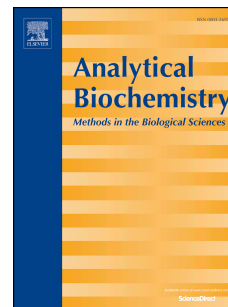


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A method to distinguish morphologically similar *Peromyscus* species using extracellular RNA and high-resolution melt analysis

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Title: A method to distinguish morphologically similar *Peromyscus* species using extracellular RNA and high-resolution melt analysis

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A method applying high-resolution melt (HRM) analysis to PCR products copied and amplified from extracellular RNA (exRNA) has been developed to distinguish two morphologically similar *Peromyscus* species: *P. leucopus* and *P. maniculatus*. *P. leucopus* is considered the primary reservoir host of *Borrelia burgdorferi*, the causative agent for Lyme disease in North America. In northern Minnesota the habitat ranges of *P. leucopus* overlaps with that of *P. maniculatus*. Serum samples from live mice of both species were collected from cheek bleeds, total extracellular RNA (exRNA) was extracted, copied using reverse transcription and amplified by PCR followed by HRM analysis. A circulating ribosomal RNA (rRNA) was identified which differed at seven nucleotides between the two species and a method of HRM analysis was developed allowing rapid species confirmation. In the future, this HRM based method may be adapted for additional species.

Key words: extracellular RNA, high resolution melt, 16S mitochondrial ribosomal RNA, *Peromyscus leucopus*, *Peromyscus maniculatus*.

Abbreviations used: HRM, high resolution melt; exRNA, extracellular RNA; *P. leucopus*, *Peromyscus leucopus*; *P. maniculatus*, *Peromyscus maniculatus*; *P. m. gracilis*; *Peromyscus maniculatus gracilis*; *M. musculus*, *Mus musculus*; rRNA, ribosomal RNA; PGSC, *Peromyscus* Genomic Stock Center; Min, minimum; Max, Maximum; qRT-PCR, quantitative RT-PCR.

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