

First Record of the Arid-Land Termite, *Reticulitermes tibialis* Banks, in Wisconsin

Rachel A. Arango

Abstract

During a survey of termites in Wisconsin, one colony was found from a different habitat than the remaining populations. This observation led to further genetic testing which resulted in a determination of *Reticulitermes tibialis* Banks. This is the first record of a termite species other than *Reticulitermes flavipes* (Kollar) to be established in the state.

Numerous records of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), have been recorded from Wisconsin, a species which has long been thought to be the only termite species established in the state. Outside of an urban environment, colonies of *R. flavipes* are often found in shady, wooded areas under logs and in decaying trees. During a survey of Wisconsin termite populations, one site at Hardscrabble Prairie in Hazel Green showed termite activity under logs and rocks in the middle of a sunny prairie with very little shade cover, prompting further evaluation.

As morphological identification of termites is often difficult, genetic-based techniques were utilized for species determination to support observed physical characteristics. Termites were collected 24 July 2013 in cardboard placed under logs in the middle of the prairie. Deoxyribonucleic acid (DNA) was extracted from three individual worker termites using the Promega Wizard genomic DNA purification kit following the protocol for isolation of genomic DNA from animal tissues. Termite DNA (~50ng/ul) was amplified using primers TL2-J-3037 (5'-ATGGCAGATTAGTGCAATGG-3') and TK-N-3785 (5'-GTTTAAGAGACCAGTACTTG-3'), which are specific for the mitochondrial COII genes as chosen by Lim and Forschler (2012). Polymerase chain reaction (PCR) was performed in a standard 50 µl reaction in a MJ 225 DNA Engine Tetrad thermocycler. PCR conditions included a denaturation step at 94°C for 1 minute followed by 30 cycles each consisting of 1 minute at 94°C, 1 minute at 51°C for annealing, and 2 minutes at 72°C for extension. The cycle finished with a post-cycle extension at 72°C for 5 minutes. PCR products were cleaned using a Promega Wizard SV gel and PCR cleanup system followed by Big Dye sequencing reaction. Samples were then cleaned by bead cleanup (Agencourt AMPure XP) prior to submission for sequencing in both directions to UW Biotech (Madison, Wisconsin—Biotechnology Center). Molecular analyses and resulting sequences were aligned using MEGA: Molecular Evolutionary Genetics Analysis Free Software (version 5) (Tamura et al. 2011) and searched using the basic local alignment search tool (BLAST). All three samples resulted in a 99% identity match to the arid-land termite, *Reticulitermes tibialis* Banks (accession number: AY168206).

Unlike *R. flavipes*, *R. tibialis* is able to withstand more arid conditions, making it less susceptible to desiccation (Pickens 1934). This explains its presence in the middle of a sunny, Wisconsin prairie. Since this species has been found only on the south-western most border of the state, it is likely that this population lies on the northern boundary of *R. tibialis* distribution. The discovery

is not substantially outside of the known distribution of this species, as it has been found as far north as Iowa and Idaho (Austin et al. 2008).

Acknowledgments

I would like to thank Thomas Meyer at the Wisconsin DNR for permitting insect collection at Hardscrabble Prairie and to Jeff Gruber for helping find this termite population. Thanks also to Joliene Lindholm for help with genetic analysis and Brian Forschler for initial morphological examination of the specimens.

Literature Cited

- Austin, J. W., A. L. Szalanski, J. A. McKern, and R. E. Gold. 2008.** Molecular phylogeography of the subterranean termite *Reticulitermes tibialis* (Isoptera: Rhinotermitidae). *Journal of Agricultural and Urban Entomology* 25: 63-79.
- Lim, S. Y. and B. T. Forschler. 2011.** *Reticulitermes nelsonae*, a new species of subterranean termite (Rhinotermitidae) from the southeastern United States. *Insects* 3:62-90.
- Pickens, A. L. 1934.** The barren-lands subterranean termite, *Reticulitermes tibialis*, pp. 184-186. *In* C. A. Kofoid, S. F. Light, A. C. Horner, M. Randall, W. B. Herms, and E. E. Bowe (eds.), *Termites and Termite Control*, 2nd ed. University of California Press, Berkeley, CA.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011.** MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.