DNA barcoding poorly documented Afrotropical vertebrate faunas

Prospects for Conservation and One Health

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What are poorly documented faunas?

- Species descriptions based on few specimens with little or no information on species distributions
- Large sampling gaps - difficulties in identifications and incomplete inventories of the species
- Often supposed high intra-specific morphological variation (many taxa are probably polyspecific)
- The designation of so-called species complexes
What are poorly documented faunas?

5) *Mus (Acomys) spinosissimus* nova spec.

* M. ferrugineofuscus; labiis, gastraeo; artuum latere interno, manibus pedibusque sordide cinereis; auriculis rotundatis, nigris, pilis brevis ferrugineis vestitis; plantis caudaque nigris; pilis singulis unicoloribus aculeatis, in capite, dorso latere, humero, femoris crurisque parte externa latioribus.

Latitudo ab apice rostri ad caudae basin 0,100; caudae 085.

Habitatio: Africa orientalis interior, Tette, Buio, a 16° ad 17° Lat. Austr.

What are poorly documented faunas?

- The taxonomic basis of many described species often insufficiently known for DNA barcode approach with reliably identified voucher specimens.
- Addressing this problem requires the development of reliable reference DNA barcode libraries by examining samples from species and species complexes from all regions where they might occur, ....
The world distribution of taxonomists according to ETI's World Taxonomist Database (November 2005)
(taken from: http://www.gti-kontaktstelle.de/taxonomy_E.html)
About African reference collections

Mostly stored and studied in Natural History Museums outside the country/continent of origin

For D.R. Congo: RMCA, RBINSc, Botanical Garden Meise

Collections mostly from National parks

No easy access for Congolese taxonomists
Afrotropical mammals <> BOLD
Unexpected examples?

- African freshwater fishes
  > 3000 described freshwater fish species (Skelton & Swartz, 2011)
- African (small) mammals
  > 1,100 described species (Musser & Carleton 2015)
DNA barcoding of South Africa’s ornamental freshwater fish – are the names reliable?

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185 traded ornamental fish taxa

Genetic identification matches the scientific name

- **43.8% (BOLD)**
- **48% (GenBank)**

No genetic ID could be assigned

- **34.3% using BOLD**
- **27% using GenBank**

Further development of publicly available databases required
Morphometry and DNA barcoding reveal cryptic diversity in the genus Enteromius (Cypriniformes: Cyprinidae) from the Congo basin, Africa

Marjolein VAN GINNEKEN¹,², Eva DECRU²,³, Erik VERHEYEN¹ & Jos SNOEKS⁴

Enteromius Cope, 1867, a problematic African fish genus

Taxonomic diversity E. miolepis/eutaenia species complex over a larger geographic scale within the Congo drainage system

A. E. cf. miolepis; B. E. cf. pellegrini; C. E. cf. brazzai; D. E. cf. atromaculatus
COI results

4 ‘à priori’ morphospecies = 23 mitochondrial COI lineages

The species richness of Enteromius in the Congo basin is severely underestimated?
DNA barcodes facilitate discovery of species

4 ‘à priori’ morphospecies = 23 mitochondrial COI lineages

A. E. cf. miolepis; B. E. cf. pellegrini
C. E. cf. brazzai; D. E. cf. atromaculatus

The species richness of *Enteromius* is severely underestimated

There are other species complexes & not whole Congo basin was studied!
Grammomys Thomas, 1915

Climbing nocturnal murines that occur in African lowland forests, scrub forests, mountain forests and savannahs.

Overall similarity with *Thamnomys, Thallomys & Oenomys* (formerly considered a subgenus of *Thamnomys*).

37 type specimens

‘Only’ 12 species (Musser and Carleton 2004)
Sampling $><$ type localities

Origin of 351 samples plotted against type specimens of 13 recognized species
DNA based OTU's

Every group contains strongly supported sublineages suggesting the existence of undescribed species.
Complex geographic distribution ranges
Results suggest a potential doubling of number of species for this genus in Tanzania alone!
DNA barcodes facilitate species discovery, but

1. Species diversity once more underestimated!
2. Many species with restricted distribution ranges
3. Sequencing of museum types would not have revealed the real species diversity
4. Challenge to describe these putative taxa in a way that allows comparison with types (e.g. craniometry)
Capacity building for (fish) taxonomists

Mbisa project

1. Université de Kisangani (RDC)
2. Institut Supérieur Pédagogique, Mbanza-Ngungu (RDC)
3. Université de Lubumbashi, (RDC)
4. Centre de Recherche en Hydrobiologie, Uvira (RDC)
5. Université de Burundi, Bujumbura (Burundi)
6. Université Marien Ngouabi, (Congo-Brazza)

Preliminary freshwater fish species data for ecoregions:
(a) species richness, (b) number of endemic species, (c) percentage endemism, and (d) species per ecoregion area  incl. DNA barcoding in RBINS
Centre de surveillance in Kisangani (CSB)

Inaugurated June 2014

Missions

1. facilitate and attract projects on biodiversity in the Congo Basin;
2. expand partnerships that will benefit CBS and DR Congo;
3. capitalize gains resulting from these activities: collections, joint publications
Centre de surveillance in Kisangani (CSB)

Surface: 2300 m²
27 scientists & 5 technicians

Ground floor: 6 collection rooms, meeting room, 4 offices, 1 storage room & 8 toilets.

1. 1st floor: library, 8 labs, 5 offices, meeting room & 4 toilets.

2. 2nd floor: 5 offices, server room (airco).

3. A zoological museum
Growing taxonomic expertise:

Mammals (bushmeat)  
birds, amphibians, reptiles,  
birds, fishes, flowering plants,  
algae, edible fungi
Mammal samples & One Health

Because each sampling is unique:

**Original samples** (allozyme work)
- blood (filter paper), heart, liver & kidneys

Recently also
- urogenital swaps, tongue, lung tissue, ...

In view of importance of small mammals in spreading zoonotic diseases

Sample with reliable taxon ID in known phylogeographic context extremely useful for studies on transmission and evolution of pathogens
Sampling efforts in zone where zoonotic diseases occur
Jean Jacque Muyembe (INRB)
Steve Ahuka (INRB)
Placide Mbala (INRB – UM)
Daniel Mukadi (INRB)
Franck Edidi (INRB)
Servet Kimbongdja (INRB)
Guy Midingi (INRB)
Simon-Pierre Ndimbo (INRB)
Jimmy Kapesthi (INRB)
Fabian Leendertz (RKI)
Ariane Düx (RKI)
Herwig Leirs (UA)
Sophie Gryseels (UA, UL, UA)
Erik Verheyen (RBINS)
Dudu Akaihe (CSB - UNKIS)
Guy Crispin Gembu (CSB - UNKIS)
Nicaise Amundala (CSB - UNKIS)
Jean-Claude Mukinzi (CSB - UNKIS)
Pascal Baelo (CSB - UNKIS)
Steve Ngoy (CSB - UNKIS)
Claude Mande (CSB - UNKIS)

Patrick Mwombo (CSB - UNKIS)
Casimir Nebesse (CSB - UNKIS)
Prescott Musaba (CSB - UNKIS)
Corneille Kahandi (CSB - UNKIS)
André Malekani Bendeke (CSB - UNKIS)
Jacques Tanzito (CSB - UNKIS)
Roger Angoyo (CSB - UNKIS)
Elie Bugentho (CSB - UNKIS)

Innocent Akonda (Ministere provinciale de santé)
Anne Laudisoi (Ecohealth Alliance)
Birgit De Smet (ITM)
Kevin Ariën (ITM)
Vincent Munster (NIH)
Robert Fischer (NIH)
Stephanie Seifert (NIH)
Trenton Bushmaker (NIH)
Martine Peeters (UM, IRD, INSERM))
Innocent Ndong Bass (IRD/CREMER)
Joseph Moudimba (IRD/CREMER)
Eitel Mpoudi Ngole (IRD/CREMER)
Eric Delaporte (UM/IRD/INSERM)
Sampling efforts in zone where zoonotic diseases occur.
Sampling efforts in zone where zoonotic diseases occur
Implications for safety during sampling procedures

Additional complication of fieldwork
1. logistically (extra equipment)
2. extra time spent during work,
3. training for all participants
Implications for safety during sampling procedures

Specific field lab area dedicated outside village

Dedicated clean and dirty sides

PPE included tyvek coveralls, gowns, double gloves and PAPR

Equipment decontamination using VIRKON and waste was burned
Sampling and the Nagoya Protocol on Access and Benefit Sharing

**Nagoya Protocol** on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity: CBD COP X, Nagoya, Japan, 2010

**50 ratifications**: July 2014; presently 94 Parties (incl. EU and Belgium)  

Many probably surmountable consequences for DNA based work on biological collections ...

However, there is **at least one unanticipated consequence**:

Financial constraints of collection management in Natural History Museums may lead management to refuse accepting/ making new collections, as these will no longer become their property after registering ....
Take home messages

In regions with poorly documented faunas

- DNA barcoding is not primarily a technological challenge

More important

- Lack of taxonomic expertise
- Adequate sample/specimen collections despite of logistic issues

Proposed remedies

- International collaborations with emphasis on local scientific teams
- The development of African taxonomic expertise
- Partnerships to strengthen local research institutes

DNA barcode species identification/discovery has potential value for

OneHealth (and conservation)

Be prepared to address Nagoya Protocol based regulations concerning export and study of biological material from abroad
THANK YOU FOR YOUR ATTENTION
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