Specimen Age and Phylogenomic Analysis of Ultraconserved Elements in Carpenter Bees (Genus: *Xylocopa*)

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Introduction

Carpenter bees are members of the genus *Xylocopa* in the family Apidae (Fig. 1), with over 500 species distributed worldwide. *Xylocopa* drill tunnels in wood, which they line with cells in which larvae develop. They are generally solitary, but some species aggregate or even share nests, with a foraging mother predominating. *Xylocopa* are recognized as agriculturally important and useful in studying the evolution of social behavior in insects. However, the evolutionary relationships among *Xylocopa* species remain poorly known.

Fig. 1: Adult male *Xylocopa tabaniformis*.

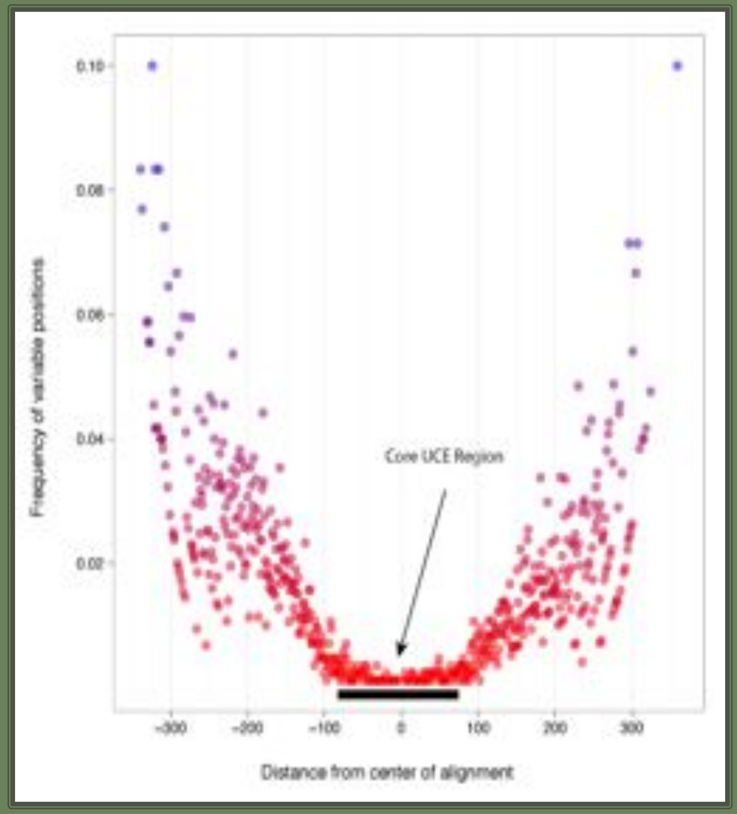

Fig. 2: Increasing variability in UCE flanking regions.

Degradation of DNA in old museum specimens makes them difficult to use in molecular phylogenetics. A novel approach involving ultraconserved elements (UCEs), which are DNA sequences with 100% fidelity across many taxa, has the potential to rectify this issue. The regions flanking UCEs accumulate variation between species over time, making them useful for phylogenetic analysis (Fig. 2). Because UCEs are short and scattered throughout the genome, they often avoid degradation. This technique has been just developed for use within insects (Faircloth et al., 2015) and has never been applied to *Xylocopa*. Our goal was to reconstruct a preliminary phylogeny of *Xylocopa* as well as to test the efficacy of UCE data from pinned insect specimens of varying ages.

Materials & Methods

We extracted DNA from 48 representatives of 24 *Xylocopa* species, selecting both the most recent and the oldest specimens present in the NMNH collections (Fig. 3). The DNA was quantified with a Qubit fluorometer and quality was assessed via gel electrophoresis. We chose 24 samples with sufficient DNA yields for library construction. After shearing via sonication the extractions with high molecular weight, we hybridized each library with Hymenoptera-specific probes targeting 1,510 UCE loci (Fig. 4). We pooled our libraries, which included sample-specific barcode adaptors, and sequenced them on an Illumina MiSeq in the LAB. Contig paralogs and those not matching UCE loci were filtered using the PHYLUCE package. The resulting species-specific UCEs were assembled and used to construct a 70% complete phylogenetic matrix which excluded two samples with <200 UCEs. RAxML was used to infer optimal and bootstrap trees under maximum likelihood. The tree was rooted with two outgroups (*Apis mellifera* and *Bombus pensylvanicus*) using data from Faircloth et al. 2015.

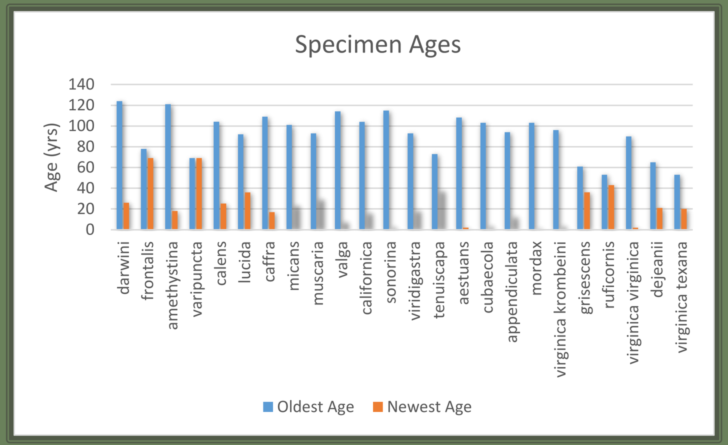

Fig. 3: Ages of the 48 extracted DNA specimens

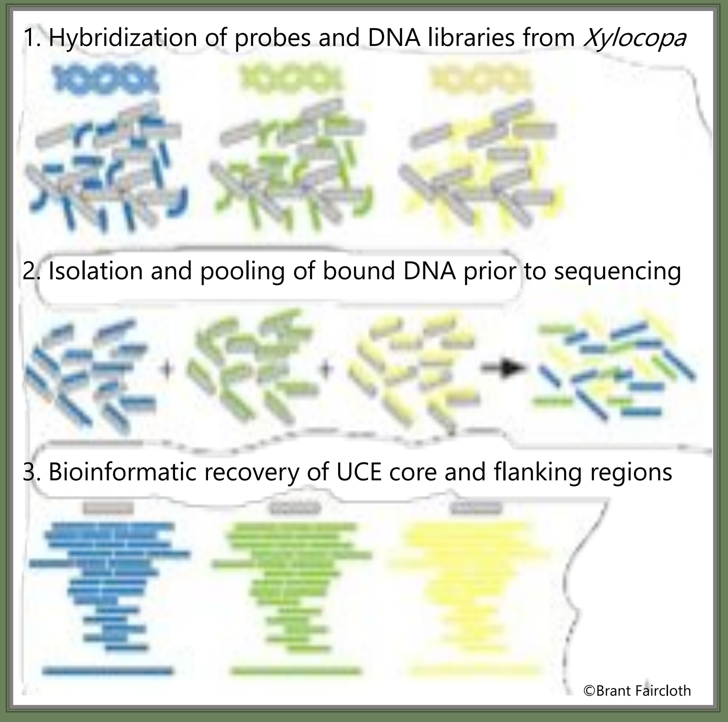

Fig. 4: Diagram of preparation of UCE data for phylogenetic analysis.

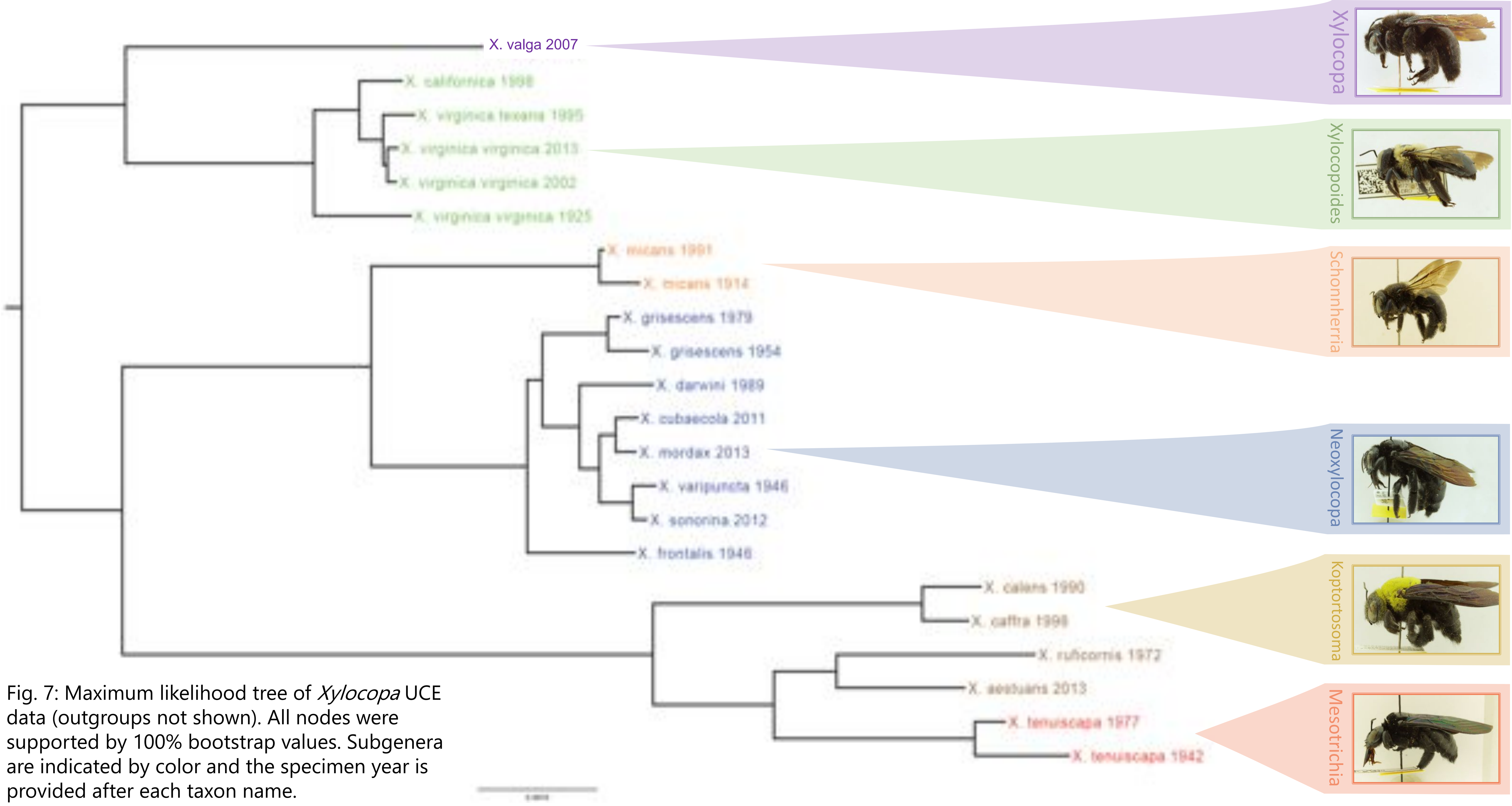

Fig. 7: Maximum likelihood tree of *Xylocopa* UCE data (outgroups not shown). All nodes were supported by 100% bootstrap values. Subgenera are indicated by color and the specimen year is provided after each taxon name.

Results & Discussion

As expected, fewer UCE loci were recovered from older *Xylocopa* specimens; however, many hundreds of loci were still recovered from specimens up to 100 years old (Fig. 5). This is the first known demonstration that UCEs work on very old insect material, although similar results have been recently reported in birds (McCormack et al., submitted). Thus, while the DNA from newer specimens was of much higher quality than that from older ones (Fig. 6), we were able to generate comparable numbers of UCE loci from all but the oldest specimens.

Phylogenetic analysis grouped together old and new specimens of the same species (Fig. 7), indicating that our UCE data from older specimens are robust. Furthermore, all subgenera represented by multiple species were monophyletic, except *Koptortosoma* whose paraphyly with respect to *Mesotrichia* was consistent with previous mitochondrial data (Leys et al. 2000).

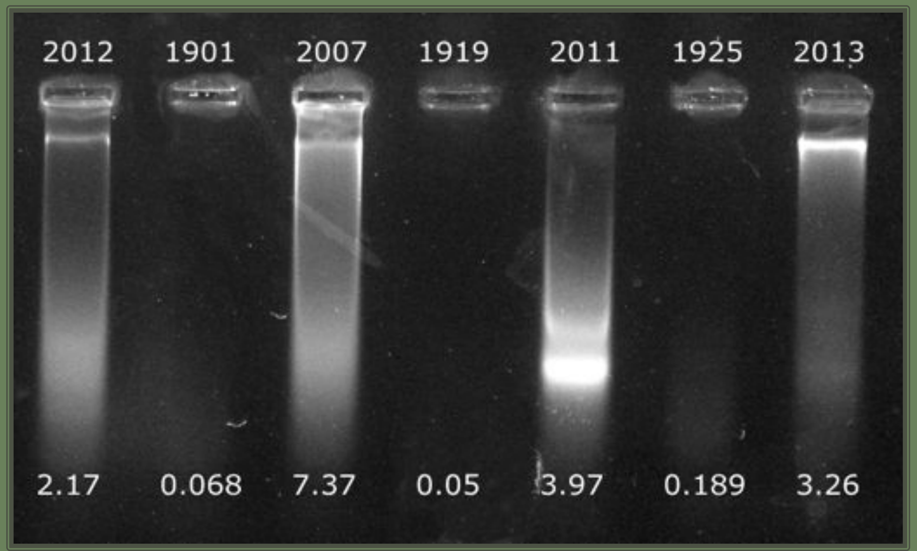

Fig. 6: Section of agarose gel demonstrating the higher quality (top, years) and quantity (bottom, ng/μg) of newer DNA samples. Higher-weight DNA from newer specimens collects in thick bands near the wells.

The ability to obtain viable phylogenomic data from very old museum specimens makes UCEs a valuable tool for collections-based research. DNA degradation renders whole-genome and traditional PCR-based methods impractical. Transcriptome analysis is also not feasible due to the ephemerality of RNA. Our results show the potential for UCEs to resolve the evolutionary history of *Xylocopa* and to provide large-scale phylogenomic data from museum specimens of many other taxa.

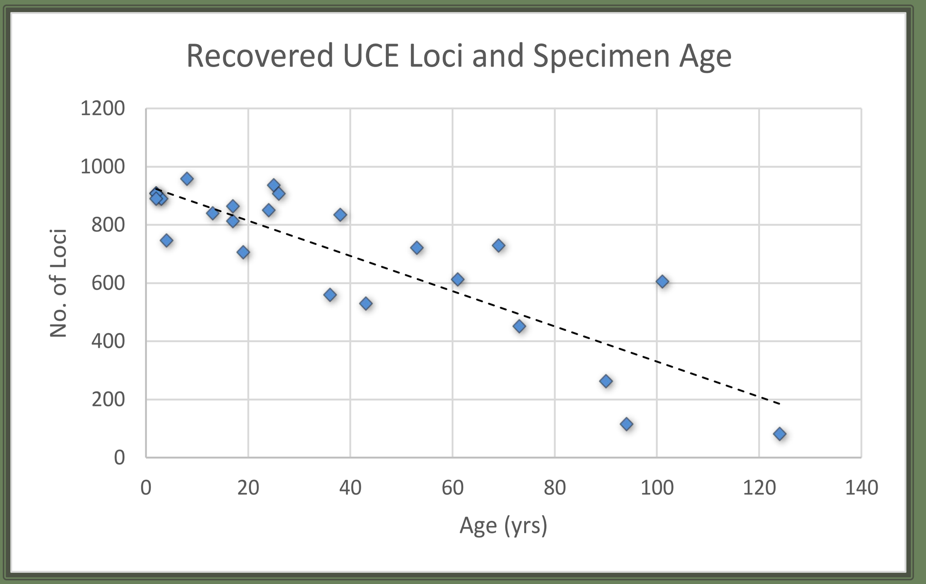

Fig. 5: Older specimens tended to yield lower amounts of viable UCE data. However, most of these specimens did provide enough data for use in the analysis.

Acknowledgements & References

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