

Long-eared bat taxonomy: Nuclear genetic evidence eliminates the species status of Keen's Myotis (*Myotis keenii*)



Cori L Lausen, Wildlife Conservation Society Canada; Doug Burles, Parks Canada emeritus; Dave Nagorsen, Mammalia Biological Consulting; Michael Proctor, Birchdale Ecological; Karen Blejwas, Alaska Dept. of Fish & Game; Purnima Govindarajulu, BC Ministry of Environment; David Paetkau, Wildlife Genetics International; Erin Harmston, Wildlife Genetics International; Laura Friis, BC Ministry of Environment emeritus.

INTRODUCTION

Four species of long-eared myotis bats are recognized in British Columbia: Keen's Myotis (*Myotis keenii*), Northern Myotis (*M. septentrionalis*), Long-eared Myotis (*M. evotis*), and Fringed Myotis (*M. thysanodes*). Accurate species description is essential given the 'vulnerable/sensitive' conservation status listing of three long-eared myotis species versus the 'secure' listing of *M. evotis*. Due to extreme morphological similarity, the taxonomic status of *M. keenii* versus *M. evotis* where the 2 species' ranges overlap has been questioned (van Zyll de Jong and Nagorsen 1994, Dewey 2006). Here, *genetic species* is defined as a group of interbreeding populations that are genetically isolated from other such population groups (Baker and Bradley 2006).

We ask: **Do *Myotis keenii* and *M. evotis* interbreed?**

METHODS

Wing tissue (2 mm biopsy) was obtained from the 4 long-eared species ($n = 257$) and 1 outgroup (*M. lucifugus*; $n = 24$) from sites in BC, Alaska, Washington, Montana, and Alberta (Figure 1).

We genotyped all samples at 14 microsatellite loci and analyzed population genetic relationships (breeding patterns) in Genetix (Belkhir 1999) and STRUCTURE (Pritchard et al. 2000).

We sequenced 750 base pairs of the mtDNA cytochrome b gene for 86 representative individuals from all putative species to examine gene flow patterns and assigned species identifications using Genbank.

We examined isolation by distance and morphological patterns using regression analysis of microsatellites and forearm lengths, respectively. We included measurements of 2 other widespread bats in BC and Alaska: *M. californicus* and *M. lucifugus*.

RESULTS

Genetix clearly delineated distinct clusters representing 4 species: *M. septentrionalis*, *M. thysanodes*, *M. lucifugus* and a mixed cluster of samples that were field identified as *M. keenii* or *M. evotis* (Figure 2). This highly mixed cluster provides clear evidence that these individuals represent a single genetics species (Figure 2b). STRUCTURE results (not presented) produced the same conclusions.

The cyt-b sequences did not align well with field identifications, with all 6 morphologically distinct *M. thysanodes* being identified as *M. keenii* or *M. evotis* when compared to known sequences in Genbank.

All long-eared samples west of the Coast Mountains were of one mtDNA haplotype complex suggesting limited female movement over these mountains. We found no evidence of limited gene flow across the Rocky Mountains.

Forearm lengths of coastal *M. keenii/evotis* are smaller than inland *M. keenii/evotis* in B.C. (t-test, $p < 0.001$). This same significant pattern was seen in *M. californicus*, another wide-ranging gleaning species of bat, but not in *M. lucifugus*.



Figure 1: Sampling locations for all species. Field identifications were based on known ranges and morphology.

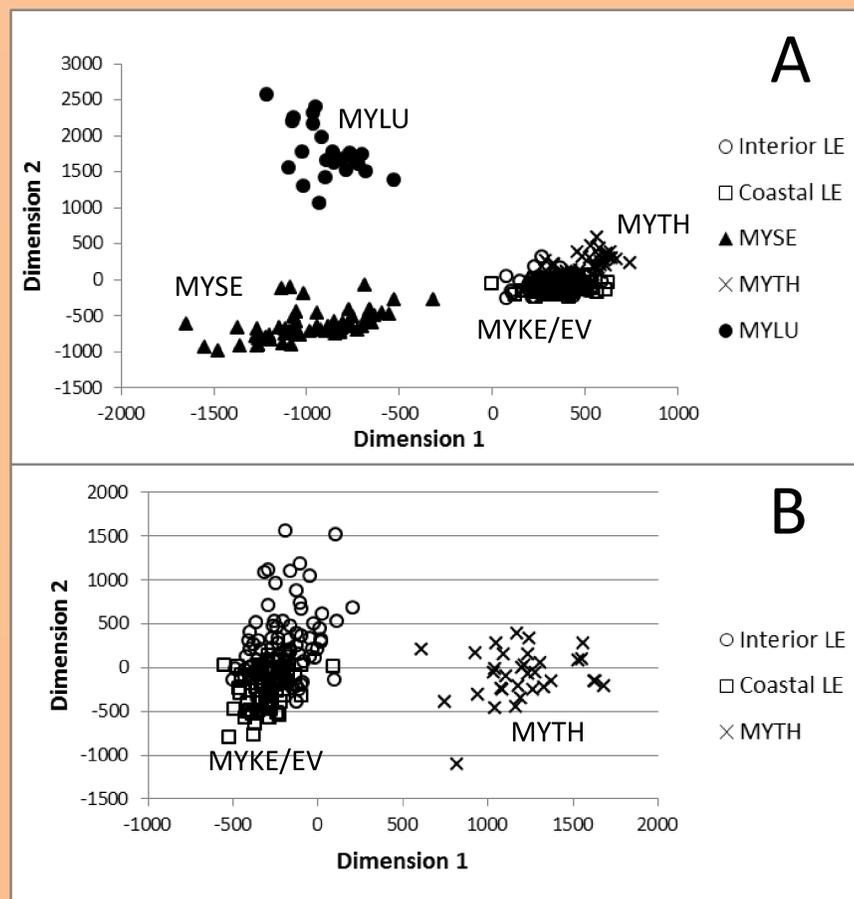


Figure 2. Cluster results from Genetix showing (A) separation of *Myotis septentrionalis* and *Myotis lucifugus* from the remaining long-eared species and (B) separation of *Myotis thysanodes* from a mixed *Myotis evotis-keenii* group.

DISCUSSION

Do *Myotis keenii* and *M. evotis* interbreed?

Based on microsatellite data (breeding patterns), *M. septentrionalis*, and *M. thysanodes* separate out from each other and from the *M. keenii* / *M. evotis* species group, but *M. keenii* / *M. evotis* were not distinguishable from each other.

Based on microsatellite data, *M. lucifugus* (easily distinguished from the long-eared myotis group by morphology) is also a genetic species, distinct from the long-eared myotis group.

Nuclear gene flow across the study area is clear based on microsatellite results. Our mtDNA data suggest partial restriction of female-mediated gene flow by the Coast Mountain range. No such limitation of gene flow by the Rocky Mountains was detected.

Taxonomic Definition and morphology – *Myotis keenii* was defined as a species, separate from other long-eared bats, based on smaller body size (van Zyll de Jong and Nagorsen 1994). We found smaller forearm lengths in coastal long-eared myotis. Geographic gaps in sampling across the range of species during taxonomic examination may have prevented observation of this cline. We propose that smaller size may be indicative of ecological pressures on gleaning bat species in coastal B.C. and Alaska. We found that this smaller coastal forearm pattern also exists in the other widespread gleaning bat, *M. californicus*.

CONCLUSION

Based on our analyses, *M. keenii* and *M. evotis* are fully interbreeding. We conclude that *M. keenii* and *M. evotis* are conspecific. The appropriate species name is *Myotis evotis* (Long-eared Myotis), and because *M. evotis keenii* was named prior to *M. e. pacificus*, we propose *M. e. keenii* be retained and *M. e. pacificus* be dropped.

Literature Cited Baker, R. J., & Bradley, R. D. (2006). *Journal of Mammalogy*, 87(4), 643-662; Belkhir K. (1999). GENETIX 4.0, Université de Montpellier II, Montpellier (France); Dewey, T.A. (2006). PhD Dissertation. [Ann Arbor (MI)]: University of Michigan; Nagorsen, D. W. & Brigham R. M. 1993. Royal British Columbia Museum Handbook. University of British Columbia Press, Vancouver. 164 pp.; Pritchard, J.K. et al. (2000). *Genetics* 155:945-959; Van zyll de Jong, C. G., & Nagorsen, D. W. (1994). *Canadian Journal of Zoology*, 72(6), 1069-1078.

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