Demonstrating the Value of Natural History Collections:
Systematics of *Crocidura* Shrews with Voucher Specimens

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Objective

- Sequence voucher specimens to clarify phylogenetic relationships and identify cryptic species among the diverse shrew genus *Crocidura*, specifically among western Indochinese taxa.

Introduction

- *Crocidura* is a genus of shrews found throughout Africa and Eurasia. Encompassing over 200 species, it is the most species-rich mammalian genus[1].
- Many of these species were differentiated using phenotypic characters, which is problematic because of the highly conserved morphology of the group[1]. Therefore, the validity of many species is uncertain.
- Many *Crocidura* shrews inhabit remote, largely inaccessible habitats that are prone to political conflict, making observation and collection of species of interest extremely difficult[1].
- This study aimed to extract DNA from USNM voucher specimens to reconstruct phylogenetic relationships within the genus and potentially reveal cryptic species.
- Particular attention was given to samples acquired during a 2003/04 field expedition to Myanmar assigned to *C. vorax* (USNM 583806, 583807, 583791, 584374) and *C. fuliginosa* (USNM 583814); cytochrome b sequencing was used to place them in a phylogenetic context.

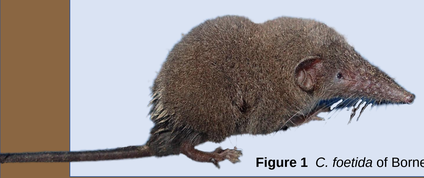

Figure 1 *C. foetida* of Borneo

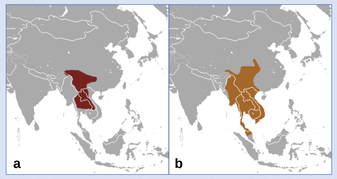

Figure 2 Range maps of *C. vorax* (a) and *C. fuliginosa* (b)*

Methods

- 52 specimens across 21 morphologically diagnosed species were sampled in the NMNH Biorepository, including four unidentified *Crocidura* shrews.
- Samples were digested with proteinase K.
- Performed DNA extraction and purification using MagBinding Beads.
- Full cytochrome *b* gene (high-quality DNA) and 255-base-pair fragments (low-quality / fragmented DNA) were amplified by PCR using a BioRad T100 Thermocycler.
- Performed Sanger sequencing using an ABI 3130 Capillary sequencer.
- Sequence quality filtered / sequences aligned in Geneious Prime using MAFFT.
- Model selection and phylogenetic inference (through a maximum likelihood approach) were conducted simultaneously with ModelFinder and IQ-TREE, using our data plus additional published *Crocidura* sequences from GenBank.

Figure 3 A selection of African and Asian *Crocidura* shrews analyzed in this study. This image highlights the conservative morphology of the genus.

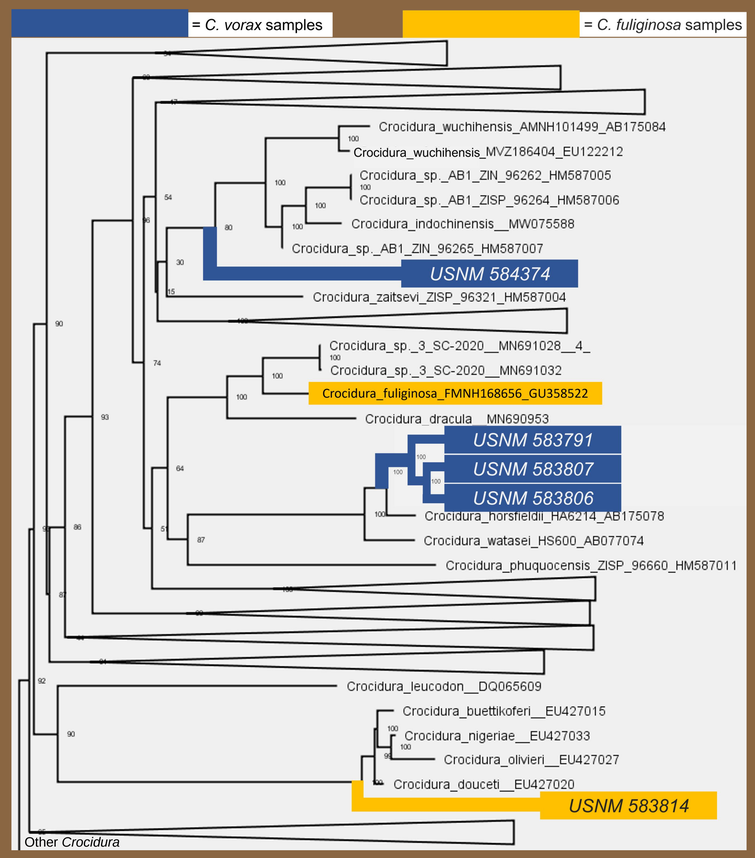

Figure 4 Placement of *C. vorax* and *C. fuliginosa* specimens based on cytochrome *b* phylogenetic reconstruction. Specimens highlighted in blue indicate *C. vorax* and yellow indicate *C. fulignosa*, revealing polyphyly in both taxa. Bootstrap support values are shown at each node, indicating high support for the position of the specimens. Several clades have been collapsed for clarity.

Results

- Out of 26 USNM specimens sampled, only two showed strong congruence between morphologically diagnosed species names and the names linked to sequence data in GenBank.

Crocidura vorax	*Crocidura fuliginosa*
• USNM 584374 was sister to a clade containing *C. wuchihensis*, *C. indochinensis*, and two unnamed clades. USNM 583806, 583807, and 583791 were in a clade sister to *C. cf. horsfieldii,* and closely related to *C. watasei*. The population of *C. vorax* closest to the type locality was found in a clade different from the USNM specimens.	• USNM 583814 was apparently sister to *C. leucodon*—a geographically distant Eurasian species—and closely related to a clade of apparently West African species, some of which were polyphyletic. *C. fuliginosa* also appeared elsewhere on the tree, indicating polyphyly.

Discussion

- Reliable sequences from vouchered Asian *Crocidura* bolsters support for inferred relationships.
- Despite high tree support, instances of polyphyly and paraphyly were common, suggesting widespread unreliable field identification and/or taxonomic instability.
- There is likely an underestimation of species diversity in Myanma *Crocidura*.
- Integrative taxonomy incorporating additional nuclear loci and morphology is the best way to approach species complexes.
- Sequences in public repositories, such as GenBank, ought to be linked to vouchered museum specimens—if not, they have little value in systematic research. The “West African” *Crocidura* clade nested within the Asian radiation demonstrates the need for this.
- The NMNH collection proved an invaluable resource in this study. Without its abundance of voucher specimens, this work would be impossible.
- Type sequencing is the only way to solve many of the issues recovered in this phylogeny, particularly for allocating proper species names to cryptic taxa.

Acknowledgments and References

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[1]Dubey S, Salamin N, Ruedi M, Barrière P, Colyn M, Vogel P. Biogeographic origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from mitochondrial and nuclear genes. *Mol. Phylogenet. Evol.* 2008; 48(3): 953–963.

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