DNA barcoding for constructive taxonomy and diversity evaluation of black fly populations from Manitoba

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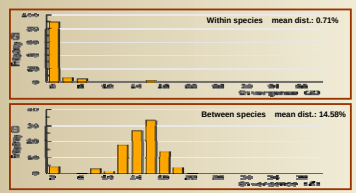
Introduction

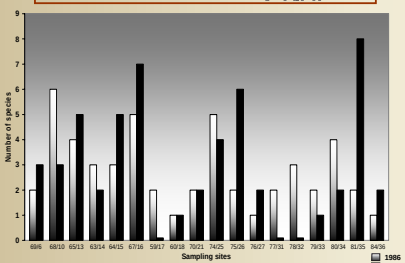
Identification of simuliids is difficult and many species can be distinguished reliably only through examination of the giant polytene chromosomes of larvae. The DNA barcoding technique (mitochondrial cytochrome oxidase subunit1) was applied to recently collected material from Manitoba to test congruence between morphological taxonomy and sequence divergence thresholds for black fly species.

Methods

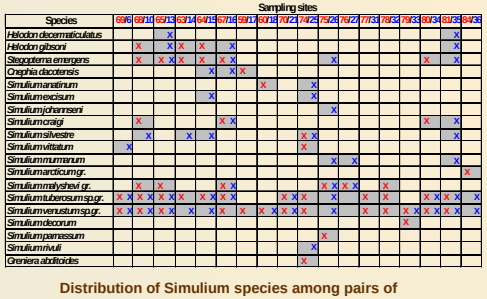
In April 2005, simuliid larvae were sampled at 38 sites, between Winnipeg, Brandon, and Duck Mt. Prov. Park (Manitoba). For each individual 30 µL of total DNA extractions were obtained with the use of SIGMA GeneElute™ Kit. The primer pairs LCO1490 and HCO12198 (Folmer et al., 1994) or LepF (TCAACCAATCATAAAGATATTGGAAC) and HCO12198 were subsequently used to amplify a 618 bp fragment of the CO1 gene. The pair of universal primers 16Sa and 16Sb (Palumbi, 1996) were used to obtain 502 bp fragments (including gaps) of the 16S gene. All PCR products were sequenced on an ABI 3730 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.



Pairwise comparisons between CO1 sequences among Simulium species which are separated into intraspecific & intrageneric differences between individuals.



Pairwise comparisons of the number of Simulium species between sites surveyed in Manitoba both in April of 1986 and 2005.



Distribution of Simulium species among pairs of sites (red-1986, blue-2005) in Manitoba.

Diversity of simullid species in the surveyed area of Manitoba did not change significantly between 1986 and 2005 in terms of average number of species per site. However the std dev. of species richness was significantly higher in 2005 than in 1986 which indicates a more uneven distribution of species in recent years.

Results & Conclusions

1. A total of 135 individual specimens were barcoded and the mt CO1sequence diversity allowed us to assign unique sets of DNA barcodes to most of the 20 simuliid species from our study. Only for a few individuals we were not able to test the discriminating power of our method at the species level as they could not be distinguished morphologically and/or required cytological study (particularly members of *S. malyshevi*-, *S. tuberosum*-, and *S.venustum* species-groups).
2. Simuliid species formed tight monophyletic clusters. Pairwise comparisons between CO1 sequences showed that conspecific K2P divergences averaged 0.71% (min 0%, max 4.02%, S.E. 0.029) while sequence divergences between congeneric species averaged 14.58% (min 2.8%, max 18.5%, S.E. 0.044).The CO1 sequence differences among congeneric species were on average almost 21 times higher than the total average difference within species. These values were slightly higher than those among some other insects, such as mosquitoes (by 0.2% and 4.2% respectively).
3. Patterns of species richness were not significantly different when using morphological taxonomy vs sequence divergence thresholds.
4. CO1 barcoding appears to be a promising technique in rapid discrimination among simuliid morphospecies.

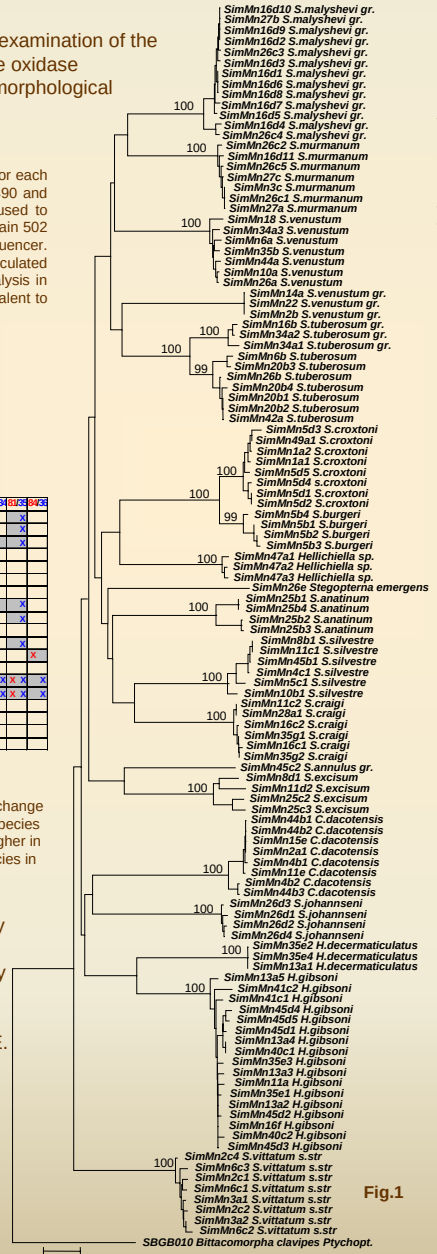


Fig.1

NJ tree of CO1 sequences from 20 Simulium species collected at 38 sites in Manitoba. There was generally high bootstrap (99-100%) and the interior branch test support (90%-99%) for the terminal branches at the species level

Fig.2

NJ tree of 16S sequences from Simulium species collected in Manitoba. 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.

Fig.3

Bayesian (character-based) consensus tree of CO1 sequences from simuliids collected in Manitoba. 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 14600 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 1.00.

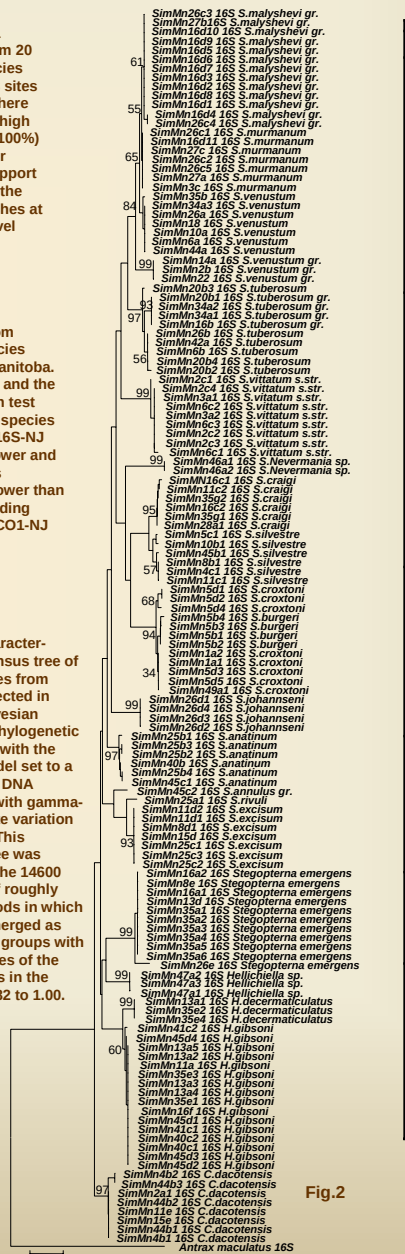


Fig.2

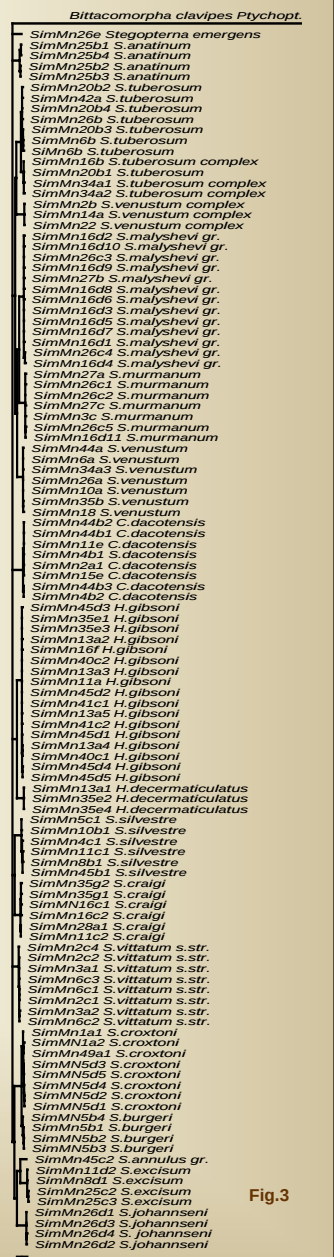


Fig.3