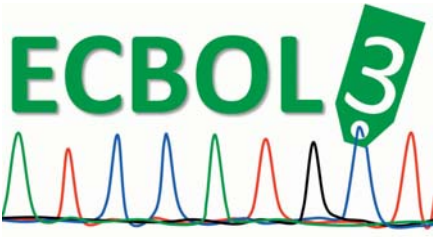


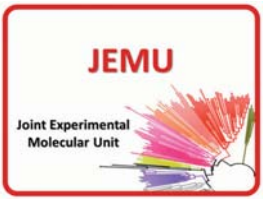
THIRD EUROPEAN CONFERENCE FOR THE BARCODE OF LIFE

"Barcoding of organisms of policy concern"

BRUSSELS, BELGIUM
17-20 SEPTEMBER 2012

PROGRAM AND ABSTRACTS

edited by the Joint Experimental Molecular Unit



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Abstracts volume ECBOL 3 – Brussels 2012

Third European Conference for the Barcode of Life, Brussels, Belgium, 17-20 September 2012

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TABLE OF CONTENTS

WELCOME ADDRESS

The organizing committee wishes to welcome all participants to the third European Conference for the Barcode of Life, in Brussels, the heart of Europe. The place of venue is the Academy of Sciences, close to the historical centre of the city and with the residence of the king of the Belgians as next door neighbour. The academy has been a site of scientific exchange with a history older than this country itself as it was established in 1772 when the southern Netherlands were part of the Austrian Empire. So we are happy and privileged to host the conference at this venue.
At the time of printing of this abstract volume we had more than 130 registered participants from 24 countries, including several outside Europe. About a decade after the term "DNA-barcoding" was coined, the whole endeavour is still alive and kicking, and enjoys an ever increasing interest. We are sure that this conference will amply illustrate this interest and we hope that this conference will further underpin the societal relevancy of DNA barcoding. In order to do so, we choose "Barcoding of organisms of policy concern" as theme of this conference. We indeed feel that it is time to focus more on day-to-day applications of DNA barcodes and how they can be instrumental in different fields of public concern and daily life. Aspects such as health, agriculture, forensics, or conservation, to name just a few, can benefit greatly from the powerful identification tools that DNA barcoding can provide. The conference program promises to demonstrate some exciting highlights in this respect. We, therefore, hope that this will be the appropriate forum for you, as a participant, to share your views and ideas with fellow colleagues from Europe and beyond, on how DNA-barcoding can play an active role in policies development and how you see the future of this research field in the forthcoming years. In this light, we also hope that during this conference we will hear more about new methodological developments and perspectives with respect to DNA barcoding and its future.

So please, do enjoy the city of Brussels and its historical setting, and take this opportunity to interact with friends and colleagues. We hope this conference will be a great success and look forward to meeting you all during these days.

On behalf of the organizing committee,

Thierry Backeljau & Marc De Meyer
Conference co-ordinators

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- providing grants for organizing international congresses in Belgium;
- mobility allowances for FWO-Postdoctoral Fellows.

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Our research focuses on **long-term trends relevant to the environment,** including changes in population, transportation, energy, land use, and industrial materials, as well as past, present, and future states of plant and animal populations on land and in the oceans.

Our approach is anchored in statistical analysis of time-series datasets using models of growth and diffusion, particularly Lotka-Volterra dynamical systems. We apply these methods to changes in the human environment over the last 10,000 years, and to look ahead to the next 1000.

ORGANISATION OF CONFERENCE

Conference co-ordinators (in alphabetical order)

Thierry BACKELJAU – Royal Belgian Institute of Natural Sciences and University of Antwerp
Marc DE MEYER – Royal Museum for Central Africa

Organizing committee (this committee comprises all members of the Belgian Network for DNA Barcoding – in alphabetical order)

Tom ARTOIS (University of Hasselt)
Floris C. BREMAN (Royal Museum for Central Africa)
Peter BREYNE (Research Institute for Nature and Forest)
Bart COTTYN (Institute for Agricultural and Fisheries Research)
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Veerle VERSTEIRT (Institute for Tropical Medicine)
Wim VYVERMAN (University of Ghent)
Filip VOLCKAERT (University of Leuven)
Annick WILMOTTE (University of Liège)

Conference secretariat

Myriam VANDENBOSCH (Royal Museum for Central Africa)

Technical assistance

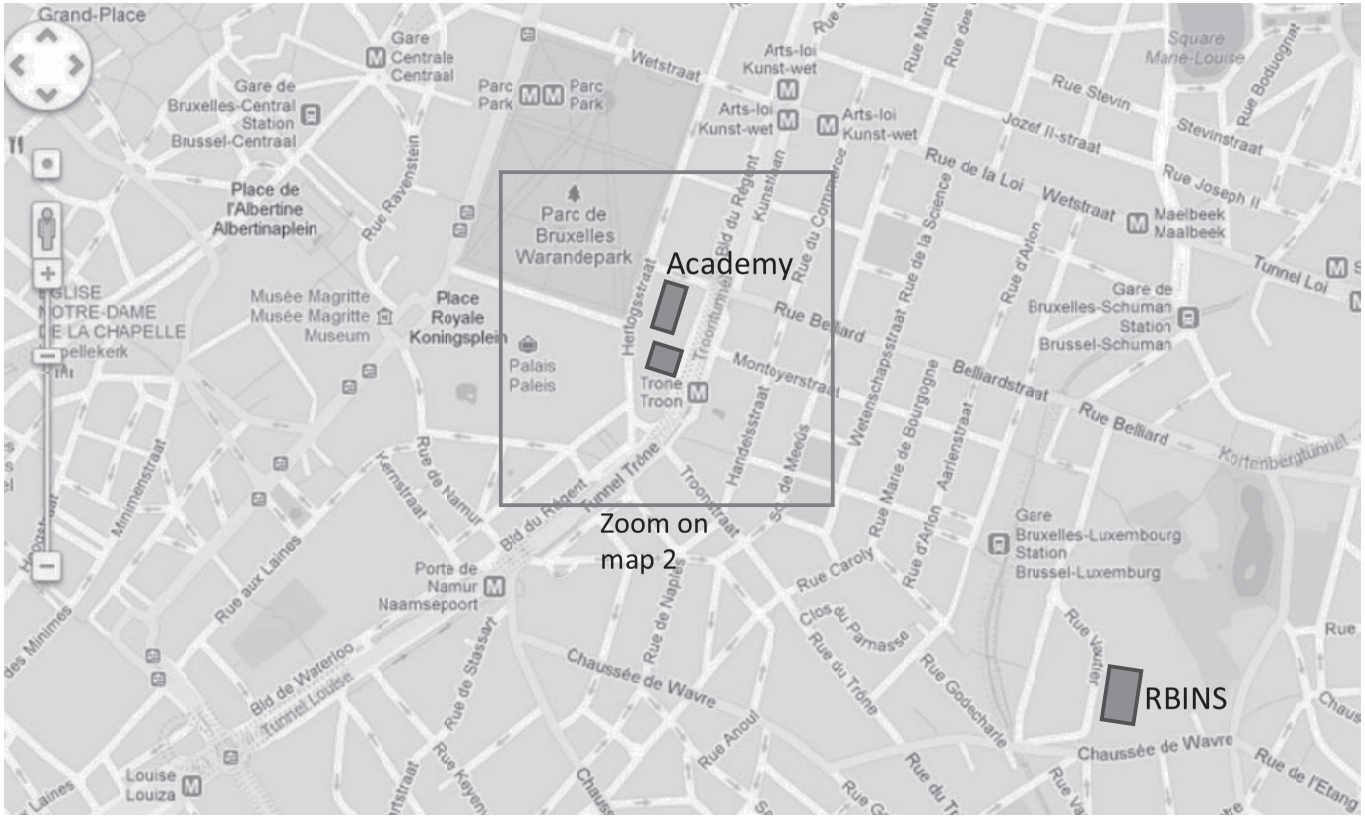
The RBINS and RMCA museum staff
Michel SWITTEN (University of Antwerp)
Kris BROSSÉ (KVAB)

CONFERENCE VENUE

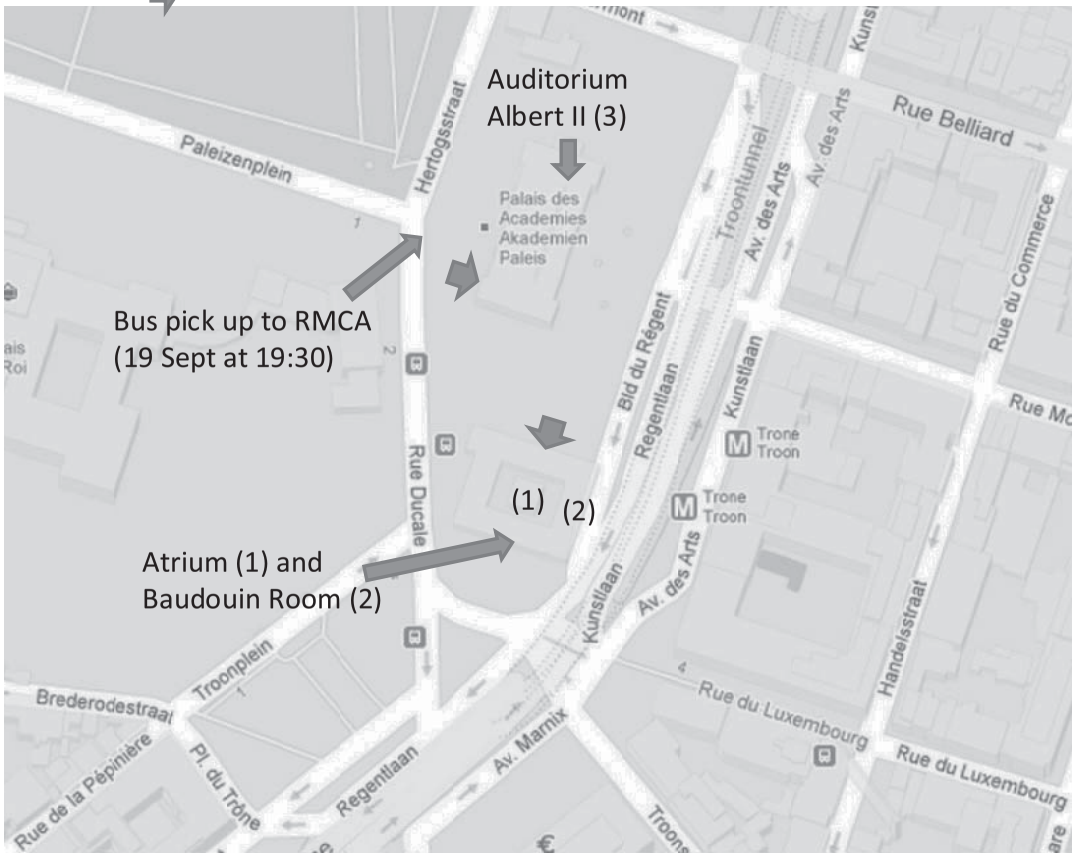
The ECBOL3 conference will start with an icebreaker on Monday, 17 September 2012 at the RBINS (18:00-20:00) which is within walking distance of the conference venue (see Map 1). The conference itself will take place at the Royal Flemish Academy of Belgium for Sciences and the Arts which is located in the historical center of Brussels, next to the Royal Palace and within walking distance (10 min) of the central railway station (see Map 1). Scientific presentations are planned in sessions on the following two and a half days (see Map 2 and the conference program). Posters will be displayed throughout the conference in the 'Atrium' where also the coffee breaks will take place and lunch will be served (see Map 2).

A conference dinner will take place on 19 September at the Royal Museum for Central Africa (RMCA). There will be a bus shuttle that will pick you up at 19:30 in front of the Academy Palace (conference venue). The shuttle service will return to the Academy Palace at 23:00 hours.

Map 1: **Royal Belgian Institute of Natural Sciences (RBINS)**, Rue Vautierstraat 29, 1000 Brussels and **Royal Flemish Academy of Belgium for Science and the Arts**, Hertogsstraat/Rue Ducale 1, 1000 Brussels



Map 2: **Royal Flemish Academy of Belgium for Science and the Arts**, Hertogsstraat/Rue Ducale 1, 1000 Brussels. ' ' indicates the main entrance of the building.



Program at a glance

	Monday 17-Sept		
12:00-16:40	registration and poster set-up at the Academy (1)		
18:00-20:00	**icebreaker at RBINS***		

	Tuesday 18-Sept	Wednesday 19-Sept	Thursday 20-Sept
08:00-08:30	Registration		
08:30-09:00	**opening session** (2) T. Backeljau & M. De Meyer		
Session:	**invasive species and open** (2)	**conservation and open** (3)	**human & animal health and open** (3)
09:00-09:40	M. Virgilio (invited talk)	N. De Vere (invited talk)	S. Morand (invited talk)
09:40-10:00	J. Marescaux ⇧	A. Ardura ⇧	E. Bellemain
10:00-10:20	I. Schön	C. D. Nwani	P. De Vos
10:20-10:40	X. Zhou	E. Alfonsi ⇧	A. Nieman
10:40-11:10	*coffee break* (1)	*coffee break* (1)	*coffee break* (1)
11:10-11:30	C. Lopez-Vaamonde	K. Yessoufou	T. Huyse
11:30-11:50	K. K. Beentjes	K. Yessoufou	D. Apolônio Silva de Oliveira ⇧
11:50-12:10	M. Geiger	M. van der Bank	J.-F. Flot
12:10-12:30	E. Verheyen	K.-D. B. Dijkstra	D. E. Schindel
12:30-12:50	S. Pietsch	A. Sueur ⇧	Discussion (12:30-13:10)
12:50-14:00	*lunch break* (1)	*lunch break* (1)	student awards (13:10-13:20) closing remarks (13:20-13:30)
Session:	**legal aspects of DNA barcoding and open** (2)	**agriculture & forestry and open** (3)	*lunch break* (1) (13:30-14:30)
14:00-14:40	R. Zehner (invited talk)	P. Crous (invited talk)	
14:40-15:00	S. Hassold ⇧	L. Lombard	
15:00-15:20	L. Schwiebbe ⇧	P. Bonants	
15:20-15:40	S. Desmyter	M. Maes	
15:40-16:10	*coffee break* (1)	*coffee break* (1)	
16:10-16:30	M. Y. Stoeckle	J.-C. Pintaud	
16:30-16:50	L. A. Galindo ⇧	O. J. Hardy	
16:50-17:10	C. O. Eche ⇧	C. Schoelinck ⇧	
17:10-17:30	J. Astrin	J. A. Nicholls	
17:30-17:50	R. Hanner	A. Zaib-un-nisa⇧	
18:00:20:00	**poster reception** (1)	19:30: bus pick up Academy Palace	
20:00:23:00		**conference dinner at RMCA***	

Academy:	Royal Flemish Academy of Belgium for Science and the Arts, Hertogsstraat/Rue Ducale 1, 1000 Brussels
(1)	Atrium, Throne building
(2)	Baudouin Room, Throne building
(3)	Auditorium Albert II, Academy Palace
RBINS*:	Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels
RMCA*:	Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren
🏛	Eligible for the student award

CONFERENCE PROGRAM

Students participating at the awards are indicated with (🏛)

MONDAY, 17 SEPTEMBER 2012

12:00-16:40: Registration (Conference venue; Royal Flemish Academy of Belgium for Science and the Arts (hereafter "Academy"), Atrium, Rue Ducale / Hertogsstraat 1, 1000 Brussels)

18:00-20:00: Icebreaker (Royal Belgian Institute of Natural Sciences, Rue Vautier / Vautierstraat 29, 1000 Brussels), welcome speech by Camille Pisani, director of the RBINS

TUESDAY, 18 SEPTEMBER 2012

Opening session (Academy, Room Baudouin)

8:30-9:00: **Backeljau**, Thierry & **De Meyer**, Marc: Welcome address

Session 1: Invasive species and open session (Academy, Room Baudouin)

Chair: Erik Verheyen

9:00 - 9:40: INVITED LECTURE – **Virgilio, Massimiliano:** Making the best out of incomplete DNA barcode libraries: simple ad hoc thresholds to reduce identification errors (p. 72)

9:40-10:00: **Marescaux, Jonathan** (🏛): Using DNA barcoding to differentiate invasive *Dreissena* species in the river Meuse (p. 41)

10:00-10:20: **Schön, Isa**: *Eucypris virens* (Ostracoda, Crustacea) – an exceptionally diverse species complex that has invaded Western Australia (p. 64)

10:20-10:40: **Zhou, Xin**: Biodiversity analysis through high-throughput sequencing without reference barcode library (p. 78)

10:40-11:10: coffee break (Academy, Atrium)

11:10-11:30: **Lopez-Vaamonde, Carlos**: Lessons from a nearly complete barcode library for European Gracillariidae leaf-miners (p. 38)

11:30-11:50: **Beentjes, Kevin K.**: Using a network of amateur specialists for the collection and identification of specimens for DNA barcoding of Dutch flora and fauna (p. 7)

11:50-12:10: **Geiger, Matthias**: Introducing the FREDIE project with notes on the freshwater fish diversity of the Mediterranean hot spot (p. 24)

12:10-12:30: **Verheyen, Erik**: The reliability of DNA barcoding in Congolese fish through the comparison of two independently generated data sets (p. 71)

12:30-12:50: **Pietsch, Stephanie**: The German Barcode of Life project (GBOL): A national library of biodiversity (p. 54)

12:50-14:00 lunch break (Academy, Atrium)

Session 2: Legal aspects of DNA barcoding and open session (Academy, Room Baudouin)

Chair: Jonas Astrin

14:00-14:40: INVITED LECTURE – **Zehner, Richard**: DNA-barcoding in forensic entomology (p. 77)

14:40-15:00: **Hassold, Sonja (🏛)**: Development and validation of a barcoding strategy to support identification, tracing and trade regulation of Malagasy rosewood (p. 30)

15:00-15:20: **Schwiebbe, Lucas (🏛)**: DNA barcoding of orchids species used in traditional Chinese medicines (TCMS) reveals illegal trade in CITES protected species, adulteration and food fraud (p. 65)

15:20-15:40: **Desmyter, Stijn**: Canine mitochondrial DNA profiling in forensics (p. 16)

15:40-16:10: coffee break (Academy, Atrium)

16:10-16:30: **Stoeckle, Mark Y.**: Frequency matrix approach demonstrates high sequence quality in avian barcodes and highlights cryptic pseudogenes (p. 68)

16:30-16:50: **Galindo, Lee Ann (🏛)**: Microwaving gastropods for DNA barcoding (p. 23)

16:50-17:10: **Eche, Christopher Oche (🏛)**: Identification of marine nematode communities through DNA barcoding: Do mitochondrial COI sequences outperform the ribosomal 18S gene? (p. 20)

17:10-17:30: **Astrin, Jonas**: Barcoding and biobanking: a synergy (p. 6)

17:30-17:50: **Hanner, Robert**: International Barcode of Life (iBOL) project: interim report (p. 29)

18:00-20:00: Poster session (Academy, Atrium)

WEDNESDAY, 19 SEPTEMBER 2012

Session 3: Conservation and open session (Academy, Auditorium Albert II)

Chair: Zoltán T. Nagy

9:00 - 9:40: INVITED LECTURE – **de Vere, Natasha**: DNA barcoding the flora of the UK: the creation and use of a national resource for biodiversity conservation and human health (p. 17)

9:40-10:00: **Ardura, Alba (🏛)**: Beyond biodiversity: fish metagenetics measured with barcoding tools (p. 5)

10:00-10:20: **Nwani, Christopher D.**: DNA barcoding discriminates freshwater fishes from southeastern Nigeria and provides river system-level phylogeographic resolution within some species (p. 51)

10:20-10:40: **Alfonsi, Eric (🏛)**: Contribution of DNA barcoding to the study of marine mammals and of their preys (p. 3)

10:40-11:10: coffee break (Academy, Atrium)

11:10-11:30: **Yessoufou, Kowiyou**: Testing discriminatory power of DNA barcodes within *Encephalartos*, a highly threatened genus endemic to Africa (p. 75)

11:30-11:50: **Yessoufou, Kowiyou**: Beneficial effects of large mammal herbivores on phylogenetic structure of plant communities (p. 74)

11:50-12:10: **van der Bank, Michelle**: Human population density correlates with phylogenetic diversity and plant vulnerability in southern Africa (p. 42)

12:10-12:30: **Dijkstra, Klaas-Douwe B.**: Dragonflies in freshwater conservation in tropical Africa: DNA barcodes support field surveys (p. 19)

12:30-12:50: **Sueur, Annabelle (🏛)** Barcoding marine benthic invertebrates: Preliminary results (p. 69)

12:50-14:00 lunch break (Academy, Atrium)

Session 4: Agriculture & Forestry and open session (Academy, Auditorium Albert II)

Chair: N.N.

14:00-14:40: INVITED LECTURE – **Crous, Pedro W.**: DNA barcoding of fungal pathogens to enhance trade and food production (p. 13)

14:40-15:00: **Lombard, Lorenzo**: DNA barcoding of fungi in the Hypocreales with cylindrical conidia (p. 36)

15:00-15:20: **Bonants, Peter**: The results of QBOL deposited in the Q-bank database to support plant health diagnostics (p. 9)

15:20-15:40: **Maes, Martine**: Bacterial barcoding applied to regulated plant pathogens (p. 40)

15:40-16:10: coffee break (Academy, Atrium)

16:10-16:30: **Pintaud, Jean-Christophe**: Species identification in the genus *Phoenix* L. (Arecaceae): A comparison of performances between barcoding with 3 plastid loci and genotyping with 20 SSR loci (p. 56)

16:30-16:50: **Hardy, Olivier J.**: How effective are DNA barcodes in the identification of African rainforest trees? (p. 53)

16:50-17:10: **Schoelinck, Charlotte** (🏛): Cryptic parasites in one fish: The microscopic species of the *Pseudorhabdosynochus cupatus* complex (Platyhelminthes, Monogenea, Diplectanidae) (p. 62)

17:10-17:30: **Nicholls, James A.**: When barcoding goes bad: Mitochondrial barcodes are diagnostic of shared refugia but not species in hybridising oak gallwasps (p. 49)

17:30-17:50: **Zaib-un-nisa, Ali** (🏛): DNA barcoding as a means of identification of flora of botanic garden GC University Lahore, Pakistan (p. 76)

19:15-19:30: meeting in the front of the Academy building

19:30: shuttle buses leave to Tervuren for the conference dinner

20:00-23:00: conference dinner (Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren), welcome speech by Guido Gryseels, director of the RMCA

23:00: shuttle buses leave back to Brussels

THURSDAY, 20 SEPTEMBER 2012

Session 5: Human & animal health and open session (Academy, Auditorium Albert II)

Chair: Tine Huyse

9:00 - 9:40: INVITED LECTURE – **Morand, Serge**: From pathogens' screening to barcoding: A continuous challenge for human and animal health (p. 45)

9:40-10:00: **Bellemain, Eva**: Environmental DNA barcoding and metabarcoding as useful tools for biodiversity assessments (p. 8)

10:00-10:20: **De Vos, Paul**: Prokaryotic systematics and barcoding: a difficult marriage (p. 18)

10:20-10:40: **Nieman, Aline**: DNA barcoding of ancient DNA reveals climate driven biodiversity changes by showing a surprisingly high fungal diversity in fossil mammoth dung (p. 50)

10:40-11:10: coffee break (Academy, Atrium)

11:10-11:30: **Huyse, Tine**: Alternative markers for the barcoding of flatworms (p. 70)

11:30-11:50: **Apolônio Silva de Oliveira, Daniel** (🏛): An integrative approach to characterize cryptic species in the *Thoracostoma trachygaster* Hope, 1967 complex (Nemtoda: Leptosomatidae) (p. 4)

11:50-12:10: **Flot, Jean-François**: DNA barcoding using haplowebs (p. 22)

12:10-12:30: **Schindel, David E.**: Institutional adoption of DNA barcoding: CBOL's new strategies (p. 61)

Concluding session (Academy, Auditorium Albert II)

12:30-13:10: Discussion round: Barcoding's second decade in Europe (moderated by David E. Schindel)

13:10-13:20: Announcement of students' awards winners

13:20-13:30: closing remarks

13:30-14:30 lunch (Academy, Atrium)

POSTERS

Students participating at the awards are indicated with (🏛)

Abdullah, Asadatun(🏛) and Rehbein, Hartmut: Application of DNA barcoding genes as universal markers for identification of commercial fish species of Indonesian origin (poster nr. 2, p. 2)

Breman, Floris C., Jordaens, Kurt, Van Steenberge, Maarten, Loix, Sara and Snoeks, Jos: DNA barcoding of the Lake Tanganyika cichlid species (Pisces, Perciformes, Cichlidae) of the rocky shores (poster nr. 5, p. 10)

Breman, Floris C., Jordaens, Kurt, Sonet, Gontran, Nagy, Zoltán T., Van Houdt, Jeroen and Louette, Michel: DNA barcoding and evolutionary relationships in *Accipiter* Brisson, 1760 (Aves, Falconiformes: Accipitridae) with a focus on African and Eurasian representatives (poster nr. 6, p. 11)

Breugelmans, Karin, Jordaens, Kurt, Kano, Yasunori, Asami, Takahiro and Backeljau, Thierry: Phylogeny and DNA barcoding of *Nipponarion* (Gastropoda: Stylommatophora: Arionidae): a bridge too far? (poster nr. 7, p. 12)

Decraemer, Wilfrida, Palomares-Rius, Juan Emilio, Cantalapiedra, Carolina, Landa, Blanca B., Duarte, Isabel, Almeida, Teresa, Vovlas, Nicolas and Castillo, Pablo: Integrative approach in identification of cryptic species of the virus vector family Trichodoridae (Nematoda) from the Iberian Peninsula, an apparent centre of speciation (poster nr. 26, p. 14)

De Groot, Arjen, Laros, Ivo, Dimmers, Wim, Beentjes, Kevin, Doorenweerd, Camiel, Faber, Jack: Monitoring mite diversity in European soils using high throughput e-DNA barcoding tools (poster nr. 8, p. 15)

Elejalde, Arantza, Breugelmans, Karin, Jordaens, Kurt, Pearse, John S., Leonard, Janet L. and Backeljau, Thierry: Applying species delimitation methods in *Ariolimax* (Stylommatophora: Arionidae) (poster nr. 9, p. 21)

Geml, József, Morgado, Luis N., Neilen, Manon(🏛) and Noordeloos, Machiel E: DNA barcoding of ectomycorrhizal agaric fungi of the flora Agaricina Neerlandica for taxonomic and ecological studies (poster nr. 19, p. 25)

Ghahramanzadeh, Robab, Esselink, Danny G., Kodde, Linda P., Duistermaat, H. Leni, van Valkenburg, Johan, Marashi, Hasan S., Smulders, Marinus J.M., van der Schoot, Hanneke and van de Wiel, Clemens: DNA barcoding of invasive aquatic plant species (poster nr. 1, p. 26)

Gombeer, Sophie, Bervoets, Lieven and Knapen, Dries: Using COI and other barcodes in the development of a tool to screen mixed environmental samples for the presence of biomonitoring taxa (poster nr. 10, p. 27)

Hanc, Magdalena, Felczak, Karolina, Kukuła, Wojciech, Kurczak, Paulina, Lipska, Agnieszka, Matysiak, Szymon, Nowak, Piotr, Okła, Agnieszka, Okraska, Radosław, Pawlak, Ewa, Rewińska, Monika, Rzyśkiewicz, Paulina, Sady, Ewa, Skarzyński, Marek, Szczerba, Jacek, Wróblewska, Joanna, Żebrowska, Paula, Mirzwa Mróz, Ewa, Zajkowski, Piotr, Schollenberger, Małgorzata and Wakuliński, Wojciech: Polymorphism of its sequences of four C*ercospora* species (poster nr. 20, p. 28)

Harnelly, Essy(⇧), Prinz, Kathleen and Finkeldey, Reiner: Suitability of the two barcoding regions *mat*K and *rbc*L to discriminate *Dipterocarpaceae* (poster nr. 21, p. 31)

Hobæk, Anders, Ekrem, Torbjørn and Stur, Elisabeth: NorBOL: Norwegian Barcode of Life network (poster nr. 30, p. 32)

Jordaens, Kurt, Sonet, Gontran, Richet, René, Dupont, Erena, Braet, Yves and Desmyter, Stijn: Towards a molecular reference database for the identification of forensically important *Sarcophaga* species (Diptera: Sarcophagidae) (poster nr. 3, p. 33)

Laiou, Angeliki(⇧), Cosimo Simeone, Marco, Piredda, Roberta and Bellarosa, Rosanna: Affirmation of DNA barcoding as a powerful tool in cataloguing medicinal and aromatic plants in Mediterranean forests (poster nr. 22, p. 34)

Litman, Jessica, Wyler, Sofia and Pawlowski, Jan: Launching SwissBOL (the Swiss Barcode of Life network) (poster nr. 31, p. 35)

Londoño, Jorge Mario, Gallego Sánchez, Gerardo, Díaz, Ana Elizabeth, Suárez-Baron, Harold and Tohme, Joe: Use of DNA barcode for identification of possible biotypes of the fruit borer *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae), an important pest of Andean solanaceous fruits (poster nr. 23, p. 37)

Lung, Oliver, Nadin-Davis, Susan, Fisher, Mathew, Erickson, Anthony, Furukawa-Stoffer, Tara, Knowles, M. Kimberly and Ambagala, Aruna: Microarray for identification of the chiropteran host species of Rabies virus in Canada (poster nr. 27, p. 39)

Mortelmans, Jonas(⇧), Nagy, Zoltán T., Sonet, Gontran and Grootaert, Patrick: Using DNA barcodes for assessing diversity of dance flies (Diptera: Empidoidea) and as a basis for phylogenetic research (poster nr. 11, p. 46)

Muñoz Colmenero, Marta(🏛), Ardura, Alba, Triantafyllidis, Alexandros, Martinez, Jose Luis and Garcia-Vazquez, Eva: Barcoding for detection of mislabeling in commercial seafood and implications for human health (poster nr. 28, p. 47)

Nagy, Zoltán T., Sonet, Gontran, Glaw, Frank, Vences, Miguel: First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar (poster nr. 12, p. 48)

Pardo, Cristina, Lopez, Lua, Peña, Viviana, Hernandez-Kantun, Jazmine, Le Gall, Line, Barbara, Ignacio and Barreiro, Rodolfo: DNA barcoding reveals a striking number of mäerl species in the Ospar area (poster nr. 13, p. 52)

Pintaud, Jean-Christophe, Ludeña, Bertha and Couvreur, Thomas: Barcoding in the genus *Bactris* (Arecaceae) with trnQ-rps16 and psbA-trnH plastid loci (poster nr. 24, p. 55)

Prévot, Vanya, Jordaens, Kurt, Sonet, Gontran and Backeljau, Thierry: Exploring species delimitation methods based on DNA barcoding in the land snail genus *Rumina* (Gastropoda: Pulmonata) (poster nr. 14, p.57)

Raspé, Olivier, Degreef, Jérôme and De Kesel, André: DNA barcoding of African boletes using the mitochondrial genes ATP6 (poster nr. 25, p. 58)

Robbins, Timothy(🏛) and Nijman, Vincent: Smaller is better (or almost as good): validating CO1 mini-barcodes for primate species identification (poster nr. 15, p. 59)

Roeder, Mareike and Slik, J.W. Ferry: Climb together: using DNA barcodes for the phylogenetic analysis of liana communities and identifying stems without canopy access (poster nr. 16, p. 60)

Schön, Isa, Shearn, R., Koenders, A., Halse, S. and Martens, Koen: A revision of Western Australian *Bennelongia* (Crustacea, Ostracoda, Cyprididae) – the complementarity of barcoding and morphological studies (poster nr. 17, p. 63)

Sonet, Gontran, Jordaens, Kurt, Braet, Yves and Desmyter, Stijn: Molecular identification of the forensically important greenbottle fly species *Lucilia caesar* and *L. illustris* (family Calliphoridae) (poster nr. 4, p. 66)

Stielow, Benjamin, Verkleij, Gerard, Groenewald, Marizeth, Robert, Vincent and Crous, Pedro: A global genetic backbone for the fungi: the CBS-barcoding project (poster nr. 18, p. 67)

Webster, Bonnie L., Huyse, Tine and Littlewood, Tim: Schistosome mitochondrial genomes: phylogeny and diagnostics (poster nr. 29, p. 73)

ALPHABETICAL LIST OF ABSTRACTS

(ABSTRACTS INDICATED WITH 🏛 COMPETE
FOR THE STUDENT AWARDS)

APPLICATION OF DNA BARCODING GENES AS UNIVERSAL MARKERS FOR IDENTIFICATION OF COMMERCIAL FISH SPECIES OF INDONESIAN ORIGIN (🏛 poster)

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Advancement of DNA-based methods for fish species identification is an important process for fisheries research laboratories and international food control authorities, as a result of species substitutions practices in commercial fish markets. 54 reference fish species from Indonesian commercial markets were identified by biological characteristics and assessed using DNA sequencing of mtDNA genes in order to estimate the applicability of these genetic markers up to species level. A 464 bp segment of the cytochrome b (cyt b) gene and a 655 bp segment of cytochrome c oxidase I (COI) gene was amplified by PCR and sequenced using universal primers. 81 original sequences were obtained and compare by BLAST to sequences available in GenBank. Comprehensive results showed that cyt b and COI sequences enabled authentication process for 90.74% and 59.25% respectively. In addition, 12 sequences of cyt b gene and 4 sequences of COI gene cannot be assigned in GenBank. Nevertheless, there was any intention of mislabeled species in the Indonesian fish market. This preliminary results show that mtDNA markers present a high possibility to act as a tool for Indonesian commercial fisheries product authentication in the future. Due to any difficulties to differentiate *Thunnus* species and *Oreochromis* species, we undertake an effort to develop other species-specific markers from nuclear DNA besides DNA barcoding genes in order to evade misidentification at species level particularly for closely related species.

CONTRIBUTION OF DNA BARCODING TO THE STUDY OF MARINE MAMMALS AND OF THEIR PREYS (🏛 oral)

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Located on the north west of France, the Brittany Atlantic coast is an area presenting a high level of marine mammal–biodiversity. More than fifteen species (cetaceans and pinnipeds) have already been observed. Since 2006, a collaborative work between the University of Brest and the Marine Mammal Laboratory of Oceanopolis has been established, with the aim of studying some molecular aspects of marine mammal biology and ecology. This work is also supported by the first French Marine Nature Park ("Parc Naturel Marin d'Iroise"), and in January 2012, the University of Brest created BioGeMME, a new laboratory with a scientific project focusing on marine mammal biology and genetics.

In this context, we used DNA barcoding with different goals. The first one is, when needed, to determine – or confirm – the taxonomic identification of stranded animals or parts of stranded animals, when visual identification cannot be performed. Some examples will be shown, including for instance a spectacular 2-3 meters long bubbler fragment stranded on the island of Sein, and identified as belonging to a *Balenoptera physalus*, the second largest living animal on the planet. The applicability of the method for different species of marine mammals will be discussed (so far, we have determined the barcode region sequence of 86 animals belonging to 16 different marine mammal species).

DNA barcoding was also used to study the diet of two species of marine mammals, the harbour porpoise and the grey seal. We analysed the residual DNA contained in soft remains of preys found in the stomach of the predators. Although only degraded DNA was generally extracted from these digested tissues, we identified around 10 fish species. The use of this simple DNA-based method, in addition to visual identification, increased the rate of prey identification by some 30%.

Furthermore, we studied the intra-specific variability of the COI barcode region in two species of marine mammals, the harbour porpoise and the grey seal, and compared it to the variability of other mitochondrial regions. The usefulness of these results in population genetics studies will be discussed. One of our projects is to further compare this intra-specific variability with samples obtained from natural history museums, including the "Musée Zoologique" of Strasbourg.

These projects, and all the sequences determined, are published on the BOLD Database.

AN INTEGRATIVE APPROACH TO CHARACTERIZE CRYPTIC SPECIES IN THE THORACOSTOMA TRACHYGASTER HOPE, 1967 COMPLEX (NEMATODA: LEPTOSOMATIDAE)
(🏛 oral)

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Nematode diversity may seriously be underestimated when taking into account cryptic speciation. *Thoracostoma trachygaster* is commonly found in kelp holdfasts along the California coastline and was recently shown to consist of at least two distinct molecular clades (I and II). Here, we provide detailed morphological analysis of both clades, based on measurements taken from video vouchers of respectively eight and 16 individuals from the previous study, as well as 80 newly collected specimens from four Californian beaches. The latter were vouchered, measured, and then subjected to molecular analyses of the mitochondrial cytochrome oxidase c subunit I (COI) gene, and the ribosomal D2D3 and internal transcribed spacer (ITS) regions. This integrative approach shows that the three molecular clades are phylogenetically and morphologically distinct species, but a combination of morphological characters is needed to distinguish them. Two new species, *Thoracostoma fatimae* sp. nov. and *Thoracostoma igniferum* sp. nov., are identified and described. The spicule length of *T. fatimae* sp. nov. is significantly shorter than that of *T. trachygaster*. *Thoracostoma igniferum* sp. nov. can be distinguished by the irregular posterior edge of the cephalic capsule and the two internal subdorsal tropis-like projections in the wall of the cephalic capsule, which are lacking in *T. fatimae* sp. nov. and *T. trachygaster*.

BEYOND BIODIVERSITY: FISH METAGENETICS MEASURED WITH BARCODING TOOLS (🏛 oral)

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Biodiversity and intra-specific genetic diversity are interrelated and determine the potential of a community to survive and evolve. Both are considered together in Prokaryote communities treated as metagenomes or ensembles of functional variants beyond species limits. Many factors alter biodiversity in higher Eukaryote communities, and human exploitation can be one of the most important for some groups of plants and animals. For example, fisheries can modify both biodiversity and genetic diversity (intra specific). Intra-specific diversity can be drastically altered by overfishing. Intense fishing pressure on one stock may imply extinction of some genetic variants and subsequent loss of intra-specific diversity. The objective of this study was to apply a metagenome approach to fish communities and explore its value for rapid evaluation of biodiversity and genetic diversity at community level. Here we have applied the metagenome approach employing the Barcoding target gene COI as a model sequence in catch from four very different fish assemblages exploited by fisheries: freshwater communities from the Amazon River and northern Spanish rivers, and marine communities from the Cantabric and Mediterranean seas. Treating all sequences obtained from each regional catch as a biological unit (exploited community) we found that metagenomic diversity indices of the Amazonian catch sample here examined were lower than expected. Reduced diversity could be explained, at least partially, by overexploitation of the fish community that had been independently estimated by other methods. We propose using a metagenome approach for estimating diversity in Eukaryote communities and early evaluating genetic variation losses at multi-species level.

BARCODING AND BIOBANKING: A SYNERGY

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As molecular markers are evolving into a very widely used tool for species identification, millions of specimens in hundreds of thousands of species are being accumulated.
In my presentation I want to show on the one hand that DNA barcoding projects, often organized as national campaigns, qualify as one of the most valuable sources for biobank specimens of wild organisms. This applies, among others, to the speed with which samples are obtained, to the underlying determinations, to vouchering requirements, or to the diversity and taxonomic coverage of samples.
On the other hand, I want to emphasize how barcoding can benefit from biobanking – e.g. for dataset expansion, for molecular vouchering / reproducibility, for barcode 'scalability' (adding other markers in the future) – and how important it is to apply proper biobanking standards for frozen sample storage, which is often neglected.

USING A NETWORK OF AMATEUR SPECIALISTS FOR THE COLLECTION AND IDENTIFICATION OF SPECIMENS FOR DNA BARCODING OF DUTCH FLORA AND FAUNA

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One of the main objectives of the DNA barcoding project of Naturalis Biodiversity Center is to determine DNA barcodes for as many species as possible of the Dutch flora and fauna, for both scientific and societal purposes. Since the museum collection of Naturalis often does not contain relatively fresh material, we have decided that newly collected specimens from the field would have to become the main source of voucher material for a selection of the DNA barcoding subprojects. To obtain fresh material, Naturalis has employed the relatively large network of enthusiastic amateur and non-professional specialists and taxonomists in the Netherlands. They are coordinated through several NGOs that specialize in flora and fauna research within the country, who ensure the proper handling of specimens and affiliated data. NGOs specializing in flora research have even designed a system for online reservation of specimens, to avoid oversampling of taxa and encourage collectors to find missing species. The amateur specialists collect, identify, register and sometimes prepare the specimens according to standardized protocols. Professional employees of the NGOs review specimens and data, take subsamples for DNA barcoding and deliver the total package of subsamples, vouchers and data to the DNA barcoding facility, for which they receive financial compensation. By operating this way, we do not only extend our capacity, but also our expertise. For certain specific groups, taxonomic knowledge is limited or absent within the museum, and amateur specialists can provide reliable determinations of the voucher specimens. An additional benefit of the project is the stronger cooperation between amateur specialists, NGOs and the museum. At this moment, non-professionals have collected over 7000 specimens in the field, with an estimated number of 6000 still to come.

ENVIRONMENTAL DNA BARCODING AND METABARCODING AS USEFUL TOOLS FOR BIODIVERSITY ASSESMENTS

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Environmental DNA (eDNA) barcoding refers to the automated identification of a target species from a modern or ancient environmental sample containing degraded DNA, such as soil, water or faeces. When using universal primers (or group-specific primers), DNA from multiple species can also be retrieved from an environmental sample: in this case, we refer to eDNA metabarcoding. The combination of next-generation sequencing and eDNA metabarcoding is revolutionizing the nature and quantity of data available for ecological assessment and monitoring applications.
In this presentation, I will first show the power of such methods by describing potential applications for 1) biodiversity assessment from modern soil or permafrost samples; 2) detection of target organisms such as endangered or alien species from water environments or 3) diet analysis. In some of those examples, the results from DNA-based methods and field methods will be compared and their respective benefits and pitfalls will be examined. I will finally present perspectives for eDNA (meta)barcoding studies in ecology and requirements for future improvements.

THE RESULTS OF QBOL DEPOSITED IN THE Q-BANK DATABASE TO SUPPORT PLANT HEALTH DIAGNOSTICS

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The rate of introduction and establishment of damaging plant pests and diseases has increased steadily over the last century as a result of expanding globalisation of trade in plant material, climate change, EU expansion, and by a recognised decline in the resources supporting plant health activities. Furthermore there is a constant decline in the number of taxonomic specialists in the different disciplines (mycology, bacteriology, etc.), capable of identifying plant pathogens, and funds to support this kind of work are very hard to obtain . Also the number of other specialists in phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are diminishing. These problems affect all members of the EU and other nations.
In this context QBOL (www.qbol.org), an EU project on DNA barcoding, started in 2009 to generate DNA barcoding data of quarantine organisms and their taxonomically relatives to support plant health diagnostics. The data are included in a database, called Q-bank (www.Q-bank.eu). Q-bank now consists of a dynamic open-access database of quarantine plant pests and look-alikes, linked to curated and publicly accessible reference collections. It contains sequence and morphological data including photographs, nomenclatural and diagnostic data of specimens available in reference collections.
Within Q-bank curators from many countries with expertise on taxonomy, phytosanitary and collection issues for the different groups have been appointed and links with other databases have been made; this in order to provide Q-bank an international role in supporting plant health agencies.
The results of the QBOL project will be presented as well as the Q-bank database.

DNA BARCODING OF THE LAKE TANGANYIKA CICHLID SPECIES (PISCES, PERCIFORMES, CICHLIDAE) OF THE ROCKY SHORES

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Three-hundred ninety nine DNA barcodes of 99 species of Lake Tanganyika (LT) cichlids from 11 tribi were analyzed and their usefulness as identification tool for LT-cichlid species was evaluated. Three datasets were analyzed, 1) a dataset with species that are currently recognized, 2) a dataset where species were identified on morphological characteristics and 3) a dataset where problematic species are grouped in species complexes. The third approach yielded the highest success rate with 78% (vs. 66 and 64% for datasets one and two, respectively). Our results show that DNA barcoding performs poorly, when compared to other fish groups, in LT-cichlids. Potential causes for this low identification success include frequent hybridization, incomplete lineage sorting and the rapid morphological evolution of several LT-cichlid taxa. The problem can be partially circumvented by lumping a number of species in species complexes.

DNA BARCODING AND EVOLUTIONARY RELATIONSHIPS IN *ACCIPITER* BRISSON, 1760 (AVES, FALCONIFORMES: ACCIPITRIDAE) WITH A FOCUS ON AFRICAN AND EURASIAN REPRESENTATIVES

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We obtained full (647bp) or mini (291bp) DNA barcodes of 140, mostly African and European, specimens of 25 *Accipiter* (Aves: Accipitridae) species. Kimura-two-parameter (K2P) distances were calculated between barcodes to calculate thresholds of intra- and interspecific species boundaries. Thresholds were comparable, or higher, to that of previous studies on birds and ranged from 2.8% to 3.0% and from 3.9% to 5.3%. Identification success was determined using the best match and best close match criteria and ranged between 84% (mini barcodes) and 90% (full barcodes). Incorrectly or ambiguously identified specimens belonged to two species that were represented by single sequences in the database (*A. madagascariensis* and *A. trivirgatus*) and three species pairs that shared at least one haplotype: viz. *A. nisus – A. rufiventris*, *A. gularis – A. virgatus* and *A. cooperii – A. gundlachi*. All 19 other species could be identified unambiguously using the full DNA barcodes. We have further studied the phylogeny on a low taxonomic level using the same DNA barcodes and found that: the studied species belong to eight traditional superspecies of which three [*gentilis*], [*cooperii*] and [*tachiro*]) were well supported. In one superspecies [*badius*], species pairs were supported but not the superspecies.

PHYLOGENY AND DNA BARCODING OF *NIPPONARION* (GASTROPODA: STYLOMMATOPHORA: ARIONIDAE) : A BRIDGE TOO FAR?

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The taxonomy and phylogeny of the terrestrial (semi)slug family Arionidae (sensu lato) are still poorly known. Particularly, the subfamilial and generic composition of this family is in a state of confusion, up to the extent that several recent authors have split the group by raising its former subfamilies to family rank. Also the assignment of genera to arionid (sub)families is often problematic. A case in point is the rare, endemic Japanese genus *Nipponarion* with its single species *Nipponarion carinatus* Yamaguchi & Habe, 1955. According to its original description, *Nipponarion* would be most closely related to the North American genus *Prophysaon* and thus would belong to either Ariolimacidae, Anadenidae or even the Polygyridae, depending on how authors interpreted *Prophysaon* relationships. Alternatively, *Nipponarion* was not considered to be closely related to *Prophysaon*, but instead was assigned to Arionidae s.s. Against this background we attempted to reconstruct the phylogenetic relationships of *N. carinatus* by determining nucleotide sequences of the COI DNA barcode fragment, a fragment of the nuclear 18S rDNA and of the ribosomal stretch including ITS-2 plus a part of the 28S rDNA. These data were aligned with similar data of a range of arionoid and stylommatophoran taxa, and subjected to phylogenetic analysis. Our preliminary results suggest that *Nipponarion*: (1) shows a remarkable COI diversity, (2) definitely does not belong to the Polygyridae, and (3) is probably somehow related to North American – Asiatic arionoids. Still, arionoid relationships remain largely unresolved and this also applies to the position of *Nipponarion*.

DNA BARCODING OF FUNGAL PATHOGENS TO ENHANCE TRADE AND FOOD PRODUCTION

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Recent changes to the International Code of Nomenclature for algae, fungi and plants have significant implications for practical plant pathology. Other than the abolishment of Latin and the implementation of name registration, asexual genera will be integrated with sexual genera of fungi, and a single name will be used to communicate about species. By employing DNA-based techniques, genera and species can be linked in the absence of all stages of their life cycle. To complicate matters, however, many genera are either poly- or paraphyletic, and many pathogens are in fact species complexes. Even though it is clear that the phenotype conveys limited information about true genealogical relationships, close to 80 % of all novelties described per year still lack DNA sequence data, which represents one of the biggest challenges facing our community. Although the internal transcribed spacer region has been selected as the universal fungal barcode, it only successfully identifies 73% of the taxa screened across kingdom Fungi to species level, suggesting that secondary barcodes will be needed to provide accurate identifications for trade in agricultural produce. This is especially true for species in many important plant pathogenic fungal genera such as *Botryosphaeria, Calonectria, Cercospora, Colletotrichum, Ilyonectria, Mycosphaerella, Phoma* and *Pseudocercospora*. Based on these findings, it is clear that even though many diseases are associated with species complexes, some of these species are in fact also complex in that they have a complicated ecology and life cycle.

INTEGRATIVE APPROACH IN IDENTIFICATION OF CRYPTIC SPECIES OF THE VIRUS VECTOR FAMILY TRICHODORIDAE (NEMATODA) FROM THE IBERIAN PENINSULA, AN APPARENT CENTRE OF SPECIATION

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Trichodoridae are polyphagous root ectoparasites occurring worldwide. Their major pest status is as vector of Tobraviruses. Trichodoridae are difficult to fix, show intraspecific variation in shape and size of diagnostic features, include several cryptic species and show co-occurrence of at least two species. Increasing trade in plants and plant products influences the nematofauna worldwide. Consultants in crop protection need to be able to identify species and be aware of their ecology, including interactions with plants and their ability to transmit viruses. Molecular analyses based on nuclear ribosomal RNA genes (D2-D3 expansion segments of 28S and partial 18S gene) together with a comparative morphological study clearly points the way ahead. Currently, the family has 109 species classified within 6 genera of which the genus *Trichodorus* is the largest in number of species (63). Surveys for trichodorids were carried out in cultivated and natural habitats in Spain and compared with the trichodorid fauna from Portugal. Characteristic for the Iberian Peninsula is the high number of morphologically closely resembling species, but clearly separated molecularly (cryptic species), characterized in males by slightly ventrally curved spicules with a mid-blade constriction with bristles and females with relatively large vaginal sclerotized pieces, quadrangular to triangular in shape. Molecular analyses demonstrated that D2-D3 expansion segments are suitable diagnostic markers for Trichodoridae. Comparative morphology and molecular analyses provide support for the Iberian Peninsula as an apparent centre of speciation.

MONITORING MITE DIVERSITY IN EUROPEAN SOILS USING HIGH TROUGHPUT E-DNA BARCODING TOOLS

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Within the EU FP7-project EcoFINDERS, various European partners collaborate to gain more insights in links between soil diversity and ecosystem services, across different soils, climate types and land uses. To allow rapid screening of many soils throughout Europe, new tools are being developed for high-throughput species identification based on the DNA contained in soil extracts. Alterra participates by developing and applying a new approach for the DNA barcoding of soil mites. For this purpose, soil samples were collected from agricultural soils and (semi-)natural grasslands around Europe, and mites were extracted from them. A reference data base was created containing CO1 sequences of as many of the local species as possible. Species discrimination reached 89%. Additional samples, to be used for diversity screening, were split in two. DNA was extracted from one part, the other part was subjected to morphological identification. A mini-barcode located within the CO1-fragment was developed for 454 pyrosequencing of the DNA extracts. First pyrosequencing results are expected near the end of 2012. Species composition as obtained via molecular and morphological identification will then be compared to test the performance of the new method.

CANINE MITOCHONDRIAL DNA PROFILING IN FORENSICS

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The interpretation of non human biological traces is gaining interest in forensics. Genetic profiling can help to identify the donor of the evidence in a direct or indirect way. Besides human hairs also canine hairs are often recovered from the crime scene. Dogs are very popular pets and live in close contact with humans. Losing a lot of hair every day, dogs can play a role as crime witness.
The genetic profiling of hairs is most often limited to the mitochondrial DNA, since nuclear DNA is only present in a minority of the roots of naturally lost hairs. Since in most cases hairs are analysed individually we're often confronted with minor amounts of DNA, which can be degraded too. Our strategy for control region amplification and sequencing is designed for meeting forensic QA standards even under challenging conditions.
If a known and unknown sample show the same mtDNA profile the matching probability should be estimated. Therefore a population study of close maternally related individuals is needed. A sampling in the Belgian canine population revealed a wider range in haplotype diversity than compared to humans. Since some haplotypes have been observed in 15% of this population sample, we are running a project to extend the mtDNA profile to the whole mtDNA genome.

DNA BARCODING THE FLORA OF WALES: THE CREATION AND USE OF A NATIONAL RESOURCE FOR BIODIVERSITY CONSERVATION AND HUMAN HEALTH

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We have DNA barcoded the native flowering plants and conifers of Wales using the plant DNA barcode markers *rbcL* and *matK*. For the 1143 species (455 genera, 95 families and 34 orders) we have 3304 *rbcL* and 2419 *matk* sequences, covering 98% and 90% of the entire Welsh flora. The majority of our samples (85%) are from DNA extracted from herbarium specimens. Recoverability of DNA barcodes is lower using herbarium specimens, compared to freshly collected material, mostly due to lower amplification success, but this is balanced by the increased efficiency of sampling species that have already been collected, identified, and verified by taxonomic experts. The effectiveness of the DNA barcodes for identification is assessed using the presence of a barcode gap, formation of monophyletic groups using Neighbour-Joining trees and sequence similarity in BLASTn searches. These approaches yield similar results, providing relative discrimination levels of 69 to 75% of all species and 99 to 100% of genera using both markers. Species discrimination can be further improved using spatially explicit sampling. Mean species discrimination using barcode gap analysis is 82% within 10×10 km squares and 93% for 2×2 km squares.
We are beginning to develop a wide range of applications that make full use of our DNA barcode research platform ranging from phylogenetic community ecology to investigating the pollination service provided by hoverflies. We are also using DNA barcoding to investigate the medicinal properties of honey. Honey samples collected from throughout the UK are tested for their ability to kill the hospital infection MRSA. The honey is then DNA barcoded using 454-sequencing in order to investigate whether the floral composition of the honey affects its antimicrobial properties.

PROKARYOTIC SYSTEMATICS AND BAR CODING : A DIFFICULT MARRIAGE

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Prokaryotic systematics covers the Archaea and Bacteria domains of the living world. As any other taxonomic system of living organisms, it concerns three major topics: i) grouping of the organisms according to certain similarities into taxa; ii) naming of these groups according to strict rules (The Bacterial Code) and iii) identification of unknown as belonging to one of the groups in i) and named in ii).
Because of very limited morphologic differentiation between various prokaryotes, their taxonomy was based on discrepancies in biochemical and physiological characterization and much earlier than in other domains of the living world on molecular characteristics i. e. differences in DNA and RNA resulting in a polyphasic bacterial taxonomy (Vandamme et al., 1996). Since the last two decennia, prokaryotic systematics and in particular their identification relies more and more on molecular data that are being generated at high speed. It became clear that bar coding for identification of e.g. quarantine bacteria (QBOL project: http://www.qbol.org/UK/) is not straightforward and can certainly not be based on the sequence of one single gene. This is in contrast to bar coding of other living organisms were mostly one or two genes are used. The genes that are used nearly always go back to a prokaryotic origin due to endosymbiosis processes. Because the diversity of the prokaryotic world is so wide we think that bar coding of these organisms must probably be based on sequence of 'gene panel' that is well chosen and that might be different from one taxon to another and must most probably be applied in a hierarchic stepwise approach.
Vandamme, P., B. Pot, M. Gillis, P. De Vos, K. Kersters and J. Swings. 1996. Polyphasic taxonomy, a consensus approach to bacterial sytematics. *Microbiol. Mol. Biol. Rev. June 1996 vol. 60 no. 2 407-438*

DRAGONFLIES IN FRESHWATER CONSERVATION IN TROPICAL AFRICA: DNA-BARCODES SUPPORT FIELD SURVEYS

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Dragonflies and damselflies (Odonata) are increasingly used as indicators of habitat integrity and biodiversity in freshwater. They are generally easily recorded, identified and applied because the taxonomy, threat status and ecological sensitivity of the species are sufficiently known, even in the tropics. Nonetheless, some uncertainties remain about species that are more easily found as larvae than adults, the life stage on which the taxonomy and most surveys are based. Nearly 4000 records of over 200 species were obtained during five months of surveys in rainforest sites in Liberia and Sierra Leone. COI-sequences from almost 700 specimens, of which 130 larvae, showed high congruence of morphological and molecular identification. The larvae of almost all species rarely encountered as adults were identified, in most cases for the first time. This added important records and allowed the first assessment of their status and ecology, as well as their future identification using larvae. Although barcodes revealed some potential species not detected as adults, their number was low. In conclusion larval sampling supported by molecular identification is an efficient tool to supplement the usually adult-based surveys, although thorough adult-only sampling does generally provide remarkably complete species lists.

IDENTIFICATION OF MARINE NEMATODE COMMUNITIES THROUGH DNA BARCODING: DO MITOCHONDRIAL COI SEQUENCES OUTPERFORM THE RIBOSOMAL 18S GENE? (🏛 oral)

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Free-living nematodes are the most abundant metazoan organisms in marine sediments. They are important in many ecological processes and are used as bio-indicators. Yet, they remain the least described taxon, because morphological diagnostic features are difficult to observe due to their small body size. DNA barcoding may overcome the problems associated with morphology and may lead to a quicker identification of marine nematodes. The nuclear ribosomal 18S gene has been the preferred locus for barcoding nematodes because it is easily amplified throughout the phylum. This fragment lacks however resolution at the species level. In contrast, only few studies have addressed the applicability of the mitochondrial COI gene for nematode identification. In this study, the amplification and sequencing success of both gene fragments were compared in a wide range of marine nematodes. We used 73 species belonging to 56 genera that were sampled from Paulina Polder (the Netherlands). Our results demonstrate that 18S is more easily amplified in marine nematodes compared to COI (57% vs 43% amplification success). The production of aspecific bands was more common in 18S than in COI, but sequencing success remained higher for 18S than for COI (61% vs 39%). Neighbor joining analysis using the K2P-model showed that both genetic markers cluster into well defined clades congruent with known taxonomic families and orders that have been delineated based on morphology. Pairwise genetic distance for the 18S sequences showed that ≈74% of intraspecific comparisons showed a genetic divergence ≤3% while about 77% of interspecific comparisons were above 3%. For COI sequences, 98% of intraspecific comparisons showed a genetic divergence of ≤8% and >8% was observed for about 94% of all interspecific comparisons. This study shows that COI sequences qualitatively outperforms the 18S gene in the delineation of marine nematode species.

APPLYING SPECIES DELIMITATION METHODS IN *ARIOLIMAX* (STYLOMMATOPHORA: ARIONIDAE)

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Banana slugs in the genus *Ariolimax* are the largest native terrestrial slugs throughtout the Pacific Northeast of North America. On the basis of genital morphology the genus has been divided in two subgenera distinguished by the presence (*Meadarion*) and the absence (*Ariolimax s.s*) of an 'epiphallus', comprising five (sub)species distinguished by the size and shape of the penis and vaginal musculature. Recent studies of the genital morphology and reproductive behavior of *Ariolimax* sp. have suggested that the genus has undergone a rapid evolutionary divergence, so that the current taxonomy of the genus needs to be re-interpreted. In this context, ongoing phylogenetic analyses based on three mtDNA gene fragments (COI, 16S rRNA and CytB) show four well supported clades in the subgenus *Ariolimax*, which were interpreted as four phylogenetic species and an undescribed species from Mount Palomar (San Diego County). The subgenus *Meadarion* comprises two clades, one including an undescribed species from Fremont Peak (San Benito County), the other one including three (sub)species. Current mtDNA, morphological and behavioral evidence suggest that there may be at least eight species level taxa in the genus.
Using the COI sequence data, we here apply a DNA barcoding approach to explore to what extent the eight putative species level taxa are indeed recognized as such by a number of alternative species delimitation methods.

DNA BARCODING USING HAPLOWEBS

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A key problem when using DNA barcoding to delineate species or identify specimens is to decide where to draw the line between intraspecific variations and interspecific differences. Whereas the first DNA barcoding studies used an arbitrary threshold, various approaches aiming to find the best threshold for a given group of species have been proposed. However, these approaches all rely on models and assumptions that may be violated in actual biological populations; moreover they perform poorly when interspecific divergence is smaller than intraspecific variability. Hence I recently proposed a graphical method, named “haplowebs”, that delineates species without using thresholds; it is based on the criterion of mutual allelic exclusivity and applies to all sexually reproducing species, without further assumption on their population structure. It also does not require species to be monophyletic. Published examples of application of this approach to scleractinian corals will be presented, followed by unpublished examples dealing with freshwater amphipods. I will conclude by unveiling a new web-based bioinformatic platform that takes as input FASTA-formatted alignments of DNA sequences and performs species delimitation using this novel approach.

MICROWAVING GASTROPODS FOR DNA BARCODING (🏛 oral)

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Extracting DNA from gastropods presents particular difficulties due the capacity of the living animal to retract into the shell, resulting in poor penetration of the alcohol into the tissues. However, because the shell is essential to establish the link between sequences and traditional taxonomic identity, cracking the shell to facilitate good fixation is not ideal. Several methods are currently used to overcome this difficulty, including chemical relaxation of the animal, drilling the shell, and boiling ("*niku nuki*"). However, most of these methods are time consuming – and sometimes hazardous in terms of safety – and, in the field, constitute a bottleneck in the preparation of large numbers of specimens. We have experimented with a method traditionally used to clean shells that involves placing the living gastropods in a microwave oven; the electromagnetic radiation very quickly heats both the animal and the water trapped inside the shell. This results in separation of the muscles that anchor the animal to the shell, and steam is then able to force the animal from the aperture. Done properly, the body can be removed from the shell entirely intact and the shell voucher is preserved undamaged. To test the method, the bodies of live-collected specimens from throughout the spectrum of gastropod clades and size classes were separated from their shell by either: (a) microwaving, or (b) anesthetizing and/or drilling. After identical extraction and PCR procedures, the gels showed no difference in DNA amount/quantity, and the resulting sequences are identical within species boundaries. The microwave technique is also effective for quickly and easily removing other shelled mollusks from their shells, i.e. bivalves and scaphopods. During several recent expeditions, the microwave technique has permitted the processing of ~10,000 mollusk specimens during 100 person-days, a 3 to 5 fold gain in productivity compared to other methods.

INTRODUCING THE FREDIE PROJECT WITH NOTES ON THE FRESHWATER FISH DIVERSITY OF THE MEDITERRANEAN HOT-SPOT

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FREDIE stands for 'Freshwater Diversity Identification for Europe' and is a nationally funded project aimed at bringing together DNA barcoding with taxonomic expertise for European freshwater organisms (www.fredie.eu). Included are freshwater fishes, mayflies and freshwater molluscs, and we aim to develop an online available identification system for species diversity of these groups in Europe. Although DNA barcoding offers the possibility to accelerate detection and monitoring of biodiversity, its quality and significance is strictly bound to availability and quality of underlying reference data, especially reliable species determinations. Resources and expertise of three core institutes and a web of associated partners are combined to create a reliable and sustainable reference system. Representatives of nearly all species of the three groups occurring between Portugal and the Ural are collected, identified by experts and sequenced to become barcode references. In selected cases a refined identification system is planned including morphological characters and other genetic markers with better taxonomical resolution. We will provide the respective voucher specimens and a DNA collection as permanent references available to the scientific community. FREDIE generates molecular and morphological estimations of species numbers of the three organism groups for Europe, as well as deeper insights into their spatial structure on a large scale. In a first sub-project we focused on the freshwater fish diversity of the Mediterranean hot-spot, which harbors about 557 extant species. We were able to obtain so far more than 90% of these species, represented by >3,500 individuals by own collecting efforts and support by European colleagues. Through intensive sampling of widely distributed taxa from several major European drainages, some possessing different levels of morphological divergence and specific designation, we evaluated the performance of barcoding in distinguishing sister-species and glacial lineages. DNA barcoding can be an extremely efficient tool for species identification across various families of freshwater fishes. In many cases, the approach identified also biological meaningful intra-specific groups. The analysis of geographic variation revealed both, previously known but also underappreciated phylogeographic breaks among different taxa. This has important conservation implications for those species and populations and further enhances our understanding of the biogeography and evolution of European freshwater fish diversity.

DNA BARCODING OF ECTOMYCORRHIZAL AGARIC FUNGI OF THE FLORA AGARICINA NEERLANDICA FOR TAXONOMIC AND ECOLOGICAL STUDIES (🏛 poster)

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The multi-volume Flora Agaricina Neerlandica provides identification keys, extensive descriptions, and illustrations of all agarics and boleti occurring in the Netherlands and adjacent regions, with data on their ecology and distribution. The detailed information provided in the Flora is generally based on vouchered specimens, many of which are deposited at the National Herbarium of the Netherlands (L). The Flora project is also linked with nation-wide mapping effort of amateur and professional mycologists that has been coordinated by the Werkgroep Paddenstoelenkartering Nederland (WPN) since 1980. The accumulated knowledge has resulted in an official national Red List of fungi published in 1996, then revised in 2008. We have recently initiated a project to generate DNA barcodes (ITS rDNA sequences) for taxa featured in the Flora Agaricina Neerlandica. Out of the vast number of species, in this initial phase of the project, we prioritize barcoding ectomycorrhizal (ECM) agaric species for the following reasons:

- Currently, progress in fungal ecology is severely halted by the shortage of reliable reference database for semi-automated identification of root- and soil samples. Our barcoding project of ECM fungi aims to reduce this gap and, therefore, to enhance ecological and biodiversity studies globally.
- Many ECM genera are species rich and are taxonomically challenging. Generating sequences for types and other well-documented collections helps us to create a reliable backbone for systematic studies.

We have begun a series of high-throughput sequencing projects to supplement long-term sporocarp records accumulated by the WPN with DNA-based species identification from environmental samples. In this presentation, we will demonstrate examples for combined use of specimen-based DNA barcoding and 454 sequencing of soil samples for fungal biodiversity assessments, mapping and conservation in the Netherlands.

DNA BARCODING OF INVASIVE AQUATIC PLANT SPECIES

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The last few decades have shown a strong increase of non-native species in the Dutch flora. The majority of these new-comers are very modest and do not lead to problems. However, some species cause problems by their invasive behaviour, such as the aquatic Floating pennywort (*Hydrocotyle ranunculoides*). The Dutch Ministry of Economic Affairs, Agriculture and Innovation provided a 4-year subsidy from the FES-programme "Versterking Infrastructuur Plantgezondheid" to build an information system on potentially invasive exotic species. The aim is to deliver information that could form the basis of an estimation of the chance that a species will develop invasive behavior upon introduction in The Netherlands, and to provide tools with which these species can be identified. Focus is on vascular plants (thus excluding algae and mosses), with special attention to aquatic plants because of acute problems in this group. The information system forms part of Q-bank, an identification and detection reference database for phytosanitary organizations (http://www.q-bank.eu/).
To distinguish invasive from non-invasive species based on morphology alone can be hard at a vegetative stage, as is commonly how material is met in inspection situations. Thus, DNA barcoding could be an alternative for reliable identifications, for which the CBOL Plant Working Group proposed the chloroplast sequences *rbcL* and *matK*. We performed a few pilot studies and additionally tested the *trnH-psbA* locus, as it is more variable than *rbcL* and more reliably amplified for sequencing than *matK*. Using *trnH-psbA* alone, we were able to distinguish the invasive *Hydrocotyle ranunculoides* from at least six other species from the genus *Hydrocotyle*. We were also successful in the genera *Ludwigia* (*L. grandiflora* and *L. peploides*), *Myriophyllum* (*M. heterophyllum* and *M. aquaticum*), *Cabomba* (*C. caroliniana*), and four species of the Hydrocharitaceae (*Egeria densa*, *Elodea canadensis*, *Elodea nuttallii*, and *Lagarosiphon major*). We foresee that in the near future determination of DNA sequences may become a matter of routine that could be performed by customs and inspectors in the field, allowing for identification of species independent of external features.

USING COI AND OTHER BARCODES IN THE DEVELOPMENT OF A TOOL TO SCREEN MIXED ENVIRONMENTAL SAMPLES FOR THE PRESENCE OF BIOMONITORING TAXA (🏛 oral)

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Macro invertebrates are frequently used for the biomonitoring of freshwater quality. Identifications of the taxa used for index calculations are often limited to the genus or family level due to a lack of time, funding and/or taxonomic expertise. In the present study the potential offered by a molecular approach to develop a biomonitoring-chip for the automated detection of macroinvertebrate families in complex samples is evaluated using the order of the Trichoptera (Caddisflies; Class: Insecta) as case-study organisms.
A database with accurate species level identifications linked to species specific sequences for three genes (COI, *wingless* and a fragment of 18S) was used to design family specific primer-sets for a real-time PCR (QPCR) assay. Single-target experiments were conducted to (1) test the specificity of the designed primers, (2) detect species that could possibly cause false positives and false negatives signals, and (3) identify reference species for each primer-set to be used as standard in mixed sample analyses. The results were used to develop a ΔCt-based method and to build decision trees which allow to interpret the QPCR output of mixed samples. Next, the sensitivity of the assay was determined by detection limit experiments and a protocol for complex environmental samples was established. Finally, the developed QPCR assay was validated using fresh field samples containing a mixture of Trichoptera families and non-trichopteran freshwater invertebrates.

POLYMORPHISM OF ITS SEQUENCES OF FOUR *CERCOSPORA* SPECIES

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Cercospora genus is a member of *Ascomycota* with putative teleomorph in *Mycospherella*. Originally, it was introduced by Fresenius for fungi with dark conidiophores, and long, acicular hyaline and multiseptate conidia with thickened scars. The genus constitute highly heterogeneous group of fungi with several described species and due to equivocal morphological diagnostic traits applied in taxonomy, proper recognition of particular taxons is very difficult, using standard mycological procedure. The aim of this work was to test possibility to use ITS1 and ITS2 gene region for *Cercospora* species barcoding. Totaly 42 isolates of *Cercospora apii, C. armoraciae, C. beticola* and *C. carotae* collected from different hosts and localities in Poland has been tested. DNA was isolated using using DNeasy Plant Mini Kit (Qiagen). Amplification of analysed region was curried out using Gen Amp PCR System thermocycler and universal primers WRIZG1 (5'GTAACAAGGTTTCCGTAGGTG-3') and Pn10:(5'-TCCGCTTATTGATATGCTTAAG-3'). Comparative analysis of amplicon sequences obtained in PCR reaction indicated on high similarity of analyzed species and constructed dendrograms did not discriminate precisely individual taxa for separate clades.
In conclusion, the studies demonstrate that ITS region, commonly explored for fungal barcoding, is not a good marker for distinguishing tested *Cercospora* species.

INTERNATIONAL BARCODE OF LIFE (IBOL) PROJECT: INTERIM REPORT

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In 2010 the International Barcode of Life (iBOL.org) project was launched with the objective of constructing a barcode reference sequence library of global scope, capable of addressing the biological specimen identification needs of diverse socio-economic interest groups. As the project nears the end of its second year, a number of advances have been made, including the release of BOLD 3.0, the online informatics workbench that supports the assembly, analysis and archival of barcode records, which now includes some 2 million sequences representing more than 200 thousand species. Participation in iBOL continues to grow but the global financial climate has also created challenges. However, new strategies suggest that economies of scale can be achieved that will still allow iBOL to meet its target of 5 million records by 2015. These and other relevant developments for the international barcoding community will be discussed.

DEVELOPMENT AND VALIDATION OF A BARCODING STRATEGY TO SUPPORT IDENTIFICATION, TRACING AND TRADE REGULATION OF MALAGASY ROSEWOOD (🏛 oral)

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The Masoala region in Madagascar is one of the world's biodiversity hotspots but is highly threatened as a consequence of illegal logging. Directly affected are precious timber species such as rosewood (genus *Dalbergia*, family Fabaceae). Selective logging of *Dalbergia* has massive indirect effects associated with human activity in the heart of the forests and the additional wood needed to transport logged stems on rivers. Despite their conspicuous hard wood, the different *Dalbergia* timber species are difficult or impossible to distinguish and identify to species once they have been logged and morphological traits used for identification, such as bark, leaves, fruits and flowers, have been removed. In addition, only five Malagasy species are currently listed on CITES Appendix III, but more species may be affected by logging. To better characterise *Dalbergia* diversity in the Masoala region, identify the species most affected by logging, and assign logged trees to species, we develop a molecular barcoding strategy using a combination of chloroplast and nuclear DNA markers.
Therefore the primary goals of this project are A) to develop and validate molecular identification tools for *Dalbergia* species from Madagascar using DNA barcoding and B) to help incorporating this technique into international trade regulation and law enforcement for Malagasy rosewood. First results obtained from sequencing multiple chloroplast markers for plant DNA barcoding showed that not all tested species could be distinguished, but that species from Madagascar could be readily distinguished from Asian species. To increase the resolution of our DNA barcoding approach we sequenced the leaf transcriptome of *Dalbergia baronii* using next-generation sequencing and developed nuclear molecular markers. We anticipate that this molecular method – once fully developed and validated – can be used as a forensic tool for species identification, trade regulation and traceability of logs on the international market.

SUITABILITY OF THE TWO BARCODING REGIONS *MAT*K AND *RBC*L TO DISCRIMINATE *DIPTEROCARPACEAE* (🏛 poster)

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The two DNA barcode regions *mat*K and *rbc*L adopted from the Consortium for the Barcode of Life (CBOL plant working group 2009) are applied to assess the feasibility of this region as a barcode region to discriminate the *Dipterocarpaceae*. Most information for the *mat*K region was available in NCBI database, but additional samples were also included in our study. In total 156 an 76 samples were studied using the *mat*K and *rbc*L region respectively. The effectiveness of barcode analysis in this study was assessed by the formation of monophyletic groups of the query sequences and the reference sequences (e.g. NCBI) using Neighbor-Joining Trees. The result showed that the *mat*K region could be a potential barcode for *Dipterocarpaceae* since many of the query sequences form a monophyletic group with the sample of the same species available in the databases. This region can also resolve the tribe and genera among the *Dipterocarpaceae* sequences with high bootstrapping value (99 % and 66 %, respectively). This indicates that this region has a potential to delineate the *Dipterocarpaceae*. For *rbc*L region, we cannot observed the affiliation of the query sequences from laboratory since only several sequences of this region are available in the databases. However, our result showed the potential of this region to resolve tribe and genera (73 % and 58 %) that this region also has discriminatory power for *Dipterocarpaceae* as well.

NORBOL: NORWEGIAN BARCODE OF LIFE NETWORK

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The Norwegian barcode of Life Network was formed after the Inaugural Workshop in Canada in June, 2007. Our aims were to establish a national network for barcoding national as well as Arctic biodiversity, to raise funding in support of barcoding and curation of barcoded material, to co-ordinate and initiate new barcoding projects, and to raise public awareness about the barcoding and barcoding results in Norway. NorBOL is a regional node within iBOL, with a particular responsibility for polar barcoding. NorBOL was initiated from and led by the Natural History Museum, University of Oslo until 2011, when leadership was taken over by the Museum of Natural History and Archaeology, Norwegian University of Science and Technology in Trondheim. At present, the network comprises 16 institutions, including all four major natural history museums as well as all major research institutes. In spite of strong support among research institutions, national fund-raising has had limited success, and only this year we have succeeded in establishing an economic basis for the network's activities 2012-2015 through a grant from the Norwegian Biodiversity Information Centre. As a result, barcoding progress lags behind original plans. The target for NorBOL is set to 20,000 species barcoded over 6 years. At present, NorBOL primarily targets barcoding of museum collections, vascular plants of the north, coastal marine invertebrates, and projects supported by the Norwegian Taxonomy Initiative that focus on various little known groups.

TOWARDS A MOLECULAR REFERENCE DATABASE FOR THE IDENTIFICATION OF FORENSICALLY IMPORTANT *SARCOPHAGA* SPECIES (DIPTERA: SARCOPHAGIDAE)

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The identification of species of the forensically important genus *Sarcophaga* is very difficult and requires strong taxonomic expertise. In this study we sequenced the mitochondrial cytochrome *c* oxidase subunit I gene (COI) of 126 specimens of 56 W European *Sarcophaga* species and added GenBank data to our database to yield a total dataset of 270 COI sequences from 99 *Sarcophaga* species to evaluate the COI gene as a molecular diagnostic tool for species identification in this genus. Using two simple criteria (Best Match and Best Close Match) we showed that the identification success using a mini-barcode region of 127 bp was very low (80.7-82.5%) and the use of this region is not recommended as a species identifier. In contrast, identification success was very high using the standard barcode region (658 bp) or using the entire COI region (1535 bp) (98.2-99.3%). Yet, there was a low interspecific sequence divergence (< 2%) in six species groups so that for 16 out of the 99 species (nine of which are of forensic importance) the use of COI barcodes as species identifier should be done with care. For these species, additional markers will be necessary to achieve a 100% identification success. We further illustrate how such reference databases can improve local reference databases for forensic entomologists.

AFFIRMATION OF DNA BARCODING AS A POWERFUL TOOL IN CATALOGUING MEDICINAL AND AROMATIC PLANTS IN MEDITERRANEAN FORESTS (🏛 poster)

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From the pre-historic era, men have been using plants as medicine and in recent years, medicinal and aromatic plants (MAPs) harvested from the wild still remain of immense importance. MAPs are clearly an important global resource in terms of healthcare but they are also an important economic resource. Consequently, MAPs are an ideal case for developing DNA barcodes. So far, more than 77 individuals belonging to 38 plant species have been collected and analyzed derived most of them from 4 different Mediterranean regions through Italy and Greece (some representatives from Western Mediterranean were included for comparison) with a total number of 22 provenances. We used "core barcode" for land plants rbcL and matK, and tested trnH-psbA as additional marker. Each marker was amplified with the universal primer pairs. RbcL displayed 100% amplification success, followed by trnH-psbA 96%, while matK was unable to amplify some certain plant groups (overall amplification success of 81). Species discrimination success was attempted using sequence character states (Blastclust), presence of barcoding-gap (uncorrected p-distances), and species monophyly through inference of RAxML dendrogram. These approaches return similar results, providing discrimination species level of about 65% using all combined markers. The trnH-psbA region showed the highest values of genetic variability, however this divergence did not allow reliable alignment across all sequences. On the whole, conserving medicinal and aromatic plants biodiversity should be given a serious attention as well as the development of an integrated DNA database. Moreover the use of a fourth marker could be necessary to obtain adequate discriminatory power.

LAUNCHING SWISSBOL (THE SWISS BARCODE OF LIFE NETWORK)

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Biodiversity in Switzerland is estimated at 50,000-70,000 species, yet DNA sequence data exist for very few groups and little is known about the genetic diversity that exists both within and among most species. In order to address these issues, Swiss partners representing universities, natural history museums, and botanical gardens have united to form the Swiss Barcode of Life (SwissBOL) network, a network committed to the use of DNA barcoding for monitoring national biodiversity and enhancing conservation strategies. SwissBOL has secured three years of preliminary funding from the Swiss Federal Office for the Environment (FOEN). All SwissBOL projects will be integrated within the ECBOL and iBOL systems; moreover, SwissBOL will contribute to existing networks for sharing biodiversity information, such as the Global Biodiversity Information Facility (GBIF), and will seek collaboration with other national and international partners in the fields of biodiversity research and management.

DNA BARCODING OF FUNGI IN THE HYPOCREALES WITH CYLINDRICAL CONIDIA

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Fungi in the order *Hypocreales* are regarded as economically important and have been shown to have high species diversity based on multigene phylogenetic inference. Contemporary phylogenetic studies using the fungal barcoding region, the internal transcribed spacer of the nrDNA operon (ITS), have shown low levels of intra- and inter-specific variation leading to incorrect species identification for this group of fungi. Morphological identification of these fungi to species level is difficult and in some instances, impossible. For rapid and accurate identification of hyporealean fungi with cylindrical conidia, the ITS as well as the translation elongation factor 1-alpha (TEF-1α) gene region were investigated with TEF-1α as possible secondary DNA barcode regions for eight genera. Distance analysis of the ITS region resulted in the identification of the isolates to generic level. Similar analysis of the TEF-1α region, however, provided a more robust resolution of the various species in the eight genera studied. This was further supported by the intra- and inter-specific pairwise distances calculated for both gene regions. Therefore, the TEF-1α gene region is proposed as secondary DNA barcode marker for these fungi.

USE OF DNA BARCODE FOR IDENTIFICATION OF POSSIBLE BIOTYPES OF THE FRUIT BORER *Neoleucinodes elegantalis* (GUENÉE) (LEPIDOPTERA: CRAMBIDAE), AN IMPORTANT PEST OF ANDEAN SOLANACEOUS FRUITS

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In Colombia, Venezuela, Ecuador, Brazil and Honduras, the tomato borer, *Neoleucinodes elegantalis*, is the most important fruit-related plague of the solanaceae family. In this research study we tried to establish whether there was genetic variability in populations of *N. elegantalis* and determine the possible occurrence of different biotypes related to wild and cultivated plants of Solanaceae in Colombia and Ecuador. A total of 233 adult individuals were characterized using molecular techniques by comparing the sequence of the gene *Cytocrome c oxidase* I (COI), following the methodology of DNA Barcoding and integrating different bioinformatic approaches and geographic information systems (GIS). The analysis of the COI sequences showed a very good sensitivity, achieving an initial differentiation of ten possible biotypes, associated to specific life zones, but without apparent relation to the host type. Divergence between groups was set at 0.1% -1.9%, and the greatest were found in group 2, which was distributed all along the Western Cordillera, and was also apparently geographically isolated from the rest of the populations of *N. elegantalis*. This group was located in an area with very high antropogenic disturbance by agrochemicals; this could be acting as a driver of increased genetic distance between the groups through local extinctions i.e. distribution gaps. In order to confirm if these groups correspond to biotypes, biological tests of reproductive compatibility to verify the existence of reproductive isolation will be performed. Ethology will be assessed through the sexual pheromone from *N. elegantalis,* Neoelegantol®, in cultivated and wild environments to verify possible differences between the captured populations. Morphologic variability will be studied by means of geometrical morphometry of the wings.

LESSONS FROM A NEARLY COMPLETE BARCODE LIBRARY FOR EUROPEAN GRACILLARIIDAE LEAF-MINERS

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Moths in the family Gracillariidae constitute one of the primary groups of plant-mining microlepidoptera. While the majority of species are leaf miners, the family shows a diversity of other life-history strategies, such as fruit mining, stem mining, leaf rolling, boring, and galling. Many species are highly invasive and serious pests of agricultural and ornamental plants. Gracillariids include 1855 described species of which 257 have been recorded in Europe. Here we present barcode data for more than 2000 specimens for 230 species representing 89.5% of the European fauna. On average seven specimens were barcoded per species. Morphology and DNA barcodes were carefully studied and compared. Preliminary results show that barcoding is capable of unambiguously discriminating ~97% of the species investigated so far, including some of closely related species notoriously difficult to identify. We highlight some interesting cases of barcode similarity, barcode sharing, and deep intraspecific splits. DNA barcoding revealed divergent clusters within several described species when extending the study area to the whole continent of Europe calling attention for closer taxonomic scrutiny. Our comprehensive DNA barcode library for Gracillariidae leaf-mining micromoths will make identification more straightforward in particular larvae and light trapped specimens for which no host plant data is known. This research highlights the importance of international cooperation among European entomologists to complete the barcoding of all the Lepidoptera of Europe.

MICROARRAY FOR IDENTIFICATION OF THE CHIROPTERAN HOST SPECIES OF RABIES VIRUS IN CANADA

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Chiropteran (bat) specimens submitted for rabies diagnosis are typically identified via morphological traits. Phenotypic plasticity, inconclusive taxonomic keys, poor sample condition, and limited material are common factors that result in an inability to identify or mis-identification. In this study, a microarray assay with associated PCR of the mitochondrial COX I locus was developed for differentiation of 14 bat species submitted to the Canadian Food Inspection Agency for rabies diagnosis from 1985 - 2012. The assay was validated with a reference collection of DNA from 159 field samples, all of which had been barcoded previously. All 158 samples that gave good amplification were accurately identified by the microarray assay, including multiple specimens of each target species. One sample that appeared to be severely decomposed failed to amplify with the "universal" PCR primers developed in this study, but amplified weakly after switching to alternative primers and was accurately typed by the microarray. Thus, the chiropteran microarray was able to accurately differentiate between the 14 species of Canadian bats targeted in this study. This PCR and microarray assay would allow unequivocal identification to species of most, if not all, bat specimens diagnosed as rabid in Canada.

BACTERIAL BARCODING APPLIED TO REGULATED PLANT PATHOGENS

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The BOL programs study the existing diversity of living species, but not of prokaryotes. Moreover, several bacteria pathogenic to plants are not represented by species but pathovars which is an infra-species division that refers to a pathological specialization that is not necessary correlated with their taxonomic position. A barcode strategy is being developed in support of the European legislation on quarantine organisms on plants. Our strategy for barcode identification of the quarantine bacteria uses several gene sequences in a step wise approach and following different flows in a decision scheme. The subsequent challenges are to compose a relevant collection of the quarantine and related bacteria, to unravel their taxonomic and pathogenic identity, to generate sequences from a selected set of candidate DNA regions and select the barcode regions based on genetic variation, inclusiveness and exclusiveness for the selected scope of Q-bacteria. Barcode sequences have been defined for a group of quarantine bacteria of the combined EU/EPPO lists, comprising the three *Clavibacter michiganensis* subspecies, *Ralstonia solanacearum*, *Xylella fastiosa* and a subset of *Xanthomonas* pathogens.

USING DNA BARCODING TO DIFFERENTIATE INVASIVE *DREISSENA* SPECIES IN THE RIVER MEUSE (🏛 oral)

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River basins are conducive to invasions because they form corridors that facilitate rapid spread of introduced species. The Meuse River is particularly vulnerable to invasive species, firstly because the river has been highly altered by anthropogenic pressures and secondly it is connected to the major European rivers Rhine and Danube and to European main ports. Biofouling invaders not only have an impact on both aquatic ecosystems and biodiversity but also have negative influence industrial activities. The best-known example is the invasion of Western Europe and North America by the zebra mussel (*Dreissena polymorpha*, Pallas 1771), which is present in the Meuse River since 1822. In the meantime, a second dreissenid species, the quagga mussel (*Dreissena rostriformis bugensis*, Andrusov 1897) recently became invasive in both the Old and New World. The quagga mussel was first reported in the Meuse River in 2007. Both species are native from the Ponto-Caspian area.
Because of high phenotypic plasticity in the genus *Dreissena*, visual identification and shell length measurements are not always reliable to distinguish both invasive *Dreissena* species. Here, based on a complete sampling of the Meuse River (Hollandsch Diep, Dutch, Belgian and French section), we propose to use both a Principal Component Analysis on specific shell characteristics and the COI mitochondrial gene as a barcode to discriminate the two invasive *Dreissena* species. Both methods allow a clear distinction between the zebra and quagga mussels.

HUMAN POPULATION DENSITY CORRELATES WITH PHYLOGENETIC DIVERSITY AND PLANT VULNERABILITY IN SOUTHERN AFRICA

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Identifying the services natural ecosystems are providing to humanity is a primary step towards their sustainability. These services termed 'ecosystem services' are generally regarded as primarily linked to species diversity. Using a molecular phylogeny reconstructed based on plant DNA barcodes; we show that human population density in southern Africa correlates better with phylogenetic diversity than with species richness. This finding indicates that the evolutionary processes that create variation between living organisms might be providing key services – 'evosystem services' - for humanity. Of concern, however, is the observation that distribution of threatened species also strongly correlates with human population density, even after correcting for distribution of species richness. Distribution of humans in sub-Saharan Africa has therefore been shaped by the evolutionary history of its flora, but represents a significant threat to its evolutionary future. Our study links evolutionary processes, ecosystem function and human population density in Africa. We therefore suggest that conservation planning should shift considerably from preserving species diversity to preserving evolutionary history, if we are to ensure full benefits for future generations.

A CONTRIBUTION TO THE MOLECULAR PHYLOGENY OF EARTHWORM DIVERSITY OF EASTERN GHAT, INDIA

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An important aspect of biodiversity is the relative importance of species in the functioning of ecosystems; this is particularly so for the soil biota which regulates organic matter and nutrient dynamics in soil. Soil bioresources have been recognized as the foundation for sustainable livelihood and food security. The importance of earthworms cannot be ignored because they have enormous potentials to improve soil condition on a sustainable basis. Most naturalists claim that all fertile areas on our planet have, at one time or another, passed through the bodies of earthworms.

Earthworms are ancient and they have been on our planet for 600 million years. They have survived through five mass extinctions, and helped life to sustain on the earth and human civilization by ploughing and fertilizing the soil. As far back as 1881, Charles Darwin, the great biologist, observed '*It may be doubted whether there are many other animals which have played so important a part in the history of the world as have these lowly organised creatures and every inch of topsoil on the entire planet had surely passed through the belly of earthworms several times'*. Earthworms constitute a major component of the invertebrate biomass (>80%) in most terrestrial ecosystems of the world contributing considerably to soil physico-chemical properties and processes.

Approximately 3700 species of earthworms are known in the world (Reynolds, 1994) but the number of estimated species may be as high as 8000 (Fragoso et al, 1997). In India, 418 species and subspecies belonging to 69 genera have been described so far (Julka and Paliwal, 2005). This number is expected to rise to about 800 with extensive surveys of large unexplored areas of 'biodiversity hotspots' of the Western Ghats and Eastern Himalaya. High earthworm diversity in our country is primarily due to its geographical location with a wide latitudinal range (between 8.4˚N and 37.6˚N), complex topography, varied climate (ranging from temperate to arctic in the Himalaya to tropical in the peninsular India) and past geological history that is linked to ancient super continent of Gondwanaland from which it separated in the late Jurassic and drifted to collide with the Asian mainland in the Eocene.

The aim of the present study area was to update the existing information on earthworm biodiversity in the study area with particular reference to vermicomposting species. A field survey of earthworm in Eastern Ghat of India was conducted in June 2011. Survey covered 130,058 sq kilometer area including 20 districts & 180 blocks of eastern ghat. The methodology adopted for earthworms' collection was based on Julka (1988). Earthworm & soil samples for taxonomic studies were collected by digging & hand sorting method, collected worms were washed in fresh water & stored in test tube in the field. Ethyl alcohol was gradually added to the test tube containing earthworms and then transferred to dish containing solution of 5% formalin for fixation. This is kept for 6-8 hrs & later preserved in 10% formalin.

Earthworms were identified with the help of monograph & other available literature on the subject (Stephenson 1923, Gates 1972, Julka 1988) at Vermiculture Research Station & later confirmed by experts of the subject. Twenty one species of earthworm belonging to seven genera & six families have been identified. *Drawida* sp. was

recorded as most prominent (51.06%) followed by *Metaphire* sp.(33.94%) and *Lampito* sp. (22.77%) While, *Perionyx* sp. & *Octochaetona* sp. were least in relative density. Thirteen taxonomic characters were considered during the study and illustrated. The familial, generic diagnostic characters, geographical distribution with DNA barcode of the reported genera were mapped in study and its close relatives were examined by molecular methods. Full sequences were of COI gene aligned & analyzed by molecular phylogenic software.

FROM PATHOGENS' SCREENING TO BARCODING: A CONTINUOUS CHALLENGE FOR HUMAN AND ANIMAL HEALTH

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Emerging and re-emerging infectious diseases pose serious threats to public and animal health, challenging prediction and surveillance. Molecular typing is an integral part of the public and animal health toolboxes with major recent evolution linked to emerging technologies for typing human or animal pathogens. However, there is a huge diversity of pathogens including viruses, prokaryote organisms and eukaryote organisms, if we consider parasitic forms such as protozoan, fungi and helminths. A similar diversity is also observed at the genomic level, which led to develop specific methodological approaches for each major group and often within. A brief overview of the barcoding approaches will be given, which shows that this term is not commonly used in the medical and veterinary literature. Examples will show recent applications in the discovery of pathogenic but also commensally organisms that may account for more than half of the living things. A discussion will open questions on the need for implementing barcoding approaches of pathogens in "hostspot" of emergence and on the improvement of collaboration between fields of biodiversity sciences (ecology and evolution), epidemiology, public and animal heath.

USING DNA BARCODES FOR ASSESSING DIVERSITY OF DANCE FLIES (DIPTERA: EMPIDOIDEA) AND AS A BASIS FOR PHYLOGENETIC RESEARCH

Mortelmans, Jonas[1], Nagy, Zoltán T.[2], Sonet, Gontran[2], Grootaert, Patrick[1] (🏛 poster)

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Empidoidea is a species-rich dipteran group with over 10.000 described species and a worldwide distribution. They are diverse not only regarding species counts but also regarding their morphology, habitat exploitation, life history and interactions with humans. The superfamily as such is well defined based on molecular and morphological evidence, but internal relationships remain unclear. As a first step, DNA barcoding was conducted for many representatives of Empidoidea which will be used for facilitating species recognition based on molecular markers. DNA barcodes assisted to identify several current taxonomic problems and also uncovered the existence of cryptic taxa. The project was part of the "Barcodes for two-winged insects (BC2W)" initiative. So far, 325 DNA barcodes were generated: 236 sequences of 117 species of Hybotidae, 29 sequences of 17 species of Dolichopodidae, and 60 sequences for 32 species of Empididae. We assessed several debated species groups where classification is unsure at generic level (e.g. *Ariasella*, *Tachydromia luang* species group), specific level (e.g. *Platypalpus culiciformis*, *Platypalpus niger*) or where distinction between sister species is unclear (e.g. *Platypalpus kirtlingensis* and *P. pictitarsis*; *Platypalpus minutus* and *P. australominutus*). Furthermore, this selection of specimens and these data will serve as a basis for phylogenetic investigations on Empidoidea.

BARCODING FOR DETECTION OF MISLABELING IN COMMERCIAL SEAFOOD AND IMPLICATIONS FOR HUMAN HEALTH (🏛 poster)

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The Barcoding initiative has had numerous applications in the field of biology, management and conservation since its inception has had. Here we highlight how its application to commercial seafood may have also beneficial consequences from the perspective of human health. Employing the Barcoding tool for identifying the species contained in a wide variety of seafood we have found mislabeling in European and American countries, at different levels and with different economic implications. Some of those errors or misidentifications may have serious consequences for human health because they involve species with different allergenic properties; for example cod and Panga generically labeled as hake. We suggest using the Barcoding tool for routine authentication of commercial fish products, improving both consumer's safety and fish market reliability.

FIRST LARGE-SCALE DNA BARCODING ASSESSMENT OF REPTILES IN THE BIODIVERSITY HOTSPOT OF MADAGASCAR

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DNA barcoding of non-avian reptiles based on the cytochrome oxidase subunit I (COI) gene is still in a very early stage, mainly due to technical problems. Using a newly developed set of reptile-specific primers for COI we present the first comprehensive study targeting the entire reptile fauna of the fourth-largest island in the world, the biodiversity hotspot of Madagascar.
Representatives of the majority of Madagascan non-avian reptile species (including Squamata and Testudines) were sampled and successfully DNA barcoded. The new primer pair achieved a constantly high success rate (72.7–100%) for most squamates. More than 250 species of reptiles (out of the 393 described ones; representing around 64% of the known diversity of species) were barcoded. The average interspecific genetic distance within families ranged from a low of 13.4% in the Boidae to a high of 29.8% in the Gekkonidae. Using the average genetic divergence between sister species as a threshold, 41–48 new candidate (undescribed) species were identified. Simulations were used to evaluate the performance of DNA barcoding as a function of completeness of taxon sampling and fragment length. Compared with available multi-gene phylogenies, DNA barcoding correctly assigned most samples to species, genus and family with high confidence and the analysis of fewer taxa resulted in an increased number of well supported lineages. Shorter marker-lengths generally decreased the number of well supported nodes, but even mini-barcodes of 100 bp correctly assigned many samples to genus and family.
The new protocols might help to promote DNA barcoding of reptiles and the established library of reference DNA barcodes will facilitate the molecular identification of Madagascan reptiles. Our results might be useful to easily recognize undescribed diversity (i.e. novel taxa), to resolve taxonomic problems, and to monitor the international pet trade without specialized expert knowledge.

WHEN BARCODING GOES BAD: MITOCHONDRIAL BARCODES ARE DIAGNOSTIC OF SHARED REFUGIA BUT NOT SPECIES IN HYBRIDISING OAK GALLWASPS

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Mitochondrial DNA barcodes provide a simple taxonomic tool for systematic and ecological research, with particular benefit for poorly studied or species-rich taxa. Barcoding assumes genetic diversity follows species boundaries; however, many processes can disrupt species-level monophyly of single-locus barcodes leading to incorrect classifications. Spatial population structure, particularly when shared across related and potentially hybridising taxa, can invalidate barcoding approaches yet few empirical data exist to examine its impacts. We tested how shared refugial population structure across hybridising species impacts upon the utility of mitochondrial barcodes. We barcoded 518 individuals from four well-delimited gallwasp species co-distributed across three Western Palaearctic refugia; these gallwasp species are reliably distinguishable based on both adult morphology and the shape of the gall they induce on oaks. Bayesian and neighbour-joining analyses clustered mitochondrial barcodes into mixed-species clades corresponding to refugia. Within- and between-species divergence was similar, with multiple species clustering together at all levels of differentiation. Four nuclear genes were also sequenced from 4-11 individuals per refugial population of each species. Multi-locus analyses of these data supported established species, with no evidence of the refugial clustering across species seen in mitochondrial barcodes. These data clearly show that mismatch between species and mitochondrial histories can lead to the inability of single-locus barcoding to delimit species; mitochondrial introgression in shared refugia results in "spatial barcodes", while "nuclear barcodes" reveal true species boundaries. Many animal groups encompass radiations over similar temporal and spatial scales as examined here, so these findings may reflect a more general pattern. Hence robust molecular barcoding approaches must sample extensively across population structure to disentangle spatial variation from species-level variation. Multi-locus methods are also essential to accommodate inter-locus coalescent variation and provide power to resolve recently diverged species.

DNA BARCODING OF ANCIENT DNA REVEALS CLIMATE DRIVEN BIODIVERSITY CHANGES BY SHOWING A SURPRISINGLY HIGH FUNGAL DIVERSITY IN FOSSIL MAMMOTH DUNG

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Little is known yet about the species richness of fungal communities of the mammoth steppe, as fossil fungi are often difficult or even impossible to identify. Using DNA barcoding, the fungal diversity of the mammoth steppe was assessed to provide better knowledge about climate-driven changes of fungal communities and associated plants and animal.
Ancient DNA was extracted from fossil mammoth dung from two sample localities (Alaska and Siberia), dated to 18,560 ± 50 BP and 12,300 ± 70 BP respectively. Sequences were obtained from these permafrost-preserved samples using massive parallel Roche 454 pyrosequencing.
DNA barcoding analyses confirm the presence of taxa identified by morphology, and identify a high diversity of dung fungi. This indicates that mammoths had a wide food choice. Furthermore, genera of dung fungi found are similar to those associated with extant boreal moose and deer. The fact that mammoths ate dung also contributes to the diversity of retrieved fungal taxa. Phylogenetic analysis shows that several fungal species identified in the fossil dung are closely related to species known to occur on elephant and horse dung; not surprising since their relatives roamed the mammoth steppe. Changes observed in the diversity of dung fungi correspond with the environmental shift of the Pleistocene-Holocene transition (15,000-11,000 years ago) in two respects. Firstly, few matches were found between dung fungi identified and recorded nowadays in both localities. Secondly, dung fungi associated with large mammalian herbivores seem to have declined after the end of the Last Ice Age. Contrary to the large diversity in coprophilous fungi identified, no bryophilous fungi were found, even though bryophytes were part of the mammoth's diet. Since bryophilous fungi are currently frequently encountered it might be that Arctic fungal coprophilous species were replaced by bryophilous and other plant associated species after mammoths and other large megafauna went extinct.

DNA BARCODING DISCRIMINATES FRESHWATER FISHES FROM SOUTHEASTERN NIGERIA AND PROVIDES RIVER SYSTEM-LEVEL PHYLOGEOGRAPHIC RESOLUTION WITHIN SOME SPECIES

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Fishes are the main animal protein source for human beings and play a vital role in aquatic ecosystems and food webs. Fish identification can be challenging, especially in the tropics (due to high diversity), and this is particularly true for larval forms or fragmentary remains. DNA barcoding, which uses the 5′ region of the mitochondrial cytochrome c oxidase subunit I (cox1) as a target gene, is an efficient method for standardized species-level identification for biodiversity assessment and conservation, pending the establishment of reference sequence libraries. In this study, fishes were collected from three rivers in southeastern Nigeria, identified morphologically, and imaged digitally. DNA was extracted, PCR-amplified, and the standard barcode region was bidirectionally sequenced for 363 individuals belonging to 70 species in 38 genera. All specimen provenance data and associated sequence information were recorded in the barcode of life data systems (BOLD; www.barcodinglife.org). Analytical tools on BOLD were used to assess the performance of barcoding to identify species. Using neighbor-joining distance comparison, the average genetic distance was 60-fold higher between species than within species, as pairwise genetic distance estimates averaged 10.29% among congeners and only 0.17% among conspecifics. Despite low levels of divergence within species, we observed river system-specific haplotype partitioning within eight species (11.4% of all species). Our preliminary results suggest that DNA barcoding is very effective for species identification of Nigerian freshwater fishes.

DNA BARCODING REVEALS A STRIKING NUMBER OF MÄERL SPECIES IN THE OSPAR AREA

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Maërl is a collective term for unattached calcified red seaweeds that, under favorable conditions, can form extensive beds. In the Oslo-Paris Convention (OSPAR) area, maërl beds have been reported from the Atlantic coasts off Norway to the south of Portugal; they are particularly abundant in Brittany (NW France) and Galicia (NW Spain). Maërl beds are considered habitats of ecological and economical importance because they harbor high biodiversity and benefit commercial fisheries. They should therefore benefit from conservation initiatives. However, maërl is sensitive to various stressors (commercial dredging, eutrophication, siltation, smothering by alien species and aquaculture) and there is consistent evidence of its decline in the OSPAR area. Furthermore, the taxonomy of maërl is fraught with uncertainties due to both phenotypic plasticity and convergence which lead to a lack of well-defined diagnostic characters. For instance, a background report on maërl beds issued by OSPAR in 2010 listed 10 maerl-forming species; however, it warned that species status and limits were often uncertain. In this context, DNA barcoding seemed to be a fruitful alternative; we have therefore undertaken a DNA barcoding study of maërl from OSPAR (Svalbard to Algarve) and adjacent (Macaronesia) areas. COI-5P was sequenced for 136 samples and further analyzed with the software ABGD. Our results highlighted the effectiveness of DNA barcodes as an identification tool for cryptic maërl-forming species. Fifteen Operational Taxonomic Units (OTUs) were detected but only 4 could be assigned to known species: *Phymatoliton calcareum, Lithothamnion corallioides*, *Lithothamnion glaciale*, and *Mesophyllum sphaericum*. Seven OTUs concentrated most of the samples (major OTUs); the other 8 were scarce and occurred in only one or a few locations. Major OTUs displayed a latitudinal gradient and some species (*P. calcareum, L. corallioides*) might be less widespread than previously thought. Only 3 OTUs matched BINs in BOLD, indicating that there is still much work to be done in the barcoding of maërl-forming Corallinales.
Financial support: Xunta de Galicia (10MMA103003PR) and Spain's Ministerio de Economía y Competitividad (CTM2010-18787). ASSEMBLE (grant agreement no. 227799) supported sampling in Brittany.

HOW EFFECTIVE ARE DNA BARCODES IN THE IDENTIFICATION OF AFRICAN RAINFOREST TREES?

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DNA barcoding of rain forest trees could help non-specialists identify species and discover unknown species. However, DNA barcodes cannot always distinguish between closely related species and the size and completeness of barcode databases is also critical to their successful application. We test the performances of *rbcL*, *matK* and *trnH-psbA* plastid DNA markers to identify rain forest trees of Atlantic central Africa under the assumption that a database is exhaustive in terms of species content, but not necessarily in terms of haplotype diversity within species. We assess the accuracy of identification to species or genus using a genetic distance matrix between samples either based on a global multiple sequence alignment (GD) or on a basic local alignment search tool (BLAST). When a local database is available (within a 50 ha plot), barcoding was generally reliable for genus identification (95-100% success), but less for species identification (71-88%). Using a single marker, best results for species identification were obtained with *trnH-psbA*. There was a significant decrease of barcoding success in species-rich clades. When the local database was used to identify the genus of trees from another region and did include all genera from the query individuals but not all species, genus identification success decreased to 84-90%. The GD method performed best but a global multiple alignment is not applicable on *trnH-psbA*. In conclusion, barcoding is a useful tool to assign unidentified African rain forest trees to a genus, but identification to a species is less reliable, especially in species-rich clades, even using an exhaustive local database. Combining two markers improves the accuracy of species identification but it would only marginally improve genus identification.

THE GERMAN BARCODE OF LIFE PROJECT (GBOL): A NATIONAL LIBRARY OF BIODIVERSITY

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The GBOL (German Barcode of Life) project is a large-scale DNA barcoding campaign designed to capture the biodiversity of Germany. GBOL has been granted a funding of approximately 5 million Euros by the German Federal Ministry of Education and Research (BMBF) for an initial period of 3 years. Launching the GBOL project is a step towards collecting, processing, data sharing and deposition of samples in conventional and molecular collections in order to facilitate the compilation of an open, validated DNA barcode database for German eukaryotic species.
GBOL is a national consortium of natural history museums and other research institutions which will provide their professional taxonomic expertise and existing infrastructure (e.g. dry and wet collections, frozen tissue and DNA collections, databases, and laboratories) to establish a comprehensive library of biodiversity.

Involved institutions:
ZFMK, Zoologisches Forschungsmuseum Alexander Koenig, Bonn
ZSM, Zoologische Staatssammlung München
SMNS, Staatliches Museum für Naturkunde Stuttgart
SMNK, Staatliches Museum für Naturkunde Karlsruhe
Senckenberg am Meer, Abt. DZMB, AG Molekulare Taxonomie mariner Organismen, Wilhelmshaven (associated institute)
BGBM, Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin
Nees-Institut, Universität Bonn
AvHI, Universität Göttingen
IEB, Universität Münster
Universität Tübingen
BSM, Botanische Staatssammlung München (associated institute)
JKI, Julius Kühn-Institut, Braunschweig (associated institute)
SMNG, Senckenberg Museum für Naturkunde Görlitz
University Bielefeld

BARCODING IN THE GENUS *BACTRIS* (ARECACEAE) WITH TRNQ-RPS16 AND PSBA-TRNH PLASTID LOCI

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The genus *Bactris* comprises ca. 80 species with a Neotropical distribution from southern Mexico, through Central America and the Greater Antilles to Bolivia and the Atlantic forest of south-eastern Brazil. Several species, like *Bactris maraja*, *B. simplicifrons*, *B. hirta*. display considerable morphological variability, challenging morphological identification and taxonomic circonscription. Moreover, *Bactris* is a major component of palm communities in tropical America, in particular Amazonia, where up to 10-15 species can be found simpatrically. In this context, there is a great need of an accurate identification tool, usable at the seedling and juvenile stage or with sterile adults, in transect studies of Neotropical palm communities.
More than half of the genus (45 species) represented by 1-10 individuals was obtained for sequencing of the chloroplast intergenic spacer trnQ-rps16, and additional data were obtained for psbA-trnH.
The results with trnQ-rps16 indicate that species extremely variable morphologically like *Bactris maraja* are very homogeneous and highly distinct at the molecular level. The rare *B. nancibaensis* from French Guiana could moreover be distinguished from *B. maraja*, confirming its status as a distinct species. An outlying population of *Bactris bifida* with a very distinct leaf structure could be confirmed of belonging to this species. Preliminary data with psbA-trnH show that this locus is also highly informative for barcoding in *Bactris*. The combination of the two loci is likely to constitute an accurate barcoding system for this difficult Neotropical palm genus.

SPECIES IDENTIFICATION IN THE GENUS *PHOENIX* L. (ARECACEAE): A COMPARISON OF PERFORMANCES BETWEEN BARCODING WITH 3 PLASTID LOCI AND GENOTYPING WITH 20 SSR LOCI

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The genus *Phoenix* comprises 14 species with a Palaeotropical distribution except Oceania. *Phoenix* includes the date palm, *P. dactylifera*, a major fruit crop of dry subtropical areas, two species of high economic value in the horticultural industry (*P. canariensis* and *P. roebelenii*), and other species of ornamental, ecological and conservation importance. While the genus *Phoenix* is instantly recognizable, species identification based on morphology is challenging due to the paucity of differential characters, intraspecific polymorphism and interspecific hybridization. Barcoding with the plastid loci psbZ-trnfM, rpl16-rps3 and atpI-atpH was performed on 60 individuals representing 13 species. Barcode performance was evaluated with the Best Match and Best Close Match tests implemented in TaxonDNA. Genotyping with 18 nuclear di-/tri-nucleotidic microsatellites and 2 plastid 12-20 bp long minisatellites was performed on 700 individuals representing all 14 species. Species clusters were depicted using the Neighbour Joining method and the D_{SA} genetic distance computed with PowerMarker software. The barcoding approach allowed to identify unambiguously 8 species. Two morphologically and geographically distant species, *P. theophrasti* and *P. rupicola* were only partially separated and three allied species, *P. dactylifera*, *P. sylvestris* and *P. atlantica* could not be distinguished at all with the plastid markers. SSR data allowed to distinguish unambiguously 10 out of the 14 species. *Phoenix atlantica* was mixed with *P. dactylifera* and *P. acaulis* was mixed with *P. loureiroi*, questioning species delimitations. Once these taxonomic problems will be solved and the barcode system improved, the two methods are likely to reach similar accuracy for species identification. However, the genotyping method has the advantage of identifying hybrids. For example, the enigmatic 'Golkoy palm' from Turkey could be characterized as *P. dactylifera* × *P. theophrasti* with *P. dactylifera* chloroplast. Moreorver, the barcoding system has to be specifically designed, since standard markers like rbcL and matK are useless in *Phoenix*.

EXPLORING SPECIES DELIMITATION METHODS BASED ON DNA BARCODING IN THE LAND SNAIL GENUS *RUMINA* (GASTROPODA: PULMONATA)

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Species is a universally accepted term, but there is still no generally accepted species concept applicable to all organisms. Several methods have been proposed to delimit species, including DNA barcoding gap analysis that relies on the separation between putative intra- and interspecific sequence divergences (DNA barcoding gap). Various thresholds to separate intra- from interspecific divergences have been proposed to interpret barcoding results and to delimit species, however none of these thresholds is universally applicable.

The present study aims at comparing DNA barcoding gap analysis with other species delimitation methods in the facultatively selfing terrestrial pulmonate snail genus *Rumina.*

To this end, we first evaluated to what extent phenotypic differentiation in *Rumina* reflects evolutionary divergence that can be interpreted under a phylogenetic species concept by performing a phylogenetic analysis of nuclear (ITS1, ITS2) and mitochondrial DNA (COI, CytB, 12S rDNA, 16S rDNA) sequences. These results yielded *a priori* haplotype groupings, which then were subjected to several species delimitation methods using COI only: (1) classical DNA barcoding gap analysis, (2) the Automatic Barcode Gap Discovery, (3) the species delimitation plug-in of the Geneious software, (4) the Genealogical Sorting Index, and (5) the General Mixed Yule Coalescent model. The different methods were compared with respect to their ability to delimit putative species within this dataset.

DNA BARCODING OF AFRICAN BOLETES USING THE MITOCHONDRIAL GENES ATP6

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Boletes are a group of fungi widespread around the globe. However, the current conceptions of genera remain mainly based on temperate species. Applying those conceptions to tropical African boletes is often problematic and unsatisfactory. Phylogenetic analyses of DNA sequences are therefore needed. For a few years now, the mitochondrial gene ATP6 has been sequenced for all new bolete specimens entering the herbarium, as well as for the numerous older collections kept at BR. ATP6 was preferred over ITS as barcoding marker because of its ease of amplification, without contamination by non-bolete DNA, and because of the absence of multiple divergent copies within a single specimen, which are often present in the ITS of boletes. Those multiple copies make the sequences difficult to assemble when they are polymorphic for insertions/deletions. The obtained data allowed correcting misidentifications of herbarium specimens at the genus level. They also allow identifying putative species and grouping them in a more natural classification for further study. So far, several hundreds of specimens have been sequenced, among which over 180 African species were detected. Interestingly, only 155 bolete species have been reported from Africa, indicating that at least 25 species remain to be described. This is an underestimate because 1) for the most species-rich genera, i.e. *Tylopilus* and *Xerocomus*, the total number of species detected from Africa was close to 70, while only 34 species have been reported to date, and 2) we did not sequence all the species reported to date (for example, we detected 7 *Phylloporus* species, while 17 have been reported). Among the new African boletes, three new species were placed in *Chalciporus*. Until recently this genus was considered mainly temperate and unknown from tropical Africa. Thanks to the DNA analyses, the deviating morphology of two of these African *Chalciporus* helped to better understand the definition and distribution of this genus.

SMALLER IS BETTER (OR ALMOST AS GOOD): VALIDATING CO1 MINI-BARCODES FOR PRIMATE SPECIES IDENTIFICATION (🏛 poster)

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While the applicability of CO1 barcoding gains universal support there is a need for even shorter sequences for the identification of species. We tested the validity of using mini-barcodes in a taxonomically well-known Order, the Primates, based on sequences deposited in GenBank. Our dataset comprised 540 sequences of 87 nominal species (or about a quarter of currently recognised species), ranging from 1-102 sequences per species. A 648bp section of CO1 was selected so that each mini-barcode would contain full codons, and a series of mini-barcodes (between 108-324 bp) were generated from both the 3' and 5' ends. Neighbour-joining trees were compiled for each section and mini-barcode, and bootstrap values were used as an indication of the accuracy of species identification. The full length of the CO1 section provided unambiguous support (bootstrap values >99%) for 65 species (74.7%). Problem taxa, that did not allow unambiguous identification, were either those that frequently hybridise in nature (baboons, gibbons) or taxa that have seen recent taxonomic revisions (with the 'older' nomenclature still used in GenBank). Efficiency of mini-barcodes ranged from 89±10% (max 96%) for the 324 bp barcodes, 81±3% (max 84%) for the 162 bp barcodes, and 65±11% (max 82%) for the 108 bp barcodes. In general, the terminal end performed marginally better than the proximal end. The proportion of parsimony informative bp at the terminal end was around 46.2% whereas at the proximal end it was 49%. We conclude that mini-barcodes in the order of 160-300 bp can be effective in identifying primate species, especially when focussing on the terminal end. This study is one of a growing number that confirms the utility of mini-barcodes in circumstances where sequencing the entire gene is not feasible. With primates mini-barcodes are particularly applicable when dealing with historic specimens, degraded samples, or samples traded as bushmeat.

CLIMB TOGETHER: USING DNA BARCODES FOR THE PHYLOGENETIC ANALYSIS OF LIANA COMMUNITIES AND IDENTIFYING STEMS WITHOUT CANOPY ACCESS

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The integration of phylogenetic data in vegetation surveys allows understanding of how plant communities assemble and if they are shaped by environmental gradients or biotic interactions. This gives insight into processes like succession, e. g. in secondary forests, however phylogenetic studies in disturbed forests are still rare, especially regarding non tree components. Lianas are particularly abundant in disturbed tropical forests and influence forest dynamics. However, their identification is often problematic, due to limited access to leaves in the canopy. We inventoried liana communities along a disturbance gradient in a montane forest, SW China. 1-3 individuals of the occurring species (ca. 148 (morpho)-species) were barcoded with 3 chloroplast markers (rbcLa, trnH-psbA, matK) and one nuclear marker (ITS) using leaf tissue. All stems that had no accessible leaves and could not be identified by morphological features were barcoded using cambium tissue. Sequencing success for all leaf samples was 99% for rbcLa, 91% for trnh-psbA and 94% for matK. For the stem samples, sequences of the matK region were obtained for 90%. DNA barcode data will be used for calculating phylogenetic diversity and relatedness indices of species in the communities. We expect phylogenetic clustered patterns in highly disturbed areas, where environmental factors should shape the communities. Intermediated disturbance should offer a suitable habitat for many liana species, therefore biotic interaction should lead to phylogenetic over dispersed communities. Unlike trees, we expect that the distribution of liana taxa is clumped in closed canopy forests, where low light availability could act as a filter.

INSTITUTIONAL ADOPTION OF DNA BARCODING: CBOL'S NEW STRATEGIES

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The Consortium for the Barcode of Life (CBOL) has pursued several goals since its creation in 2004, most of which have been accomplished. The BARCODE data standard has been established and implemented through GenBank, EMBL and DDBJ and linkages to voucher specimens and trace files are required. Standard BARCODE regions have been approved for land plants and fungi, and a Protist Working Group is making progress toward this goal. There are active participants in barcoding projects around the world and the rate of publication is increasing rapidly. The remaining challenges are to put barcoding on a sustainable trajectory through long-term financial and administrative support by users. CBOL's Executive Committee recently established a new two-pronged strategy: to establish a sustainable management plan for the BARCODE data standard, and to gain institutional recognition and support for barcoding in several strategic areas. CBOL is now focusing on five areas of potential adoption by national and international organizations and the private sector. These target areas are: agricultural pests, endangered species, water quality indicators, medicinal plants, and food sources. A range of potential business models are being explored, including research grants, service contracts, licensing arrangements, and foundation support.

CRYPTIC PARASITES IN ONE FISH: THE MICROSCOPIC SPECIES OF THE *PSEUDORHABDOSYNOCHUS CUPATUS* COMPLEX (PLATYHELMINTHES, MONOGENEA, DIPLECTANIDAE) (🏛 oral)

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Parasites represent a substantial portion of global biodiversity. However, due to a lower taxonomic effort and the lack of easily recognised morphological features the level of hidden diversity may be very high. Monogeneans and especially the genus *Pseudorhabdosynochus* are probably one of the best candidates to observe the presence of cryptic species. With a body length (after flattening for microscopy study) of 300 to 700 µm, species description in *Pseudorhabdosynochus* is not effortless and species are distinguished by very small characters: copulatory organs (20-100 µm) and attachment organs. Within *Pseudorhabdosynochus*, the 'cupatus group' includes five morphologically close species from five fish species. Species delimitation within this complex is even more difficult than in other *Pseudorhabdosynochus* species: it can be done only by detailed study of the scale arrangement of the lamellosquamodisc (a special attachment organ), at the expense of long microscopic observations on perfectly flattened and stained collections specimens. An integrative approach was applied to first constitute primary species hypotheses (PSH) based on the COI diversity, then challenged by the analysis of phylogenetic trees obtained with three independent markers (COI, ITS1 and LSU) to define secondary species hypotheses (SSH). Molecular results underlined the presence of cryptic species. In particular, we identified two additional cupatus species in two fishes, where only two were described. However, because the whole body of the parasitic worms was used for DNA extractions, voucher specimens could not be kept and SSH could not be linked to the type specimen conserved in slide. The International Code of Zoological Nomenclature requires a diagnose for new species. DNA was thus considered as voucher and a DNA species description was proposed. In addition, several strategies were proposed to link molecular species hypothesis to describe species and resolve this nomenclatural impediment.

A REVISION OF WESTERN AUSTRALIAN *BENNELONGIA* (CRUSTACEA, OSTRACODA, CYPRIDIDAE) – THE COMPLEMENTARITY OF BARCODING AND MORPHOLOGICAL STUDIES

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Ostracods are small, bivalved crustaceans with the most extensive fossil record of extant arthropods and a variety of reproductive modes (Martens et al. 2008). Both features make them ideal model organisms for evolutionary research. Here, we report on our ongoing revision of the genus *Bennelongia* De Deckker & McKenzie, 1981; endemic to Australia and New Zealand. Originally, only two morphospecies of this genus were described from Western Australia but we meanwhile identified 9 new species (Martens et al. 2012). By applying DNA barcoding and the Evolutionary Genetic Species concept (Birky et al. 2010, Pons et al. 2006), we confirmed species status for six of these new species genetically. Subsequent, more detailed morphological studies based on new and micro characters, such as position and shape of the label on the right ostracod valve, supported the genetic species boundaries (Martens et al. 2012). Our study emphasizes the need of geneticist and taxonomists to work closely together to unravel cryptic diversities and develop adequate measures for the protection of our biodiversity.

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UCYPRIS VIRENS (OSTRACODA, CRUSTACEA) – AN EXCEPTIONALLY DIVERSE SPECIES COMPLEX THAT HAS INVADED WESTERN AUSTRALIA

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Bode et al. (2010) found that *Eucypris virens*, an ostracod with mixed reproduction, geographic parthenogenesis and Holarctic distribution, forms a species complex with more than 35 cryptic species, making it the crustacean morphospecies with the highest cryptic diversity yet reported. Here, we analysed COI and LSU DNA sequence data from Western Australian *E. virens* to test the hypotheses that (1) vicariant processes have led to the formation of Australian *E. virens* species or (2) that these ostracods have been introduced into Western Australia.
All our genetic analyses based on phylogenetic trees, parsimonious networks and estimates of genetic distances show clearly that Western Australian and European *E. virens* are either genetically identical or very closely related. Some haplotypes are identical, others only separated by one or two mutational steps. We could distinguish three phylogenetic clades among the Western Australian representatives of *E. virens.* Four European cryptic species were identified as ancestors for two of the Western Australian clades and another was found to be a close relative to the third Western Australian clade. We therefore conclude that *E. virens* has been introduced into Western Australia, most likely from Western Europe, and did not diverge there (Koenders et al. 2012). In Europe, *E. virens* shows a typical pattern of geographic parthenogenesis while we found only asexual populations in Western Australia.

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DNA BARCODING OF ORCHIDS SPECIES USED IN TRADITIONAL CHINESE MEDICINES (TCMS) REVEALS ILLEGAL TRADE IN CITES PROTECTED SPECIES, ADULTERATION AND FOOD FRAUD (🏛 oral)

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Many CITES-listed Dendrobiinae orchids are used in Traditional Chinese Medicines (TCMs) which makes them popular targets for illegal collecting in the wild. Identification of species processed in TCMs is of primary importance since concerns exist about legality and safety of TCMs. The aim of this research project was to identify species in 29 TCMs (dried pseudobulbs, tablets, capsules, flakes, patches and herbal teas) confiscated by the Dutch customs office at Schiphol airport. Both Sanger and Next Gen (Ion Torrent) sequencing were applied targeting nrITS. Nuclear ribosomal ITS sequences were obtained by Sanger sequencing and compared to data from various reference datasets. Additionally nrITS sequences of 10 TCMs were obtained by Next Gen (Ion Torrent) sequencing and compared to both the Sanger sequencing results as well as reference datasets. Presence of Dendrobiinae was confirmed in only 3 TCMs. Besides Dendrobiinae, DNA barcoding analysis retrieved 22 other plant species and 23 fungal species. Many of these plant species and a small portion of the fungal species are widely used in Chinese Medicine. Results show that concerns about legality and safety of TCMs are justified. First of all, many species detected were not mentioned on the labels violating (inter)national food fraud laws. Secondly, endangered, trade-restricted species of Dendrobiinae were discovered violating Convention on International Trade in Endangered Species (CITES) legislation. Thirdly, several fungal species potentially harmful to human health were detected. Screening of TCMs using either Sanger sequencing or Next Gen sequencing based barcoding seems an efficient method to monitor illegal trade in endangered species and potential threats to public safety.

MOLECULAR IDENTIFICATION OF THE FORENSICALLY IMPORTANT GREENBOTTLE FLY SPECIES *LUCILIA CAESAR* AND *L. ILLUSTRIS* (FAMILY CALLIPHORIDAE)

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Greenbottle flies (Calliphoridae: *Lucilia*) are among the first insects to colonise dead bodies. Based on their development times, species of this genus can therefore be useful for forensic entomologists to estimate the postmortem interval (PMI). Two close-related species *Lucilia caesar* and *L. illustris* are difficult to identify, not only based on morphology, but also based on DNA. Previous studies obtained mixed results with the mitochondrial cytochrome *c* oxidase subunit I (COI) marker and revealed that a few European specimens of *L. caesar* and *L. illustris* had identical haplotypes. Here, we sequenced the COI fragment of 58 new European specimens of *L. illustris* and *L. caesar* and obtained two other mitochondrial (cytochrome *c* oxidase subunit II and 16S) and two nuclear (internal transcribed spacer 2 and 28S ribosomal RNA) markers for a subset of these samples. For each marker, both species shared at least one haplotype/genotype and could therefore not be distinguished from each other. This conclusion was made possible by a more comprehensive sampling and by the use of several DNA fragments, including standard markers that allowed comparison with previous studies.

A GLOBAL GENETIC BACKBONE FOR THE FUNGI: THE CBS-BARCODING PROJECT

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The CBS collection currently hosts more than 90.000 strains of filamentous fungi and yeasts. This number is steadily increasing at the rate of close to 3000 new acquisitions per year. Identification, delimitation and classification of all 'new' fungal strains (or species) are routinely subjected to DNA barcoding. Considering the heritage of this world renowned collection to the scientific community and customers of CBS, DNA barcode data for 90.000 fungi will reflect a milestone in understanding fungal evolution and in quality assurance for furnished strains. Thus, the primary aim of this project is to generate two standardized DNA identifiers (ITS/partial 28S) of the entire fungal collection. In whatever way, scientifically or strategically, the generated 'barcodes' will tremendously improve our possibilities to provide guidance to future projects and innovative research at the CBS based on genetically selected fungal species of interest.

Since December 2009, significant progress was made towards achieving this challenging goal. With support of lab robotics, a fully automated pre-PCR sequencing pipeline was set up which currently produces up to 10.000 individual sequences per month (since early summer 2012). With these promising prospects, more than 80.000 produced Sanger reads and 35.000 DNA extracts (2/3 of the public collection) a global genetic backbone for the fungi appears to be achievable in the near future.

An outlook on major achievements of the project will be presented, such as barcodes for all CBS yeast and fungal ex-type strains, several smaller side projects and progress towards novel, easy PCR amplifiable fungal barcodes.

FREQUENCY MATRIX APPROACH DEMONSTRATES HIGH SEQUENCE QUALITY IN AVIAN BARCODES AND HIGHLIGHTS CRYPTIC PSEUDOGENES

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The accuracy of DNA barcode databases is critical for research and practical applications. Here we apply a frequency matrix to assess sequencing errors in a very large set of avian BARCODEs. Using 11,000 sequences from 2,700 bird species, we show most avian cytochrome *c* oxidase I (COI) nucleotide and amino acid sequences vary within a narrow range. Except for third codon positions, nearly all (96%) sites were highly conserved or limited to two nucleotides or two amino acids. A large number of positions had very low frequency variants present in single individuals of a species; these were strongly concentrated at the ends of the barcode segment, consistent with sequencing error. In addition, a small fraction (0.1%) of BARCODEs had multiple very low frequency variants shared among individuals of a species; these were found to represent overlooked cryptic pseudogenes lacking stop codons. The calculated upper limit of sequencing error was 8×10^{-5} errors/nucleotide, which was relatively high for direct Sanger sequencing of amplified DNA, but unlikely to compromise species identification. To our knowledge, these are the first estimates of sequencing error rate and pseudogene prevalence in a large BARCODE dataset created by multiple researchers. Our results confirm the high quality of the avian BARCODE database and demonstrate significant quality improvement in avian COI records deposited in GenBank over the past decade. This approach has potential application for genetic database quality control, discovery of cryptic pseudogenes, and studies of low-level genetic variation.

BARCODING MARINE BENTHIC INVERTEBRATES: PRELIMINARY RESULTS (🏛 oral)

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The marine invertebrates barcoding effort at the MNHN has resulted in several fruitful projects. We have barcoded an unprecedented diversity of Antarctic crinoids that has revealed likely cryptic species throughout many different taxa. For example, seven sympatric COI lineages can be distinguished within the broadcaster *Promachocrinus kerguelensis,* and two cryptic species are suspected. Within brooders like *Notocrinus virilis* or *Isometra graminea*, a strong geographic segregation of COI haplogroups suggests a lack of gene flow between populations, and is also indicative of cryptic speciation. Many Antarctic and sub-Antarctic starfish species have also been barcoded, revealing phylogeographic patterns as well as the discovery of many cryptic taxa.

Morphological variation often creates problems in attributing specimens to an established taxon. Our barcoding initiative has clarified taxonomic boundaries for many problematic taxa from the Atlantic, Southern Ocean and Indo-west Pacific regions. Taxonomic improvements aided by our work has helped to establish the first comprehensive crinoid phylogeny using 4 genes and 200 species spanning 90% of known crinoid families.

The barcode initiative at the MNHN was also used to explore the megaepibenthic diversity in a single region, the Bay of Biscay. Results show that the crinoid *Antedon bifida* described from the upper slopes of the Bay of Biscay should in fact be attributed to *A. petasus*, a northern relative of *A. bifida*.

ALTERNATIVE MARKERS FOR THE BARCODING OF FLATWORMS

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Some taxonomic groups are less amenable to mitochondrial DNA barcoding than others. Due to paucity of molecular information of understudied groups and the huge molecular diversity within these evolutionarily old lineages, primer design has been hampered. In case of flatworms, all attempts to develop flatworm-specific cox1 markers have failed so far. Therefore, molecular identification methods had to rely on universal markers. In the case of Monogenea (a very diverse group of platyhelminth parasites, mostly infecting fishes) and Rhabdocoela (representing one-fourth of all free-living flatworm taxa) this has led to a database of nuclear ITS and 18S/28S rRNA sequences. These regions provide ideal markers for taxonomic purposes as i) universal markers are available, ii) amplification success is high due to its multicopy structure, and iii) interspecific variation is high and intraspecific variation is low due to concerted evolution operating in these cassette regions. We therefore support the use of these nuclear markers as an alternative for barcoding in these taxonomic groups.

THE RELIABILITY OF DNA BARCODING IN CONGOLESE FISH THROUGH THE COMPARISON OF TWO INDEPENDENTLY GENERATED DATA SETS

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The biggest challenge for the FISH-BOL project in Africa is to collect, curate and correctly capture information in databases, and deliver vouchers and samples to sequencing facilities. Especially in Africa, geographically representative collection of species remains problematic due to inaccessibility of field sites, a lack of local capacity and logistical support. Of particular concern is the situation in the Congo Basin with one of the highest freshwater fish diversity and levels of endemism in Africa.
In this context, the University of Kisangani, the Royal Belgian Institute of Natural Sciences and the Royal Museum for Central Africa have started a sampling programme that uses DNA barcodes to inventory the fish biodiversity of the Congo River, its tributaries and neighbouring watersheds. The DNA barcodes used for species identification are obtained for as many species as possible through a joint effort. They constitute a composite reference library, which needs to be validated by taxonomists to ensure unambiguous species identification. In theory two types of incompatibilities could impair the reliable identification of fish species with barcodes: (1) (nearly) identical sequences are linked to different species, (2) the same species names are assigned to significantly different COI sequences. Interestingly, the results of an independent survey in the same region allowed us to compare both DNA barcode databases, and the taxonomic assignments made by independent taxonomic teams for taxa present in both studies. It also allowed us to evaluate to what extent essentially comparable species surveys in the Congo Basin result in similar species lists. To ensure that DNA barcoding can be used as cost-efficient complement to traditional taxonomy for the inventory of the Congo Basin fish fauna, it will be required to carefully check species assignments of independently compiled DNA barcode data.

MAKING THE BEST OUT OF INCOMPLETE DNA BARCODE LIBRARIES: SIMPLE *AD HOC* THRESHOLDS TO REDUCE IDENTIFICATION ERRORS

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One of the main constraints of DNA barcoding is the difficulty to assemble reference libraries that comprehensively represent the taxonomic diversity of the group to be identified. Here a general working strategy is proposed to deal with incomplete reference libraries of DNA barcodes. Starting from the observation that (1) queries with a large genetic distance with their best DNA barcode match are more likely to be misidentified, and (2) imposing a distance threshold profitably reduces identification errors, we modeled relationships between identification performances and distance thresholds in four DNA barcode libraries of Diptera (4270 DNA barcodes), Lepidoptera (7577 DNA barcodes), Hymenoptera (2067 DNA barcodes) and Tephritidae (602 DNA barcodes). In all cases, more restrictive distance thresholds produced a gradual increase in the proportion of true negatives, a gradual decrease of false positives and more abrupt variations in the proportions of true positives and false negatives. More restrictive distance thresholds improved precision, yet negatively affected accuracy due to the higher proportions of queries discarded (*viz*. having a distance query-best match above the threshold). Using a simple linear regression an *ad hoc* distance threshold producing an estimated relative identification error <0.05 was calculated for the tephritid library (relative ID error = n. of false positives /n. of not discarded queries). As expected, when this threshold was used for the identification of 188 independently collected tephritid specimens, less than 5% of queries with a distance query-best match below the threshold were misidentified. *Ad hoc* thresholds can be easily calculated for each particular reference library of DNA barcodes and should be used as cut-off mark defining whether we can proceed identifying the query with a known estimated error probability (*e.g.* 5%) or whether we should discard the query and consider alternative/ complementary identification methods.

SCHISTOSOME MITOCHONDRIAL GENOMES: PHYLOGENY AND DIAGNOSTICS

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Schistosomiasis (also known as Bilharzia) is one of the important 'neglected diseases' according to the World Health Organization, affecting over 200 million people worldwide. It is caused by digenean flatworms belonging to the genus *Schistosoma*. Complete or nearly complete mitochondrial genomes are obtained for *Schistosoma mansoni*, *S. haematobium*, *S. spindale*, *S. guineensis*, *S. incognitum, S. japonicum*, *S. mekongi*, *S. malayensis*, *Orientobilharzia turkestanicum* and *Trichobilharzia regentii.* An independently derived phylogeny of the schistosomatids reveals the patterns of evolutionary change within the mt genomes. Non-Asian *Schistosoma* species are characterized by a unique gene order revealing a split within the *Schistosoma*. Our data show that this gene rearrangement evolved with the speciation of *S. incognitum,* enabling us to clarify different theories for the origin and subsequent radiation and dispersal of the schistosomes providing a better understanding of the evolution, distribution and possible future spread of schistosomes. Complete mitochondrial genomes also allow assessment of the phylogenetic utility of mitochondrial genes, individually and combined, over a broad evolutionary time-scale. Additionally, comparison between genomes indicates regions of high and low sequence variability, thus suggesting genes and gene regions best suited as molecular markers for a variety of purposes such as barcoding and diagnostics (species, strain and life cycle stage identification) and phylogeographic analysis.

BENEFICIAL EFFECTS OF LARGE MAMMAL HERBIVORES ON PHYLOGENETIC STRUCTURE OF PLANT COMMUNITIES

Yessoufou, Kowiyou[1], Davies, T. Jonathan[2], Maurin, Olivier[1], Kuzmina, Maria[3], Schaefer, Hanno[4], van der Bank, Michelle[1] and Savolainen, Vincent[5,6]

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There has been much debate on the impact of large herbivores on biodiversity, especially given that these mammals are becoming locally extinct. The use of evolutionary information on community structure has typically been limited to evaluating assembly processes, for example, competition or habitat filtering, whereas a lack of long-term experiments has precluded the test of predictions considering more complex biotic interactions. Reconstructing the complete phylogenetic tree of the trees and shrubs of the Kruger National Park from DNA barcode data, we compared the structure of communities under various pressures of herbivory, using experimental plots spanning several decades. We show that exclusion of large herbivores results in impoverished species diversity in restructured communities. Surprisingly, we also show that reduction in species diversity coupled with community reorganisation does not necessarily result in a decrease in phylogenetic diversity. Extinction of large mammal herbivores will have cascading effects on plant diversity; however, impacts on plant community structure are contingent on initial conditions. This research has implications for best practice in managing large herbivores and natural habitats in the face of global change.

TESTING DISCRIMINATORY POWER OF DNA BARCODES WITHIN *ENCEPHALARTOS*, A HIGHLY THREATENED GENUS ENDEMIC TO AFRICA

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Extant cycads are living fossils and members of naked-seed plants that diverged 12 millions years ago. The morphological stasis and recent diversification with remarkably short terminal branches on the phylogeny coupled with the horticultural appeal suggest that cycad genera might be over-split. Here we focus on *Encephalartos*, a genus entirely endemic to the African continent with 65 currently defined species. Conducting a thorough taxon sampling, and testing the discriminatory power of different DNA regions, we showed that *rbcL* + *matK* + ITS provided the highest barcode potential for the genus. However the discriminatory power is less than 50%. We subsequently tested the discriminatory power on a new dataset where the 64 species included in this study was reclassified using both geographical and morphological affinities. We found the discriminatory power increases significantly to 75%. We therefore suggest that a classification system based on geography and morphology should be adopted for African cycads if we are to better control the ongoing illegal pressure and preserve the recent evolutionary diversity within the relics of extinct cycads on the African continent.

DNA BARCODING AS A MEANS OF IDENTIFICATION OF FLORA OF BOTANIC GARDEN GC UNIVERSITY LAHORE, PAKISTAN
(🏛 oral)

Zaib-un-nisa and Zaheer-ud-din

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One hundred plant species of GCU Botanic Garden were analysed for their DNA Barcoding using rbcl gene marker by automated DNA Extraction on Beckman/ Coulter Biomek FX (U.Guelph) for the purpose of reconfirming the identification of these plant species at the molecular level and to build up their DNA library. The plant species analysed for their DNA Barcoding were already identified on the basis of their morphological characters described in Flora of Pakistan. The identification was reaffirmed on the basis of the DNA sequences. Moreover, the phylogenetic relationship between the different taxonomic taxa of these plant species was worked out and in most of the cases, the phylogenetic relationship derived on the basis of classical or morphological characters agreed with the results obtained in the present study, i.e. DNA Barcoding, except in few cases, where it was suggested that their DNA Barcoding may either be repeated or should be carried out using other available genes in DNA Barcoding such as mat. K and ITS region of all the plant samples, before making any conclusion.

DNA-BARCODING IN FORENSIC ENTOMOLOGY

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DNA-Barcoding has become an important tool in Forensic Entomology, which is used to determine a post mortem interval in criminal casework. Forensic Entomology is used when the classical parameters of PMI estimation like the occurrence of livores or rigor mortis have become unusable, which takes part approximately 48 h after death. Necrophagous flies colonize a corpse immediately after death, they feed on it and deposit eggs. Therefore the PMI of a certain corpse cannot be longer than the lifetime of these colonizing flies, the estimation of the lifetime points at the minimal post mortem interval.
The lifetime can be estimated by measuring the length of the hatched maggots and comparison of this value with the data of standard development curves. Because the development time of various fly species may differ, even between species of the same genus, the relation of maggot length to the maggots age is dependent on the particular species, a correct species determination is crucial. But species determination on a morphological base is often difficult if not impossible in on the larval stage because differences in morphological characters are very tiny and hard to recognize or are lacking in the larval stage. Rearing to the adult stage may overcome this, but cannot help in every scenario. E.g. for the genus Sarcophaga determination of adults is very difficult, experts on this field are very rare. In addition their determination is restricted to males, because the character used is the phallus. DNA-barcoding can overcome problems on morphological characterization and is used on a regular basis in forensic entomological expertises.
My lecture will demonstrate the powerful use of this technique but will also point on difficulties in analyzing and interpretation the flies genome, especially when closely related species have to be resolved.

BIODIVERSITY ANALYSIS THROUGH HIGH-THROUGHPUT SEQUENCING WITHOUT REFERENCE BARCODE LIBRARY

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Classic DNA barcoding protocols have been optimized for individual processing of biological samples. Next Generation Sequencing (NGS) technologies are considered as a potential solution to overcoming hurdles in data collection with accurate analysis of environmental samples of mixed taxa. Among current NGS platforms, Illumina sequencers have the potential to provide an efficient solution to analyzing environmental samples at much lower cost per sequence read.

In this study, we first sequenced mixed amplicons for the standard barcode region obtained from insect samples collected by Malaise traps. Additionally, we developed a PCR-independent protocol to overcome taxonomic bias introduced by PCR amplifications. Our results show that the improved sequence length and the large sequencing capacity currently available on Illumina NGS has ensured its application to environmental biodiversity analysis, with several technical advantages: higher sensitivity for taxa with low abundance, higher recovery ratio for true positives, lower running cost per sample, and the capability to eliminate PCR bias. Additionally, our study shows that a barcode reference library will improve taxonomic recovery rate of the NGS protocols and reduce the overall laboratory and computational costs. However, when such reference libraries are unavailable, biodiversity analysis through NGS is still feasible when using mOTU as a close proxy of the morphological species concept.

In summary, the application of Illumina platforms in bioassessment is promising, yet modifications of protocols in collection, preservation, and molecular lab work seem critical for this implementation.

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Working on environmental samples is always challenging. Sometimes scientists have to dive deep into the sea several hundred meters down in search for an archeobacteria, or dig up tons of soils for acquisition of mere several nanograms of DNA. What is worse, DNA samples extracted from such origin are usually not intact, lack of integrity, and contains impurities which give great difficulty in subsequent analysis. So, for an ecology institution like NIOO in Wageningen a DNA sequencing is always an extreme challenge, and a stable, reliable and reproducible, but at the same time, affordable sequencing analysis is a mandatory.

Macrogen has been working together with the scientists in NIOO over many years and provided DNA sequence data of highest quality and reliability. Macrogen's technology and knowhow accumulated over decades of years could provide such high quality data, and that's what Macrogen was their choice over many years.

Macrogen Europe
The DNA sequencing specialists

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