA), University of Minho, Braga, Portugal
Presenting author's email: sduarte@bio.uminho.pt

Articulospora tetracladia (teleomorph: *Ombrophila tetracladia*) is an aquatic hyphomycete species discovered by Ingold in 1940. This species play a key role in the decomposition of plant litter and it has been documented as dominant sporulating species in streams of Northwest Portugal (56-79%, Souto stream; 6-20%, Ave river; 31.7-81.4%, Este river). Traditionally, identification of aquatic hyphomycetes has been based on the morphology and development of conidia. The morphology of conidia was shaped by natural selection (convergent evolution) mainly to facilitate attachment to substrata in running waters. *Articulospora tetracladia* have 2 conidial forms: Y-shaped conidia known as forma angulata, and tetraradiate conidia, described as forma tetracladia. The spores with forma angulata are generally smaller than those with forma tetracladia. Recently, aquatic hyphomycete species have been differentiated by barcodes. However, our knowledge about the diversity within the species is limited. The *A. tetracladia* isolates used were collected from various types of plant litter or foam in streams from North to Central Portugal between 2000 and 2010. First, the diversity of *A. tetracladia* isolates was analyzed by denaturing-gradient gel electrophoresis (DGGE) after PCR amplification of the Internal Transcribed Spacer (ITS) 2 region. The possibility of using ITS1-5.8S-ITS2 barcodes to discriminate the strains was further explored. The ITS barcodes were also compared with those published at the National Centre for Biotechnology (NCBI) or at the National Institute of Technology and Evaluation Biological Resource Centre (NBRC). The DGGE bands appearing at the same position on a gel were considered as the same operational taxonomic unit (OTU). Seven OTUs were recognized after DGGE, suggesting the occurrence of different populations / strains of *A. tetracladia*. The OTUs consisted of random isolates of *A. tetracladia* and were not clustered based on date, source (leaves, twigs or foam) or location of collection. The Neighbor Joining tree (Kimura 2-parameter distance) constructed with ITS1-5.8S-ITS2 sequences from Portugal, NCBI and NBRC also yielded 7 major clades corroborating results from DGGE. Out of the 7 identified clades, some of the strains from Portugal appeared to be cosmopolitan but others tend to have a localized distribution. The maximum evolutionary divergence observed between the isolates was 1.8%.

S4P4.
DNA barcoding Mediterranean Boletales

Marques G.M.[1], Pinto C.C.[1] and Muñoz J.A.[2]

[1]Dept. of Agronomy, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal [2]Sociedad Micologica Barakaldo, Barakaldo – Vizcaya, Spain
Presenting author's email: gmarques@utad.pt

Discrimination of Mediterranean Boletales relaying on morphological diagnosis is a difficult task due to their phenotypic plasticity in the characters employed for species recognition, which can lead to incorrect classifications. Morphological diagnosis can also overlook cryptic species, which are common in many fungal groups. In addition, since morphological characters are effective only for fungal sporocarps, fungal mycelia cannot be identified, for example in mycorrhizal structures. The limitations inherent in morphology-based identification systems, requires the use of other approaches to taxon recognition. Several studies showed that fungal species discrimination through the analysis of genomic variability in the internal transcribed spacer (ITS) of the nuclear ribosomal genes represents a powerful approach to the diagnosis of fungal diversity. In the present study, we evaluated the use of ITS region as a taxonomic tool for discriminating species of Mediterranean Boletales, namely members of genera *Boletus* and *Suillus*. *Boletus impolitus* was also included in the analysis despite its recent inclusion within the genus *Xerocomus* (Ladurner & Simonini, 2003). Samples analysed were collected from various geographic regions and habitats in Portugal and Spain and maintained in herbarium collections. ITS sequences from sporocarps belonging to 24 different species of *Boletus* and 9 species of *Suillus* were determined and some of them were deposited in the GenBank for the first time. Two additional species from section *Luridi* are being analysed and probably represent new taxa. Other European species were retrieved from UNITE and GenBank. Phylogenetic trees were generated using neighbor-joining and maximum parsimony methods. Species of section *Luridi* split into three groups and the other sections are monophyletic and confirm the accuracy of current taxonomic classification (Muñoz, 2005). Especially fungi from sections *Luridi* and *Appendiculati* provides challenging cases for morphology-based species diagnosis. However, results showed that the ITS region is an effective genomic approach for species discrimination and phylogenetic analysis in the studied fungal taxa.

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S4P5.
ITS as DNA meta-barcode for fungi: an *in silico* approach reveals PCR biases

Bellemain E.[(1)], Carlsen T.[(2)], Brochmann C.[(1)], Coissac E.[(3)], Taberlet P.[(3)], Marthinsen G.[(1)] and Kauserud H.[(2)]

[(1)]National center for Biosystematics, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway [(2)]Microbial Evolution Research Group (MERG), Department of Biology, University of Oslo, P.O. Box 1066 Blindern, N-0316 Oslo, Norway [(3)]Laboratoire d´Ecologie Alpine (LECA), CNRS UMR 5553, University Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France
Presenting author's email: g.m.marthinsen@nhm.uio.no

The nrDNA ITS region has become the selected marker for fungal DNA barcoding. In this study we explored the amplification biases that various commonly utilized ITS primers might introduce during amplification of environmental samples (amplification length, mismatches between primers and template and taxonomy). For this purpose we performed *in silico* PCRs, from fungi and plant databases, with commonly used primer combinations using the software EcoPCR. We revealed that primers ITS1F, ITS1 or ITS4b were hampered by a high degree of mismatches, i.e. an increased fungal diversity is revealed when reducing the stringency level of the PCR. Primers ITS2, ITS3, ITS4 and ITS5 gave more consistent amplification results. Taxonomic biases were also apparent. Overall, using primers amplifying the ITS2 region preferentially amplified ascomycetes compared to basidomycetes or other fungi. In addition, ascomycete amplicons were significantly shorter than basidomycete fragments both for the whole ITS region and the ITS2 region, which might increase the taxonomic bias since shorter fragments are more readily amplified. The results we obtained gave insight into the relative performance of ITS primer pairs, which will be of great interest to the fungal community. We also demonstrate the usefulness of using a bioinformatic approach for selecting the primer pair(s) according to the goals of the study.

S4P6.
Identification of fungi isolated from the olive moth (*Prays oleae* Bern.) based on ITS region

Oliveira I.[(1)]*, Pereira J.A.*[(1)]*, Bento A.*[(1)]*, Lino-Neto T.*[(2)] *and Baptista P.*[(1)]

[(1)]CIMO, Instituto Politécnico de Bragança, Bragança, Portugal [(2)]BioFIG, Departamento de Biologia, Universidade do Minho, Braga, Portugal
Presenting author's email: ivo.oliveira@ipb.pt

The internal transcribed spacer (ITS) region is one of the proposed DNA regions for barcoding fungal species. This region is extensively used for molecular systematic and identification of species, being probably the most widely sequenced DNA region in fungi. This fact arises from the simplicity of the amplification, related to the multicopy nature of the rDNA; the possibility of using universal primers; and the high level of sequence variation that occurs even between species closely related. Furthermore, a significant number of identified sequences is available for comparison in the GenBank database. However, some limitations can be pointed out, when using this region to perform species identification. Our work intends to exemplify the application of the ITS region in the identification of the fungal species associated to one of the major pests affecting the olive groves, the *Prays oleae* Bern., in the Portuguese region of Trás-os-Montes (Northeast of Portugal). For this purpose, larvae and pupae of the three annual generations (phyllophagous, antophagus and carpophagus) of *P. oleae* were collected from several olive groves from Trás-os-Montes region. When a fungus was associated to the cause of death of the moth, pure cultures of the fungus where prepared. Following DNA isolation, the corresponding ITS regions were amplified and sequenced, using the universal primers ITS1FO and ITS4RE. The obtained DNA sequences were analysed and fungal identification was performed by comparison with deposited sequences on NCBI database. Results concerning fungal species identification on different generation of olive moth will be presented. Overall results showed that from the total obtained sequences, 34.5% didn't allow identification to species level, only to genus level, when submitted to the NCBI database, and no further sequence handling was performed. However, as referred before, alignment comparison can provide useful information, which can, ultimately, allow the identification of all the sequenced fungal species, using the ITS region. In this work it will be discussed the percentage of sequences that were able to achieve identification to species level, the genera that demonstrated to be more difficult to disclose their species, as well as the limitations encountered will be discussed.

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S4P7.
Potential use of barcoding to identify *Tetracladium* species

Letourneau A.[1], Seena S.[2], Marvanová L.[3] and Bärlocher F.[4]

[1]Department of Biology, Carleton University, Ottawa, Canada [2] Centre of Environmental and Molecular Biology (CBMA), University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal, [3]Czech Collection of Microorganisms, Institute of Experimental Biology, Faculty of Science, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic [4]Department of Biology, Mt. Allison University, Sackville, NB E4L1G7, Canada
Presenting author's email: seena.sahadevan@gmail.com

Aquatic hyphomycetes are a phylogenetically heterogeneous group with worldwide distribution. *Tetracladium* is a common aquatic hyphomycete genus, whose taxonomy has been based on the morphology and development of asexual spores. Ecological surveys have relied almost exclusively on spore morphology of aquatic hyphomycetes. Since selective pressures have resulted in convergent shapes, misidentifications are a concern. We determined COX1, ITS and D1/D2 sequences as potential barcodes on 21 strains belonging to 7 described *Tetracladium* species and an unidentified strain. Attempts to amplify the IGS region were unsuccessful. The ratio of intraspecific to interspecific variability was optimal with ITS, which also provided the intuitively most acceptable cladogram. Typical conidia and their variability for the seven described species are illustrated. Internal node reliability depended less on total sequence length and more on the mixture of conserved and variable regions used to build cladograms. This finding can be exploited for quickly increasing phylogenetic accuracy without greatly increasing the amount of amplification and sequencing. The results have important implications for identifying freshwater hyphomycetes.

S5P1.
High diversity of deep-sea *Gromia* revealed by small subunit ribosomal DNA sequence analysis

Aranda da Silva A.[(1)]*, Pawlowski J.*[(2)] *and Gooday A.*[(3)]

[(1)]CESAM, University of Aveiro, Aveiro [(2)]Department of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland [(3)]National Oceanography Centre Southampton, UK
Presenting author's email: ana.aranda@ua.pt

The genus *Gromia* includes large marine protists ('gromiids') with filose pseudopodia and sack-like organic tests. The first deep-water species were discovered in the 1990s on the Oman Margin of the Arabian Sea and subsequently found on the Pakistan Margin, Ross Ice shelf and Weddel Sea. Although deep-water *Gromia*-like morphospecies were discovered in the 1990s, their relations to the shallow water species have been established only recently. Moreover, very little is known about the diversity within *Gromia*, reflecting the fact that these morphologically relatively simple protists have few characters useful for species identification. Their identification as gromiids is confirmed by analyses of partial small subunit ribosomal DNA (SSU rDNA) gene sequence. Although *Gromia* was always described as a "large" marine protist here we report the first tiny gromiid. This species was first thought to be an allogromiid, organic-walled foraminifera, based purely on morphological characterization. However, analysis of partial SSU rDNA gene using *Gromia* specific primers revealed it to be a gromiid instead. The discovery of this tiny gromiid species raises the possibility that these relatives of the foraminifera are more common and widely distributed in the deep sea than currently appreciated.

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S5P2.
DNA barcoding with ITS sequences reveals great heterogeneity within *Dunaliella salina* (Chlorophyceae, Dunaliellales)

Assunção P.[(1)]*, Jaén-Molina R.*[(2)]*, Caujapé-Castells J.*[(2)]*, de la Jara A.*[(1)]*, Carmona L.*[(1)]*, Freijanes K.*[(1)] *and Mendoza H.*[(1)]

[(1)]Departamento de Biotecnología, Instituto Tecnológico de Canarias (ITC), Pozo Izquierdo, Gran Canaria, Spain
[(2)]Departamento de Biodiversidad Molecular y Banco de ADN, Jardín Botánico Canario Viera y Clavijo, Tafira Alta, Gran Canaria, Spain
Presenting author's email: pclemente@itccanarias.org

The unicellular biflagellated green alga *Dunaliella salina* (Chlorophycea, Dunaliellales) is the most halotolerant eukaryotic photosynthetic organism known. This organism is able to accumulate large amounts of carotenes under extreme stressful conditions such as high salinity, low nitrogen levels and/or high solar radiation. Interestingly, it was the first microalgae to be used commercially to produce fine chemicals. Nowadays it is the best commercial source of natural Β-carotene and it has also been recommended as an important source of glycerol. Nevertheless, the enormous geographical, physiological, morphological and genetic variability described within *D. salina* is still intriguing. In an attempt to clarify the structure of this complex taxon we analyzed the Internal Transcribed Spacer (ITS1+ITS2) of 35 *D. salina* strains from different geographic origins. Thirteen ITS1+ITS2 sequences correspond to recent *D. salina* isolates from different Spanish and French saltworks. The phylogenetic analysis (Bayesian Inference and Maximum parsimony Analysis) revealed the occurrence of two major clades within *D. salina* strains, which were divided in several subclades, demonstrating the great genetic heterogeneity among this group. Statistical analysis (Mann-Whitney, Kruskal-Wallis) based on distance and DNA diagnostic characters supported the division of *D. salina* into two biological groups. Moreover, both the phylogenetic and the statistical analyses suggest that one of the *D. salina* Spanish isolates (ITC5105, Janubio) should be considered as a distinct species. Although most *D. salina* isolates from the same country were grouped in the same clade, the ITS region was not geographically informative overall. Based on these findings, we feature that the ITS1+ITS2 region should be considered for DNA barcoding in microalgae.

S5P3.
Exploring macroalgal diversity along Italian coasts: a first approach by DNA barcoding.

Morabito M., Manghisi A., Fiore V., Bertuccio C., Armeli Minicante S., Le Gall L. and Genovese G.

Life Sciences "M. Malpighi" - BOTANY, University of Messina, Messina, Italy
Presenting author's email: morabitom@unime.it

The algal flora of Italy has been subject of interest since at least two centuries. Early knowledge dates back to XIX century, thanks to the works of many phycologists, whose findings are admirably synthesized in Sylloge algarum by De Toni (1889-1924). Afterwards, works on specific areas, such as Venice Lagoon, Adriatic, Sicily and the Bay of Naples, lead to modern studies of living Italian phycologists. In macroalgae, simple morphology and anatomy, rampant convergence, remarkable degrees of phenotypic plasticity in response to environmental factors, and incompletely understood life histories tend to confound attempts at identification. Therefore, algal systematists rely increasingly on DNA sequences to discriminate taxa. Although there is ample justification for the development of multiple and divergent molecular markers for phylogenetics, a standard marker, a DNA barcode, is a powerful tool for the purposes of quick and accurate species identification. At present, the study of macroalgal diversity in the Mediterranean by molecular tools is incomplete, only a few studies are available dealing with specific genera, while a molecular assisted floristic list is definitely missing. The location of Sicily in the middle of the Mediterranean gives the island utmost importance for biodiversity studies. Furthermore, the diversity of geomorphologic and hydrological features and substratum types along its shores accounts for many different habitats. The Straits of Messina, in particular, due to its peculiar geomorphology and hydrodynamics, has rich benthic communities. The Phycological Lab at the University of Messina, Italy, has undertaken a program of census of macroalgal species in the area of the Straits of Messina including neighbouring brackish coastal lakes. The aim of the program is to compile a DNA barcode inventory and to integrate morpho-anatomical data in order to compile an updated florist list and eventually a taxonomic revision of most critical taxa. Preliminary data allowed us to identify two newly introduced species of Rhodophyta, namely *Hypnea cornuta* and *Agardhiella subulata*. Possible vectors of introduction have been evaluated, and shellfish importation and storing seems to be the main cause. A revision of *Porphyra* species is also undertaken, whose discrimination is problematic as world-widely acknowledged. In particular, species going under the name *P. leucosticta* seems to actually represent distinct taxa.

S5P4.
Molecular divergence within *Ralfsia verrucosa* (Ralfsiales, Phaeophyceae) indicates cryptic species

Parente M.I.(1), Rousseau F.(2), de Reviers B.(2), Fletcher R.L.(3), Costa F.(4) and Sanders G.W.(1)

(1)1Centre for Environmental & Molecular Algal Research, University of New Brunswick, Fredericton, NB, E3B 5A3, Canada (3)3UMR 7138, Systématique, adaptation, évolution, Muséum national d'histoire naturelle, 57, rue Cuvier, CP 39, 75231 Paris cedex 05, France (4)4Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Ferry Road, Eastney, Portsmouth PO4 9LY, Hampshire, UK (2) Centre of Environmental and Molecular Biology (CBMA), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
Presenting author's email: nelaparente@hotmail.com

Ralfsia verrucosa (J. E. Areschoug) J. E. Areschoug is characterized by a crustose and orbicular thallus, verrucose and relatively easy to raise from the substratum, with upwardly directed erect filaments, punctuate sori obvious in surface view and plurilocular sporangia with only one sterile terminal cell. This species is generally reported to be widely distributed in the Northeastern Atlantic and Mediterranean. Morphological and molecular examinations were performed using isolates from different regions including Azores, the northern and central coasts of mainland Portugal, the Atlantic and Mediterranean coasts of France, as well as isolates from Helgoland and England. In order to study DNA divergence and phylogenetic relationships within the species, analyses of mitochondrial (cytochrome c oxidase 1) and nuclear (internal transcribed spacer 1 and 2) sequence data were performed. Preliminary molecular analyses revealed the presence of divergent lineages corresponding to putative cryptic species that challenge the concept of *Ralfsia verrucosa*.

S6P1.
Barcoding Pipeline at the NHM London

Llewellyn-Hughes J.

Zoology, Natural History Museum, London, UK
Presenting author's email: julb@nhm.ac.uk

The Sequencing Facility at NHM London sits between Guelph and the Smithsonian in barcoding pipeline throughput capacity. Therefore we plan to set up a smaller scale barcoding pipeline at the NHM, which extends the existing pipeline (PCR purification to sequence data) to cover field/specimen collection to data analysis. Our Facility will continue to process all samples by our own optimised methods, many of which differ from those at other institutions (including Guelph and Smithsonian) as we believe these offer enhanced sequence quality to end users. We hope to offer a niche product to others in the barcoding community, i.e., quality barcode data from samples that have failed elsewhere, which will become more valuable over time as the barcoding database is populated. Our poster will present our optimised methodologies using our state of the art robotics.

S7P1.

Hidden diversity uncovered: examples of DNA barcoding applied to marine communities

Barreiro R., Couceiro L., Maneiro I., Ruiz J.M., Cremades J. and Bárbara I.

Biología Animal, Biología vegetal y ecología, Universidade da Coruña, A Coruña, Spain
Presenting author's email: rodbar@udc.es

Marine communities seem prone to cryptic diversity. Marine taxa often combine some phenotypic plasticity with a lack of distinctive, species-specific morphological traits. Under these circumstances, species discrimination becomes cumbersome and some taxa may even go unnoticed with unknown consequences for various fields of research. DNA barcoding has provided us with a convenient tool to address many of these difficult cases. Here, we describe several examples of its application derived from our own experience working with coastal benthic communities. Involving both seaweeds (Chlorophyta, Rhodophyta) and marine snails (Gastropoda), these examples show how DNA barcodes were pivotal to conclusively solve cryptic species issues in research fields as diverse as biological invasions (*Ulva pertusa*, Chlorophyta), endangered species (*Ahnfeltiopsis pusilla*, Rhodophyta), taxonomy (*Plocamium* spp., Rhodophyta), or pollutant biomonitoring (*Nassarius nitidus*, Gastropoda). These four examples add to a growing number of cases in which DNA barcoding has emerged as a versatile tool and may encourage other marine investigators to adopt DNA barcodes as an almost common procedure for field research.

S7P2.
Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding

Richard B., Decaëns T., James S.J., Rougerie R., Porco D. and Hebert P.D.N.

Lab ECODIV, University of Rouen, Mont Saint Aignan, France
Presenting author's email: thibaud.decaens@univ-rouen.fr

Species identification of earthworms is usually achieved by careful observation of morphological features, often sexual characters only present in adult specimens. Consequently, juveniles or cocoons are often impossible to identify, creating a possible bias in studies that aim to document species richness and abundance. DNA barcoding, the use of a short standardized DNA fragment for species identification, is a promising approach for species discrimination. When a reference library is available, DNA-based identification is possible for all life stages. In this paper, we show that DNA barcoding is an unrivaled tool for high volume identification of juvenile earthworms. To illustrate this advance, we generated DNA barcodes for specimens of *Lumbricus* collected from three temperate grasslands in western France. The analysis of genetic distances between individuals shows that juvenile sequences unequivocally match DNA barcode clusters of previously identified adult specimens, demonstrating the potential of DNA barcoding to provide exhaustive specimen identification for soil ecological research.

S7P3.
A method to discriminate species of virus vector trichodorid nematodes exploring 18S rDNA region

Duarte I.M.[(1)], Duarte M.M.[(2)] and Almeida M.T.[(3)]

[(1)]Departamento de Ciências do Ambiente, Escola Superior Agrária de Coimbra, Coimbra [(2)]Departamento de Virologia, Laboratório Nacional de Investigação Veterinária, Lisboa, Portugal, [(3)]Departamento de Biologia, Universidade do Minho, Braga, Portugal
Presenting author's email: iduarte.esac@gmail.com

The Trichodoridae nematodes are polyphagous root-feeding ectoparasites, represented by more than 100 species currently included in six genera. *Monotrichodorus*, *Allotrichodorus* and *Ecuadorus* contain 12 monodelphic species and have only been recorded from Central and northern America. The other didelphic genera, *Trichodorus*, *Paratrichodorus* and *Nanidorus*, include 13 species that are natural vectors of specific tobraviruses strains, commonly of Tobacco rattle virus, that have deleterious effect upon economically important crops, such as ornamental bulbs, potatoes and tobacco. In Portugal, 16 species were reported, some known as TRV vectors. When field populations of trichodorids become viruliferous, virus can persist for many years, acting the infected plants as virus reservoirs. Due to the specificity of virus-vector transmission, it is required to identify the virus and the vector at strain and species level, respectively. Accurate techniques to test suspicious soil and plant material are imperative for effective tobravirus management, namely for pre-planting risk assessment. Morphological identification of the vector nematode is time-consuming and requires well-trained specialists. Moreover samples frequently contain few specimens, and at immature stages, impossible to identify. We developed an alternative DNA barcoding method for a clear detection and identification of *Trichodorus*, *Paratrichodorus* and *Nanidorus* species, regardless of their life stage and geographical origin. A 500 bp region, located at the 3' end of the 18S gene exhibiting species-specific nucleotide variability was found suitable to be used as a trichodorid barcode tool. This region was identified based on the alignment of 12 nucleotide sequences of morphologically well-characterised specimens, representing the three didelphic genera. The selected region is flanked by two highly conserved sequences, which were used to design 2 primers for the PCR amplification of a 615 bp fragment. Direct sequencing of the amplicon allows a clear species identification. The typeability, reproducibility and the high discriminatory power of this approach was demonstrated with 21 populations, six of which non-indigenous. The method resolved individuals of different species but did not discriminate different populations of the same species. Our results suggest that this genetic region is adequate and effective for barcoding of virus vector trichodorids and seems very promising with soil environmental samples.

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S7P4.
Food safety and fish species identification through the DNA barcode

Schiavetti I.[1], Goria M.[2], Guercio A.[3], Fazio G.[4] and Ferrari A.[1]

[1]Istituto Zooprofilattico Sperimentale PLV, Genova, Italy [2]Istituto Zooprofilattico Sperimentale PLV, Torino, Italy [3]Istituto Zooprofilattico Sperimentale Sicilia, Palermo, Italy [4]ASL 2, Savona, Italy
Presenting author's email: angelo.ferrari@izsto.it

Since the '70-'80's there has been a strong depletion of fish stocks in Mediterranean waters, which has recently climaxed in the decline in catches and in a decrease of national fish production. In this context the present market of fish products is changing. Globalization is expanding in the fish industry too: “made in Italy” products supply and imports are covering over 68% of the current global resources. Thus standardized productions and counterfeiting issues are becoming increasingly common and it is difficult to recognize foreign species that are morphologically similar to more valuable and expensive Mediterranean ones. Often, protection of consumers' interests guaranteed by surveillance analysis fails in case of substitution commercial frauds and in case of sanitary frauds. Recently, molecular biology has been developing new PCR and sequencing protocols. The genetic profile of a species, despite its morphological identification, allows also the recognition of unhealthy or processed products. In particular, the “DNA Barcode” method uses a short DNA sequence from a particular region (citocrome oxidase I) of the genome like a “molecular signature” to identify a lot of taxa. Many research organizations are cooperating to create a universal barcode database. In particular the Istituto Zooprofilattico Sperimentale (IZS) of Genoa joins the FISH-BOL, Fish Barcode Of Life campaign, an international research collaboration that assembles a standardized reference DNA of fish for identification. Through membership to the international FishBol project, the IZS of Genoa, Torino and Palermo, the Museum of Natural History 'G. Doria' and the Minho Univ. are cooperating in a research funded by the Health Ministry. About 60 fish species were collected from the Mediterranean and European Atlantic, and now they are stored at -20°C.Seasoned professional in the recognition of fish species provided for the morphological identification of each sample. Then, following the quality standards required by the Consortium for the Barcode of Life, we proceeded with taxonomic identification through “PCR & sequencing based" methods. As the IZS is an official foodstuffs controlling entity, the aim is to create a standardized and reproducible method for routine checking of seafood to ensure the products traceability, and to evaluate the transparency of information provided to consumers. In addition it may increase support to value the impact of commercial fishing on fish stocks in terms of biodiversity.

S7P5.
Second-generation environmental sequencing unmasks marine metazoan biodiversity

Fonseca V.G.[1], Carvalho G.R.[1], Sung W.[2], Johnson H. .[1],Power D.M.[3], Neill S. .[4], Packer M.[5], Blaxter M.L.[6], Lambshead P.J.D.[7], Thomas W.K.[2] and Creer S.[1]

[1]Molecular Ecology & Fisheries Genetics Laboratory School of Biological Sciences Environment Centre Wales, Bangor University, Deiniol Road Gwynedd, LL57 2UW, UK [2]Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Rd, Durham, NH 03824, USA [3]Centre of Marine Sciences, CCMAR-CIMAR Associate Laboratory, University of Algarve, Gambelas, 8005-139 Faro, Portugal, [4]4School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, UK [5]Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK [6]Institute of Evolutionary Biology, Ashworth Laboratories, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK [7]School of Ocean and Earth Science, National Oceanography Centre, European Way, Southampton, SO14 3ZH, UK
Presenting author's email: veragfonseca@yahoo.com

In response to contemporary threats to biodiversity, accurate and objective methods of species or operational taxonomic unit (OTU) assessment are fundamental to our understanding of mechanistic links among ecosystem components and resilience. Second generation sequencing has been insightful for biodiversity analyses of the microbial biosphere, but such approaches have not been used to study metazoan phyla. Here, we reveal the relative richness of multiple metazoan phyla inhabiting the marine benthos using second-generation techniques. We identify substantial levels of unrecorded richness that refute currently accepted paradigms of phylum rank abundance, and variability in ecosystem-level community composition even at fine-level spatial scales. Additionally we describe sequences derived from putative metazoan taxa that bear little resemblance to any phylum for which sequence data are available. These findings show that such techniques will provide a rapid, objective and cost-effective taxonomic framework for exploring links between ecosystem structure and function of hitherto intractable, but ecologically important communities.

S7P6.
Use of DNA Barcode Sequences in Wildlife Forensics – A Case Study

George S., Suresh Kumar U., Ratheesh R., Santhoshkumar K. and Thomas G.

RFDF, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India
Presenting author's email: sgeorge@rgcb.res.in

Present paper reports a case in which a wildlife range officer had suspected that some people had killed a sambar deer. Cooked meat and dried skin were seized from the suspects and forwarded to our laboratory for DNA testing to resolve the identity of the animal. Mitochondrial Cytochrome C Oxidase subunit 1 (CO1) analysis revealed that the cooked meat and skin of the animal were of a sambar deer by comparing the sequences with the reference sequence of sambar deer generated in our lab. The study illuminates the use of barcode sequences and reference database in wildlife forensic applications.

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S7P7.
The use of the ITS region in marketable mushrooms authenticity

Nogueira L., Oliveira I. and Baptista P.

CIMO / Escola Superior Agrária, Instituto Politécnico de Bragança, Bragança, Portugal
Presenting author's email: ivo.oliveira@ipb.pt

Edible mushrooms, due to their flavour and nutritional characteristics, are very popular in many dishes. Some species are high valuated and reaching high market values. There are frequent reports of adulteration of these kinds of products due to the presence of fungal species less expensive among others with high-value market. This adulteration occurs especially in products in which the flavour is not prominent and in which the mushrooms are difficult to examine. In this work we utilized the internal transcribed spacer (ITS) for the identification of marketable mushrooms species in order to detected fraudulent addition of cheaper species. The products analysed were labelled as *Agaricus bisporus* (known as white mushroom), Portobello, Shiitake, Maitake, Enoki, Eringi, *Auricularia auricula-judae*, *Cantharellus cibarius*, *Craterellus cornucopioides*, shimeji and *Boletus edulis*. Following DNA isolation, the corresponding ITS regions were amplified and sequenced, using the universal primers ITS1FO and ITS4RE. The obtained DNA sequences were analysed and fungal identification was performed by comparison with deposited sequences on NCBI database. The results obtained shown that the ITS region is a suitable method of identifying mushroom species. Furthermore it was verified that the great number of the mushrooms analysed were correctly identified. However, one of the products, the shimeji, is uncorrected labelled. The advantages and disadvantages of the use of the ITS region to detect frauds in marketed mushrooms will be present and discussed.

S7P8.
Barcoding invasives: a new tool for invasion monitoring in soil

Porco D.[(1)], Thibaud D.[(2)], Deharveng L.[(1)], James S.[(1)], Butt K.[(1)], Stevens M.[(1)], Rougerie R.[(1)], Richard B.[(1)] and Hebert P.[(1)]

[(1)]Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada [(2)]UMR 5202 CNRS Origine, Structure et Evolution de la Biodiversité, Muséum National d'Histoire Naturelle, Paris, France [(3)]Division of Biology, University of Kansas, Kansas, USA [(4)]South Australian Museum, Adelaide, Australia [(5)]EA 1293 ECODIV, Université de Rouen, Mont-Saint-Aignan, France [(6)]School of Natural Ressources, University of Central Lancashire, Preston, UK
Presenting author's email: dporco@uoguelph.ca

Biological invasions are gaining substantial attention from policies makers. All biotas are or are about to become impacted by global changes and the soil fauna is no exception. However, the difficulties inherent in taxonomic surveys of the soil-dwelling organisms hamper the monitoring of this ecosystem compartment. Collembolans and earthworms are two key groups of the soil fauna that have been targeted for barcode analysis. Ten earthworm species and 6 collembolan species were used to test the efficiency of barcoding to monitor biological invasions in soil: European invasive earthworms in North America and European invasive collembolans in North America and Oceania. Present results establish the usefulness of DNA barcoding for the quick and low cost surveys of soil biodiversity.

S7P9.

A new approach towards documenting host-parasitoid relationships through DNA barcoding: Post-metamorphosis persistence of host-DNA in the gut-contents of parasitoid wasps

Rougerie R.[(1)], Smith M.A.[(1)], Fernandez-Triana J.[(1)], Lopez-Vaamonde C.[(2)], Ratnasingham S.[(1)] and Hebert P.D.N.[(1)]

[(1)]Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada [(2)]Zoologie Forestière, Institut National de la Recherche Agronomique, Orlèans, France
Presenting author's email: rrougeri@uoguelph.ca

While field observations, rearing or dissections have long been the only available means to unravel food-webs, molecular analysis of predator-prey relationships is a powerful emergent technique in the field. In particular, DNA-based identification through the use of selective primers and DNA sequencing permits thorough documentation of the prey consumed by predators or herbivores. However, this approach has never been applied to identify the larval food source from adult holometabolous insects, presumably because of internal anatomical restructuration during metamorphosis and the assumed degradation of DNA between the last larval meal and the emergence of the adult. Here, we challenge this presumption by demonstrating that the DNA of the host used by a parasitoid wasp during its larval development can be detected when sequencing genomic DNA extracts made from abdominal contents of the adult wasp. Using parasitoid wasps reared from known hosts and targeting a portion of the standard animal DNA barcode, we show that through the strategic selection of exclusionary primers, host-DNA can be selectively amplified and sequenced from wasp abdomens. Our strategy led to unambiguous identification of the host. Preliminary test in wild-collected parasitoid wasps was successful, although the overwhelming knowledge gap in host-parasitoid relationships makes data validation problematic for potential new records, emphasizing the need for developing strict sampling and processing protocols. In addition to the possibility of application to other holometabolous insects, our results offer an alternative to the current low-paced documentation of host-parasitoid relationships, and will improve the strategic use of parasitoid wasps as agents of biological control.

S7P10.
DNA-barcoding for commercial shellfish in Europe

Utge J.[(1)], Chaignon C.[(2)], Buge B.[(3)] and Samadi S.[(2)]

[(1)]MNHN, Dept. Syst & Evol UMS2700, Paris, France [(2)]MNHN/CNRS/IRD/CNRS, UMR 7138, Paris, France [(3)]MNHN, Service des collections, Paris, France
Presenting author's email: sarah@mnhn.fr

Resents studies used DNA barcoding to authenticate seafood. However, this was applied only for fish samples. The present study extends this technique to the shellfish species (Gastropoda, Equinodermata, Bivalvia, Cephalopoda, Crustacea, Tunicata) from the European's markets. More than 400 samples representing 63 species were collected, out of which 36 are the news items for The Barcode of Life Data Systems (BOLD). The morphological and the molecular approaches were confronted to the commercial labels to detect potentially mislabeled samples. Our results demonstrate that DNA barcodes are a powerful tool for the identification of seafood to the species level and have applications to detect mislabeling of products transformed from shellfish species.

S7P11.
SSM – The MNHN molecular platform

Boisselier M., Tillier A., Utge J., Lambourdiere J., Bonillo C., Pasquet E. and Samadi S.

Systématique et Evolution, MNHN, Paris, France
Presenting author's email: sarah@mnhn.fr

The "Service de Systématique Moléculaire" is the MNHN molecular platform which is part of a CNRS-MNHN unit "Outils et Méthodes de la Systématique Intégrative" (UMS 2700) directed by Eric Pasquet. This platform welcomes students, researchers and all users needing to develop molecular techniques for their taxonomic and phylogenetic studies and is opened to develop barcode projects. The MNHN is an international training lab of the Consortium of the Barcode of Life. The SSM has a long experience in molecular analyses for various taxa (Animals, Plant, Algae, Fungi…) and its technical staff is composed of 4 technicians. One of them is dedicated to the barcoding. Production capacities are now extended by automation of DNA extractions and amplification processes. Numerous Barcoding programs are developed at the SSM from molecules to data processing and analysing; we are developing electronic databases to trace all the data and link them both to the MNHN collections and online databases.

S8P1.
DNA barcodes for identification of European species of marine wood boring organisms, the 'termites of the sea'

Borges L.M.S.[1], Sivrikaya H.[2], Le Roux A.[3] and Costa F.O.[1]

[1]Centre of Environmental and Molecular Biology (CBMA), University of Minho, Braga, Portugal, [2]Faculty of Forestry, Bartin University, Bartin, Turkey [3]Vannes, France
Presenting author's email: luisa.borges@bio.uminho.pt

Marine wood borers are a heterogeneous group which includes Isopoda, Limnoridae; Amphipoda, Cheluridae and Bivalvia of the families Teredinidae and Pholadidae. Many species appear to be widely spread throughout the world's oceans, although, variation in morphological features have been documented for some. The main species occurring in European coastal waters belong to the families Limnoridae and Teredinidae. Despite the destruction they cause in wooden maritime structures – evaluated in millions of euros each year – in the last forty years wood borers have been relatively little studied in Europe. One of the reasons might be because in general surveys these organisms are missed due to their specific habitat. One of the key works on the family Teredinidae reported the occurrence of six teredinid species in Europe [1]. However in a later survey carried in 15 different sites in Europe, ranging from Iceland to Turkey, some of the species mentioned in [1] were not found and new ones were reported [2]. An annotated check-list of the family Limnoridae mentioned the occurrence of 4 to 6 species of limnoriids in Europe but in the Mediterranean 4 of the species have uncertain taxonomic status [3]. Identification of teredinids and limnoriids using morphological characters is difficult, which highlighted the need for more accurate identification tools. Therefore the aim of using DNA barcoding is to generate more rigorous and accessible identifications to facilitate research in wood borers diversity, evolution, ecology and wood protection in the marine environment. Herein we present some original DNA barcode data on a number of wood boring species which occur in Europe.

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S8P2.
A first step toward the DNA barcoding of African lymantriids

Nagy Z.T.[1], Breman F.C.[2] and Dall U.[3]

[1]JEMU, Royal Belgian Institute of Natural Sciences, Brussels, Belgium [2]JEMU, Royal Museum for Central Africa, Tervuren, Belgium [3]Lepidoptera laboratory, Entomology Section, Royal Museum for Central Africa, Tervuren, Belgium
Presenting author's email: floris.breman@africamuseum.be

Lymantriidae is a moth family showing a worldwide distribution. The highest diversity on species level can be found in the Afrotropics. In this region, about 1500 currently recognized species occur, they are assigned to approximately 110 currently recognized genera. We used an intergrative approach of morphological taxonomy and DNA barcoding to confirm or reject the hypotheses of the taxonomic status of samples collected in 2008 and 2009. Thereby, material was collected during four expeditions in Africa including the localities Lukolela (Democratic Republic of Congo), the Bia Forest Reserve (Ghana) and the Arusha and Uluguru regions (Tanzania). Tissue samples were either taken in the field and preserved in absolute ethanol or they were removed from dry preserved specimens. DNA barcoding confidently assisted in the identification in most cases, results were concordant with the morphological analyses in 67 out of the 70 species tested in this study. Inconsistencies were observed in the genus *Euproctis*, which topic needs further studies. Several cases will be presented where DNA barcoding may assist in solving taxonomic questions (e.g. a suspected case of synonymy in the genus *Stracena*). Our work makes a contribution to the DNA barcoding effort by supplying DNA barcodes to a previously underrepresented group.

S8P3.
DNA barcoding as identification tool for Congolese freshwater fish

Breman F.C.[1], Sonet G.[2], Nagy Z.T.[2], Kurt J.[1], Van Houdt J.[1], Hanssens M.[3], Vreven E.[3], Snoeks J.[3] and Verheyen E.[4]

[1]JEMU, Royal Museum for Central Africa, Tervuren, Belgium [2]JEMU, Royal Belgian Institute of Natural Sciences, Brussels, Belgium [3]Zoology / Vertebrates, Royal Museum for Central Africa, Tervuren, Belgium [4]Vertebrates, Royal Belgian Institute of Natural Sciences, Brussels, Belgium
Presenting author's email: floris.breman@africamuseum.be

The Congo basin in Central Africa is one of the largest hydrographical basins of the world and is considered to be a biodiversity hotspot. For instance, the region is known for its bewildering diversity of freshwater fish with more than 1050 described species, of which about 75% are endemic. During several expeditions more than a thousand specimens were sampled and afterwards identified using morphological tools. Currently, we evaluate the use of DNA barcoding as a potential identification tool for these taxa. DNA barcoding uses a short DNA sequence of the mitochondrial COI gene and can provide a valuable identification tool in cases where a good taxonomic sampling is available. A DNA barcode library with more than 900 sequences, comprising approximately 80 freshwater fish genera, has been compiled. The evaluation of this dataset is ongoing but preliminary results show that DNA barcodes can provide a quick assistance in the identification of the freshwater fish species of the Congo basin

S8P4.
The genus *Antedon* (Crinoidea, Echinodermata): an example of evolution through vicariance

Hemery L.[(1)], Eléaume M.[(1)], Chevaldonné P.[(2)], Dettaï A.[(3)] and Améziane N.[(1)]

[(1)]DMPA-UMR5178, Muséum National d'Histoire Naturelle, Paris, France [(2)]DIMAR-UMR6540, Centre d'Océanologie de Marseille, Marseille, France [(3)]DSE-UMR7138, Muséum National d'Histoire Naturelle, Paris, France
Presenting author's email: adettai@mnhn.fr

The crinoid genus *Antedon* is, at best, paraphyletic and assigned to the polyphyletic family Antedonidae. This genus includes ~16 species separated into two distinct groups. One group is distributed in the north-eastern Atlantic and the Mediterranean Sea, the other in the western Pacific. Species from the western Pacific group are more closely related to other non-*Antedon* species (e.g. *Dorometra*) from this area than to *Antedon* species from the Atlantic-Mediterranean zone. Species from the Atlantic-Mediterranean area show a geographical structure probably linked to the events that followed the Messinian salinity crisis, ~5 Myr ago. To test this hypothesis, a phylogenetic study of the *Antedon* species from the Atlantic-Mediterranean group was conducted using a mitochondrial gene (cytb). The analysis included ~20 specimens representing the seven nominal species described from the region. Phylogenetic hypotheses were inferred using the maximum parcimony and the maximum likelihood criteria with two outgroups sampled within the antedonid sub-family Heliometrinae. Results show that the genus *Antedon* in the Atlantic-Mediterranean zone is composed of five species. *Antedon* bifida is distributed in the Atlantic Ocean and the westernmost part of the Mediterranean (Alboran Sea) while *Antedon mediterranea* is distributed in the rest of the Mediterranean. These results suggest that the divergence between *Antedon bifida* and *Antedon mediterranea* may have occurred by vicariance after the Gibraltar Strait dried up at the onset of the Messinian salinity crisis and has since been maintained by gyres in the Alboran Sea, acting as an ecological barrier to larval dispersal.

S8P5.
Diversity of Freshwater Fishes in Europe, North Africa and the Middle East

Freyhof J.

Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany
Presenting author's email: freyhof@igb-berlin.de

The global diversity of fishes includes at least 14,000 species in freshwaters. Freshwater fishes remain the least well known among the vertebrates and 100-200 new species are scientifically described and named every year. Many freshwater fish provide important ecosystem services especially for poor communities. In Europe and the wider Mediterranean, 954 species are actually recognized. While 585 species are actually known from Europe, additional 250 species occur in Anatolia, 39 in North Africa and 67 in the Middle East. Within catchment species diversity is highest in the northern Black and Baltic Sea basins, where up to 65 species might co-occur. Endemic species diversity is highest in Iran and the Eastern Mediterranean, especially in Dalmatia, Greece and Anatolia where many species with very small ranges exist. Freshwater fishes of this area are not well documented within the barcoding record as many species have rarely been collected and species are difficult to identify by non-specialists.

S8P6.
DNA barcoding discriminates between potentially cryptic species related to *Taenia taeniaeformis* harbored by italian wild and domestic cats

Galimberti A.[1], Romano D.[1], Genchi M.[2], Paoloni D.[3], Vercillo F.[3], Bizzarri L.[3], Barbuto M.[1], Ferri E.[1], Bellati A.[2], Ragni B.[3] and Casiraghi M.[1]

[1]Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milan, Italy [2]Dipartimento di Biologia Animale, Univesrità di Pavia, Pavia, Italy [3]Dipartimento di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy
Presenting author's email: a.galimberti3@campus.unimib.it

Species identification through DNA barcoding is really welcomed for parasites, in which morphometrics are often difficult or impossible due to the poor quality of samples, and for which morphological experts are rare. The present work provides for the application of an integrated approach, based on both DNA barcoding and morphological analysis, on Cestoda belonging to the genus *Taenia*, for which biodiversity is still underestimated at both molecular and morphological level. In particular, we characterized the cestodes spectrum of Italian wildcat (*Felis silvestris silvestris*), free-ranging domestic cat (*F. s. catus*) and their hybrids. The sixty-two samples of adult cestodes were collected by post-mortem examinations of the hosts, and they were all morphologically identified as *Taenia taeniaeformis*. Amplification and sequencing of a 450 bp fragment of mitochondrial gene coxI was performed from few proglottids of each parasite. Sequences obtained were aligned with those of individuals belonging to the genus *Taenia* detected in GenBank and, in order to evaluate the performance of a DNA barcoding approach on taeniids, the strength of correlation between species identification based on morphological characters and molecular divergence of coxI sequences was measured. At this purpose, an optimum threshold (OT) value of genetic divergence was calculated. The DNA barcoding analyses performed showed a high strength of correlation in the identification of taeniids combining molecular and classical morphological-based approaches. However, we detect three distinct molecular groups within the whole panel of specimens morphologically identified as *T. taeniaeformis*. Two of these molecular lineages, encompassing all the GenBank sequences of *T. taeniaeformis* and only one sample of the present work, were already identified by other authors and considered a potentially cryptic species. The third molecular group is constituted by Italian samples analyzed in the present study, and it could represent third cryptic species.

S8P7.
Integrated approach for the identification of Oniscidea isopods

Barbuto M.[1], Montesanto G.[2], Alfano N.[1], Galimberti A.[1], Bellati A.[3], Caruso D.[2], Lombardo B.[2] and Casiraghi M.[1]

[1]Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milan, Italy [2]Dipartimento di Biologia Animale "Marcello La Greca", Università di Catania, Catania, Italy [3]Dipartimento di Biologia Animale, Università degli Studi di Pavia, Pavia, Italy
Presenting author's email: a.galimberti3@campus.unimib.it

In the last years molecular techniques, like DNA barcoding, become more and more popular. It is remarkable that its outstanding value is mainly reached when DNA barcoding is integrated with traditional morphological approaches. In the present study we focused our attention on the terrestrial isopods (suborder Oniscidea) collected in Italian chestnut grove (Sicily, Etna Mount). All samples were previously identified to species level using a morphological approach and subsequently a fragment of 600 bp of mitochondrial gene for the coxI was amplified and directly sequenced following standard protocols. After checked for the presence of pseudogenes, the obtained barcodes were unambiguously aligned using GenBank sequences as references. To perform an integrated approach to taxonomy, DNA barcoding sequences were combined with morphological data and an optimum threshold value of genetic divergence was calculated. This parameter represents the value of genetical divergence that maximizes the coherence between morphological and molecular identification and minimizes, at the same time, the cumulative error in taxonomic assignment. Forty-four sequences of 12 morphospecies, belonging to six genera of Oniscidea, were produced and in all cases they represent first entries in GenBank. The optimum threshold value showed a relatively high strength of coherence between morphological and molecular identification of the species analyzed, even if some inconsistencies were detected. In the case of Italian samples, in particular for the species *Porcellio imbutus* we found a peculiar situation with a rather high genetic divergence among the specimens. This feature can be explained with the existence of a cryptic species complex, as a matter of fact for *Porcellio imbutus* in the past it has been already identified as constituted by almost three cryptic species (*P. siculoccidentalis*, *P. hyblaeus* and *P. baidensis*).

S8P8.
Barcode database for veterinary important arthropod vectors

Garros C., Vial L. and Rasplus J.

UMR CMAEE, Cirad, Montpellier, France
Presenting author's email: claire.garros@cirad.fr

Ticks (Acari: Ixodida) and *Culicoides* biting midges (Diptera: Ceratopogonidae) are two major groups of arthropods involved worldwide in the transmission of pathogens to domestic and wild animals, respectively cowdriosis, virus of African swine fever, and bluetongue virus. However, our capacity to clearly understand the epidemiology of such diseases greatly suffers from taxonomic and systematic difficulties. Difficulties come from (1) the numerous synonymies or remaining hypotheses of conspecificities, (2) the taxonomic impediment with limited international experts, (3) the age of voucher collections or the limited number of species in collection, (4) the absence of accurate phylogeny leading to a complex systematic imbroglio. The opportunities given by on-going barcode applications and massive sequencing facilities are valuable to regain interest into taxonomy and systematic of these groups. Today, there is no database with accurate molecular data for ticks and *Culicoides*. For the first time, the development of a barcode reference database for arthropod vectors involved in animal health is presented in strong interactions with pest barcode database (R-Syst). For now, ticks and biting midges are covered. This database will help clarifying the systematic schemes and taxonomy of these groups, as well as improving our understanding of host-pathogen co-evolution.

S8P9.
Chimerical biology: a plea for good taxonomy as exemplified by the common nightcrawler (*Lumbricus terrestris* L.)

James S.W.[1], Decaëns T.[2], Porco D.[3], Richard B.[2], Erséus C.[4], Meusnier I.[3], Rougerie R.[3] and Hebert P.D.N.[3]

[1]Biodiversity Institute, Kansas University, Lawrence, USA [2]Lab ECODIV, Rouen University, Mont Saint Aignan, France [3]CCDB, Biodiversity Institute of Ontario, Guelph, Canada [4]Department of Zoology, Gothenburg University, Göteborg, Sweden
Presenting author's email: sjames@ku.edu

In spite of a critical contribution to global biodiversity, soil organisms have weakly engaged the attention of taxonomists compared with epigeic taxa and, even in groups intensively studied such as lumbricid earthworms, the use of DNA barcodes has recently revealed an unsuspected number of cryptic species often impossible to separate on a morphological basis. In this study, we used morphology and COI sequences to assess phenotypic and genetic variability among: (1) 181 specimens of the common night crawler (*Lumbricus terrestris* Linnaeus, 1758) freshly collected in European and North American populations; (2) three specimens of the neotype series of *L. terrestris* collected in Upsala (Sweden) in 1973; (3) one paratype of *Lumbricus herculeus* (Savigny, 1826) (a species recently synonymized with *L. terrestris*) collected in Versailles (France) in 1826; (4) specimens of five related *Lumbricus* species. We obtained full-length barcodes from most of the freshly collected specimens, and a series of smaller fragments (150bp) assembled in a sequence of 480bp for the co-type of *L. herculeus*. In addition a mini-barcode of 150bp was produced from one of the three specimens of the *L. terrestris* neotype series. The fresh specimen sequences highlight the existence of two different groups of *L. terrestris*, which are slightly different by morphology (body size and weight), but have strongly divergent COI sequences (mean distance of 17.6%). A Neighbor-joining tree of all sequences indicates that *L. terrestris* s.l. is paraphyletic with *L. festivus* the sister group to the *L. terrestris* clade containing the *L. herculeus* co-type specimen sequence. The sequence from the specimen of the *L. terretris* neotype series was in the other *L. terrestris* clade. These results indicate that *L. terrestris* as traditionally identified is composed of two cryptic species that have not been discriminated in the literature. One of these species is the now valid *L. herculeus*. Based on a detailed meta-analysis of the scientific literature dedicated to "*L. terrestris*", we demonstrate that this taxonomic confusion may dramatically alter the reliability of published data. The attribution to a single scientific name of characteristics measured on two cryptic species may in fact lead to the description of a kind of chimerical organism, which finally does not correspond to the true properties of any of the real species.

S8P10.
A DNA barcode library for marine fish of Portugal: first hundred species

Landi M.[(1)], *Martins R.*[(2)], *Costa M.E.*[(3)], *Carneiro M.*[(2)], *Alves M.J.*[(4)], *Costa M.H.*[(5)], *Carvalho G.R.*[(6)] *and Costa F.O.*[(1)]

[(1)]Departamento de Biologia, Universidade do Minho, Braga, Portugal, [(2)]IPIMAR, Lisboa, Portugal, [(3)]Faculdade de Ciências do Mar, Universidade do Algarve, Faro, Portugal, [(4)]Centro de Biologia Ambiental, Museu Bocage, Lisboa, Portugal, [(5)]IMAR - Instituto do Mar, Universidade Nova de Lisboa, Monte de Caparica, Portugal, [(6)]School of Biological Sciences, Bangor University, Bangor, UK
Presenting author's email: mlandi@bio.uminho.pt

Species level identifications in fisheries catch is of utmost importance for fish stock assessment and fisheries management on a regional and sometimes global scale. However, as much as 40% of the fisheries catch worldwide fails to be identified to species (1), and usually there is no system implemented to verify the rigorousness of the identifications in the other 60%. In order to contribute to cover this species identification gap, and to bring more accuracy to identifications, we initiated the creation of a reference library of cytochrome oxidase 1 (COI) barcodes for fishes of Portugal. Data collected for 657 specimens yielded COI barcodes for 109 species, represented by 1 to 18 individuals, spread across 77 genera, 52 families, and 21 orders. 96.33% of the 109 species in our dataset were distinguishable from all other species. *Macroramphosus scolopax* and *Macroramphosus gracilis* were not distinguishable, which is agreement with Robalo et al., 2009 (2) that based on morphological and molecular data claim it is in fact a single species. Deep intra-species genetic divergence was observed for *Scorpaena notata*, for which COI barcodes clustered into two different clades. Given that taxonomy keys enable to identify just one species common to both haplotypes, this observation may indicate the presence of cryptic species. Using the BOLD identification engine to verify our species assignments we confirmed the identifications in 92.66% of the species. Typically the mis-assignments highlighted groups offering particular identification difficulties or with current incomplete taxonomy. Based on available information in the literature, we discuss the results in face of three alternative hypothesis: 1) Morphological misidentifications, 2) presence of cryptic species and 3) insufficient discrimination of the CO1 barcode.

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S8P11.
More than 95% of decapod species still lack COI barcodes

Matzen Silva J.[1,2], Creer S.[1], Santos A.[3], Costa A.C.[4], Cunha M.R.[2], Costa F.O.[5] and Carvalho G.R.[1]

[1]Biology, Bangor University, Bangor, UK [2]Biologia, Universidade de Aveiro, Aveiro, Portugal, [3]Instituto Nacional de Recursos Biológicos, Lisboa, Portugal, [4]Biologia, Universidade dos Açores, Ponta Delgada [5]Biologia, Universidade do Minho, Braga, Portugal
Presenting author's email: bss405@bangor.ac.uk

In recent decades, the loss of biodiversity has been recognized as a major global environmental problem and much effort has been targeted at biodiversity conservation. Barcoding datasets are rapidly accumulating as part of the worldwide campaign for inventories of global biodiversity. Decapods (crabs, shrimps, crayfishes, lobsters, etc) are the most recognizable of all crustaceans and comprise one of the dominant groups of benthic invertebrates of the European continental shelf and slope. Establishing a robust DNA barcoding framework is particularly relevant because the Decapoda contains over 17,000 morphologically described freshwater and marine species, some of which support fisheries and other industries worth billions of dollars each year. From over 17,000 species, only 4.8% are represented by COI sequences in Genbank and 0.5% have a well annotated reference library in the Barcode of Life Database. There is currently no global campaign to barcode all Crustacea, and it therefore remains a challenge to compile regional databases that identify and analyze decapod species diversity. We contributed 101 decapod species from the North East Atlantic, the Gulf of Cadiz and the Mediterranean Sea, of which 81 species represent novel COI records. Here, we examine the combined COI barcode projects from the Barcode of Life Database and our novel Decapoda framework, generating the most comprehensive COI data set so far examined. The collective dataset provides barcoding coverage from 1910 sequences of 604 species, 224 genera and 68 families, allowing a broad overview of intraspecific variation (0% to 4.6% K2P distances) and DNA composition (31% to 50% GC content). Even with our partial data, observations reveal considerable molecular diversity within families (6.7% to 48.3% K2P distances). Biodiversity management will depend on understanding species boundaries, distributions, and population structure at large geographical scales. Its success relies on a good understanding of the genetic structure of populations since genetic diversity is the basis for evolutionary potential to adapt to changing environments. We highlight the urgency of developing regional Decapoda COI barcode libraries in order to better detect environmental, biological and evolutionary factors affecting the genetic diversity of decapods in Europe.

S8P12.

Molecular biodiversity inventory of the ichthyofauna in the Czech Republic

Urbánková S.[1], Halaèka K.[1], Vetešník L.[1], Papoušek I.[1], Bartoòová E.[1], Šanda R.[2], Koníèková M.[1] and Mendel J.[1]

[1]Department of Fish Ecology, Institute of Vertebrate Biology, v.v.i., Kvìtná 8, 603 65 Brno, Czech Republic
[2]Department of Zoology, National Museum, Václavské námìstí 68, 115 79 Praha 1, Czech Republic
Presenting author's email: jmendel@seznam.cz

This project is based on international collaboration and has contributed significantly to the recognition and description of species diversity in all Czech fishes by using a comprehensive approach (literary data search, morphology, DNA barcoding, nDNA analysis). Indigenous and non-indigenous species of fish and lampreys living in the natural waters of the Czech Republic are the subject of recent inventory-taking and subsequent cataloguing. The acquired results are used for intercontinental comparison using DNA barcode within the BoLD platform. The study has contributed to the updating of information for Natura 2000 monitoring and has provided information and recommendations to the Agency for Nature Conservation and Landscape Protection of the Czech Republic. New designs for species collection of type specimens and the development of detailed vouchers are an important contribution to the National Museum (Prague). This study was carried out within the framework of research project no. M200930901.

S8P13.
Testing DNA Barcoding to discriminate Echinodermata from Portugal

Micael J.[(1)], *Costa A.C.*[(1)] *and Alves M.J.*[(2)]

[(1)]CIBIO-Azores, Department of Biology, University of the Azores, Ponta Delgada [(2)]CIBIO-Azores, Department of Biology, University of the Azores, Ponta Delgada
Presenting author's email: jfmicael@yahoo.com

The phylum Echinodermata contains some of the most charismatic benthic marine invertebrates and has become a symbol of marine life. However, growing global pressures on the collection of echinoderms for various commercial enterprises (e.g. fishing, home aquaria, souvenirs and biomedical products) have put these enigmatic invertebrates under threat. To compound the issue, the knowledge about the majority of echinoderm species general biology, ecology and life history is very scarce. Echinodermata larvae can rarely be identified morphologically to family, let alone genus or species. Adults also can be hard to discriminate, being morphologically plastic within species and sometimes of variable colour. Therefore DNA barcoding can be particularly useful to discriminate between these species. DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's mitochondrial DNA to identify it as belonging to a particular species. A growing number of studies have shown that COI sequence variability is very low (generally less than 1-2%) and that the COI sequences of even closely related species differ by several percent, making it possible to identify species with high confidence in a cost-effective manner. DNA barcode sequences (¡Ö 900 bp segment) were collected from 7 species of Echinodermata that occur in Portugal, with the purpose to test the use of DNA barcoding in this group of animals. Three classes were represented: Asteroidea, Echinodea and Holothuroidea. All species were represented by multi-geographic samples.

S8P14.

Cryptic diversity in two arthropod species in Iceland; colonization patterns and subglacial refugia

Palsson S.

Biology, University of Iceland, Reykjavik, Iceland
Presenting author's email: snaebj@hi.is

The Icelandic biota is characterized by low species diversity, which can be explained by the short period since the island was covered by glacier (about 12000 years ago) and its geographic isolation. Analyses of mtDNA variation in species at high latitudes have often shown little variation, reflecting bottlenecks and a colonization from southern refugia. A study of sequence variation of the mtDNA gene COI in one groundwater amphipod (*Crangonyx islandicus*), endemic to Iceland, and of one circumpolar caddisfly (*Apatania zonella*) in Iceland show large amount of variation in Iceland. An estimate of the time to common ancestor within species in Iceland is 1-5 million years. The patterns of genetic variation reflect different histories of the two groups. The divergence within the amphipod occurred within Iceland during Ice-age in several distinct subglacial refugia, defining five monophyletic groups. The caddisfly diverged elsewhere two distinct lineages colonized Iceland, one from North-America and the other from mainland Europe.

S8P15.
Continental archipelagoes and variety: Contribution of the genetic and morphometric data on the systematics of *Urticicola* (Gastropoda: Hygromiidae) of summits of Mercantour.

Schoelinck C., Gargominy O., Baylac M., Brisset J. and Samadi S.

UMR7138, MNHN, Paris, France
Presenting author's email: schoelinck@mnhn.fr

Extending over 685 km^2 in the occidental Alps, the National park of Mercantour is considered as one of the hotspot of the biodiversity for the Mediterranean zone. At the end of the 90s, a forgotten species was rediscovered within territories. *Urticicola mounierensis* (Caziot, 1909) is a snail endemic to the Mercantour. Two subspecies of different sizes are recognized: *Urticicola mounierensis mounierensis*, which is large and whose type locality is the top of the 'Mont des Moulines' in the limestone massif of Mont Mounier (2000m high) and *Urticicola mounierensis maynardii*, which is small and whose type localities are the top of Mont Mounier (2800m high) and the Démant plateau (2400m high). In this study, we combined molecular (genes: COI, 16S, ITS, 28S) and morphometric (Fourier's ellipsis or EFA) analyses and confirmed the monophyly of *U. mounierensis*, while also identifying 5 clades within this species. Correlations between population structure and altitude were revealed. Morphological and genetic divergences between high-altitude populations (between 2500 and 2900m) and those living between 2100 and 2300m were also observed. Habitat fragmentation associated with the relief may be responsible for the differentiation observed. The morphometric analysis showed that high altitude individuals were smaller and presented higher spiralling. There is often considerable phenotypic plasticity in molluscs, which concerns mainly shell size. However, differences in shell conformations were also revealed in this study. Also, non-synonymous mutations in the protein encoded by the COI gene were also detected in one of the two high-altitude (2900m) individuals. This environment may create an important selective pressure, which may have triggered genetic and morphological adaptations.

S8P16.
Barcoding Mediterranean fish species

Shirak A.[1], Seroussi E.[1], Ron M.[1], Hulata G.[1] and Golani D.[2]

[1]Molecular genetics, Institute of Animal Science, Bet Dagan, Israel [2]Department of Evolution, Systematics and Ecology, The Hebrew University of Jerusalem, Jerusalem, Israel
Presenting author's email: shiraka@agri.gov.il

The conserved sequence of the 5' region of the mitochondrial gene cytochrome oxidase subunit I (COI) was proposed as a platform for the universal DNA barcoding of life. More than 65,000 (9.7% existing species) are already formally characterized with DNA barcodes and registered in the Barcode of Life Data System (BOLD, www.barcodinglife.org). The Fish Barcode of Life initiative (FISH-BOL, www.fishbol.org) focuses on fish species accounting for nearly half of the vertebrate species. Currently, barcodes of more than 7,400 out of 30,000 known fish species (24.7%) are included in the database. We optimized the primer cocktail for DNA barcoding of 40 marine and 25 freshwater fish species. In the present study we selected 350 individuals from fishermen catch near the port of Jaffa. These samples belong to 38 Mediterranean fish species classified by morphological taxonomy. Thirty two species were represented by at least 2 individuals. All samples were photographed and sequenced for the 663 bp fragment of COI. Through sequence alignment and analysis we detected 90 unique sequence variants. In 35 out of 38 species intra-specific sequence polymorphism did not exceed 1%, which is the lower limit of inter-specific variability. In each of the remaining three other species we detected two subgroups of sequences that may indicate the existence of two subspecies or two closely related species.

S8P17.
DNA barcoding of soldierless termites from South America: the *Anoplotermes* group (Termitidae)

Sonet G.[1], Nagy Z.T.[1], Vermeulen J.[2], Dery A.[3] and Bourguignon T.[3]

[1]Joint Experimental Molecular Unit, Royal Belgian Institute of Natural Sciences, Brussels, Belgium [2]Gezondheidszorg en Technologie, Katholieke Hogeschool, Leuven, Belgium [3]Sciences Biologiques, Université Libre de Bruxelles, Brussels, Belgium
Presenting author's email: gontran.sonet@naturalsciences.be

Termite taxonomy is traditionally based on morphological characters of soldiers and winged imagos. In the case of soldierless termites, species identification implies a close examination of the worker's digestive tube, a time-demanding procedure which has discouraged many taxonomists. In order to give insight into this termite group - estimated to represent one third of the termite species found in neotropical rainforests - ca. 400 *Anoplotermes* samples were collected in French Guiana and classified in 34 morpho-species based on the morphology of workers. By applying the DNA barcoding approach on representatives of the different morpho-species, we wanted to know whether morphological characters used here for species recognition are supported by genetic data. Although all samples were freshly collected, amplification and sequencing success rates of the standard DNA barcode fragment (first part of the COI mitochondrial gene) were moderate. The removal of the abdomen prior to DNA extraction and the use of bovine serum albumin during PCR considerably increased the success rate at every further step suggesting that contaminants from the soil or the digestive tract could interfere with amplifications. Preliminary genetic comparisons of the obtained data set of 60 sequences representing about 20 morpho-species supported the delimitation of most morpho-species. According to this result, the used morphological characters appear as valuable in the taxonomy of soldierless termites and will facilitate the study of the underexplored *Anoplotermes* group.

S8P18.
A molecular approach to complement traditional morphospecies delimitation: the case of the Triphoridae

Vanmaele S.[(1)]*, Bouchet P.*[(1)]*, Puillandre N.*[(1)] *and Strong E.*[(1)]

[(1)]USM 603/UMR 7138 " Systématique, adaptation, évolution", Muséum National d, Paris, France [(2)]Department of Marine Biology, University of Ghent, Krijgslaan 181/ s8, 9000 Gent, Belgium [(3)]Muséum National d'Histoire Naturelle, 57 rue Cuvier75231 Paris cedex 05, France
Presenting author's email: vanmaele@mnhn.fr

The microgastropod family Triphoridae is one of the five most diverse molluscan families and are generally identified by the characteristics of their shell (the majority is sinistral). Identification to species level can be tricky because of their extremely high diversity, their small size and their strong superficial morphological Around 23,000 specimens of *Triphorids* were collected during the Santo 2006 expedition organized by the Muséum National d' Histoire Naturelle (MNHN) of Paris. A division in morphospecies was made for both empty shells and living animals using the characteristics usually taken into account for morphological analysis (protoconch, teleoconch, shell sculpture and aperture). More than 250 morphospecies were found in the dead samples of which 70% are supposed to be new to science. The "barcode" collection (living animals) consisted of 549 live specimens that were divided in 72 morphospecies. Pictures were taken of the protoconch, aperture, teleoconch and operculum. Foot tissue was extracted from cracked specimens only for DNA analyses. Shells and operculum were kept intact as much as possible for identification and morphological comparison afterwards. Two gene fragments were amplified: the mitochondrial cytochrome oxidase I fragment and the nuclear 28S gene. Preliminary analyses of ~100 specimens sequenced for both genes and using Maximum Likelihood and Bayesian approaches confirms the hypotheses proposed by the morphological approach, as each defined morphospecies correspond to a well-supported clade including very short branches but separated from the other clades by long branches. The DNA-barcode approach was thus efficient to confirm the species delimitations based on the shell morphology, but also helped to define the phylogenetic relationships between the species and furthermore facilitate the taxonomic expertise, so far limited to only a few taxonomists.

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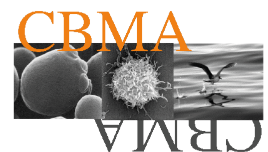

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