

Diagnostics of mosquitoes from E. Canada with the use of the DNA barcoding method

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Introduction

The ability of DNA barcodes to identify species reliably, quickly and cost-effectively has particular importance in medical entomology. Since the recent arrival of West Nile virus (WNV) in North America, mosquito identification and ascertainment of vector status has gained renewed significance on this continent. It has become imperative to be able to correctly identify genetic varieties of mosquito vectors of WNV. Although biting insects have been studied more extensively than most other animal groups, our taxonomic knowledge of mosquitoes is far from being complete; ongoing taxonomic research still regularly leads to the discovery of new species. In fact, since Edwards's (1932) outline for the modern system of mosquito classification, the number of described mosquito species has more than doubled from 1400 to almost 3200 (Harbach & Kitching, 1998; Zavortink, 1990). Many additional species await description and reliable natural system of classification is yet to be agreed upon. Because of the longstanding difficulty in gaining species identifications for mosquitoes (particularly for larvae and pupae) through morphological analysis, genetic approach has been used to test usefulness of sequence variation in the barcode region of cytochrome c oxidase subunit 1 (CO1) for the identification of mosquito species from eastern Canada.

Goals

- i) to test the utility of DNA sequence divergence in mt CO1 and 16S rRNA as molecular correlates to well defined morphologically circumscribed mosquito species from Ontario and N.Scotia, Canada; ii) to evaluate the range of their intra- and inter-specific molecular divergence; iii) to compare NJ distance method with Bayesian character-based method;

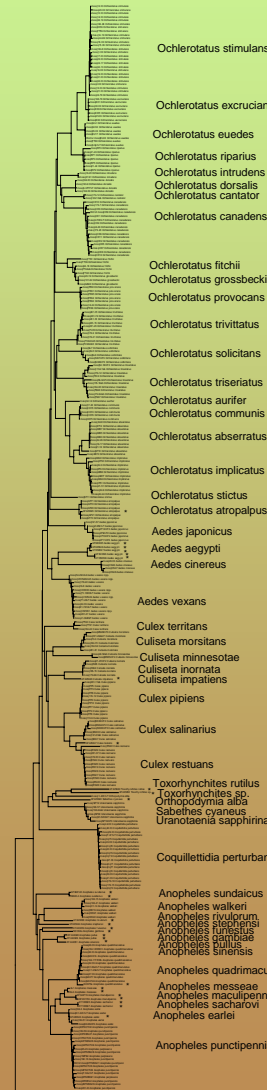
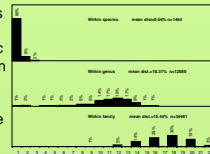
Methods

For each individual 30 µL of total DNA extractions were obtained with the use of SIGMA GeneElute™ Kit. The primer pairs LCO1490 and HCO12198 (Folmer et al., 1994) or LepF (TCAACCAATCATAAAGATATTGGAAC) and HCO12198 were subsequently used to amplify a 617 bp fragment of the CO1 gene. The pair of universal primers 16Sa and 16Sb (Palumbi, 1996) were used to obtain 488 bp fragments (including gaps) of the 16S gene. All PCR products were sequenced on an ABI 377 automated sequencer. Electropherograms were aligned with Sequencher™. A distance matrix of pairwise nucleotide sequence divergence was calculated for both genes, using the Kimura 2-parameter model, and used to construct phenograms in the neighbor-joining (NJ) analysis in Mega 2.1. Also a direct multiple alignment of all sequences was applied within MrBayes v.3.0B.4 programme which is equivalent to the Maximum Likelihood method.

Conclusions

1. With the use of the mt CO1 barcoding method we were able to assign unique sets of sequences to most of the mosquito species. Specimens of single species formed barcode clusters with tight cohesion, except for the *Ae. fitchii*/*Ae. grossbecki* complex which may reflect case of hybridization, incomplete lineage sorting or inadequate taxonomy. These results were basically confirmed by the 16S-NJ profile.
2. CO1 sequence differences among congeneric species were on average almost 20 times higher than the total average difference within species.
3. There is no difference between NJ distance method and Bayesian character-based method in discriminating species of mosquitoes recognized through prior taxonomic work
4. This study has established effectiveness of CO1 barcodes in discriminating species of mosquitoes. Notice: the ts saturation at around 7.5% sequence divergence suggests caution in the interpretation of pairwise comparisons of mosquito CO1 sequences at the congeneric and intergeneric levels, unless silent sites are excluded from analysis.

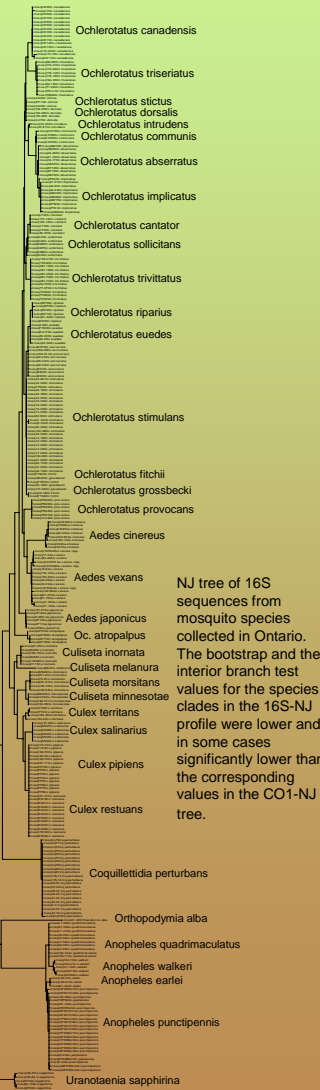
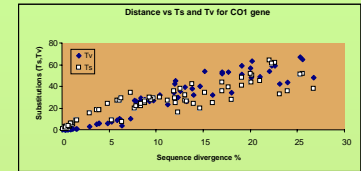
Pairwise comparisons between CO1 sequences among mosquito species which are separated into three categories: intraspecific, intrageneric and intergeneric differences between individuals. Intraspecific divergence averaged 0.55% (range 0 - 3.9%), intrageneric averaged 10.4% (range 0.2 - 17.2%), intergeneric averaged 16.0% (range 7.2 - 26.3%)



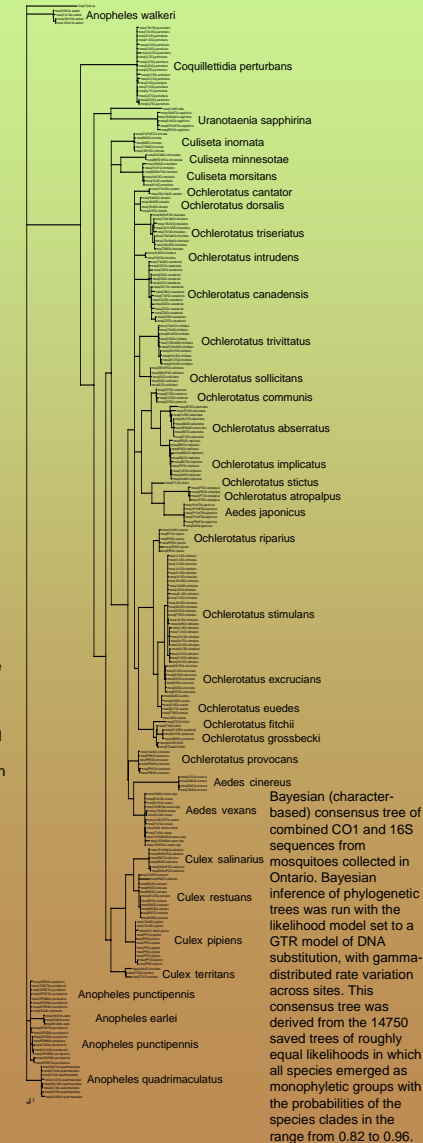
NJ tree of CO1 sequences from 37 out of 58 mosquito species known from Ontario and 15 species from outside Ontario, obtained from the GenBank (with asterisks). There was generally high bootstrap and the interior branch test support (90%-99%) for the terminal branches at the species level

Observed numbers of transitions and transversions for the CO1 fragment plotted against sequence divergence. The ts saturation begins to level off around a 7.5% sequence divergence, which suggests that caution should be taken when interpreting the pairwise comparisons at the congeneric and intergeneric levels, unless the silent sites are excluded from the reconstruction of mosquito phylogenies.

Results



NJ tree of 16S sequences from mosquito species collected in Ontario. The bootstrap and the interior branch test values for the species clades in the 16S-NJ profile were lower and in some cases significantly lower than the corresponding values in the CO1-NJ tree.



Bayesian (character-based) consensus tree of combined CO1 and 16S sequences from mosquitoes collected in Ontario. Bayesian inference of phylogenetic trees was run with the likelihood model set to a GTR model of DNA substitution, with gamma-distributed rate variation across sites. This consensus tree was derived from the 14750 saved trees of roughly equal likelihoods in which all species emerged as monophyletic groups with the probabilities of the species clades in the range from 0.82 to 0.96.