Similarity is Relative: Hierarchy of Genetic Similarities in the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) in Hawaii

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ABSTRACT Termites (Isoptera) have a strong hierarchical structure due to their social organization. To interpret genetic differences within and among termite populations it is first necessary to create a quantitative scale to determine the meaning of "different." To accomplish this, the entire hierarchy of genetic similarities in a Hawaiian population of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) was defined by multilocus DNA fingerprinting using the oligonucleotide $(GTG)_5$ as a probe. The genetic background similarity in the population, based on the genetic similarity between unrelated termites, was 0.21 and falls in the range of other natural invertebrate and vertebrate populations. The mean genetic similarity of termites collected from the same collection site was 0.64 (range, 0.58–0.72). This equals an average relatedness of 0.54 within collection sites. The genetic similarity among the offspring of an artificially outbred laboratory colony lies in the same magnitude (0.65). However, genetic similarity within field colonies was significantly lower than the genetic similarity within laboratory colonies derived from pairs of siblings (mean 0.79). This indicates moderate inbreeding within most of the field colonies. This wide range of genetic similarities defines the basis for a detailed description of the population structure as well as the identification of individual colonies of *C. formosanus*.

KEY WORDS *Coptotermes formosanus*, genetic structure, DNA fingerprinting

DESCRIPTION OF THE genetic structure of termite populations, i.e., the relationship within and between colonies, is a necessary prerequisite for understanding their biology. In the expanding field of termite genetics, efforts have been made to describe their genetic structure using such techniques as alloenzyme analysis, multilocus DNA fingerprinting, and polymerase chain reaction based methods (Pamilo et al. 1997). Not only have species and populations been genetically identified (e.g., Clément 1981, Korman and Pashley 1991, Wang et al. 1992, Miura et al. 1998, Jenkins et al. 1999a), but successful attempts have been made to differentiate between colonies (Reilly 1987, Husseneder et al. 1998). Furthermore, the presence of different genetic lines was suggested within colonies (Broughton 1995, Atkinson and Adams 1997, Husseneder et al. 1998, Jenkins et al. 1999b), which may lead to kin-biased foraging (Kaib et al. 1996). Even single termites can be assigned to colonies or parental lines by their individual specific DNA fingerprint (Husseneder 1998; Husseneder et al. 1998, 1999).

However, a common problem in most of the studies to date is the decision upon which level differentiation occurs. In other words, does differentiation indicate isolated gene pools and therefore separate populations, different colonies within the same population, or different genetic lines within colonies (Thompson and Hebert 1998; Jenkins et al. 1999a, 1999b)? Is the difference significant in light of the variation within groups? Interpretations cannot leave the realm of speculation so long as the degree of "difference" is not quantified. To solve this problem, we initiated a study to define the entire hierarchical genetic structure of *Coptotermes formosanus* Shiraki on the island of Oahu, HI.

Coptotermes formosanus is a severe pest species worldwide and in the United States, especially in Hawaii (Tamashiro et al. 1987). The cryptic nesting and foraging habits of this termite and the limited number of alloenzyme and mitochondrial DNA markers capable of detecting polymorphism have allowed only limited insight into the genetic structure of *C. formosanus* (Strong and Grace 1993; Broughton and Grace 1994; Wang and Grace 1995, 2000a). However, the technique of multilocus DNA fingerprinting has proven to be a powerful tool for detecting genetic structure in rhinotermitids (Husseneder et al. 1998). This method was therefore chosen to characterize the genetic structure of *C. formosanus* on the island of Oahu, HI, according to the following three parameters: (1) Genetic background similarity between unrelated individuals within the population. (2) Genetic similarity of individuals collected from the same collection sites in the field. (3) Genetic similarity within artificially inbred and outbred laboratory colonies. This defined hierarchy of genetic similarities provides the neces-

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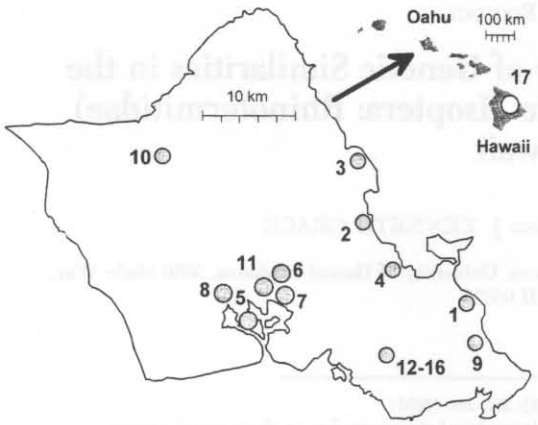

Fig. 1. Collection sites (naturally infested wood, aggregation traps containing cut wood) for *C. formosanus* on the island of Oahu. Workers and soldiers were collected on a large scale at distances from 3 to 45 km (locations 1–11 in Table 1) and on a small scale at 120–540 m apart on the Manoa campus of the University of Hawaii (locations 12–16 in Table 1). An additional collection site was on the island of Hawaii (location 17 in Table 1).

sary basis for future research on population structure and colony identification in *C. formosanus*.

Materials and Methods

Genetic similarities within and between collection sites (naturally infested wood, and aggregation traps containing cut wood) were studied by collecting workers and soldiers of *C. formosanus* from five locations on the University of Hawaii at Manoa campus (120–540 m distance) and 11 additional locations on the island of Oahu (3–45 km; Fig. 1) in November 1998. The five campus collections were repeated once after 9 mo to test for temporal variance. A single collection from the island of Hawaii served as an outgroup (Fig. 1). Comparative genetic similarities were derived from five laboratory colonies raised from pairs of siblings (inbred) or from unrelated alates from separate regions ≈20 km apart (Table 1).

Multilocus DNA fingerprinting was modified from the methods of Kaib et al. (1996) and Husseneder (1998). DNA was extracted from specimens preserved in liquid nitrogen or 190 proof ethyl alcohol (Quantum, Anaheim, CA) using vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for phenol-chloroform extraction. DNA extraction from individual workers (2.8–3.8 mg) and soldiers (2.7–3.6 mg) of *C. formosanus* yielded 3–5 μg of high molecular weight native DNA.

After digestion with the restriction enzyme *Hae*III (Roche Molecular Biochemicals, Indianapolis) the DNA samples were applied in random order on 20 by 25-cm 0.8% agarose gels. The fragments were separated through electrophoresis at 45 V for 72 h. Using the southern blot technique the DNA was transferred onto a positively charged nylon membrane (Roche, Molecular Biochemicals, Indianapolis, IN) through an alkaline capillary transfer and hybridized with the digoxigenated oligonucleotide probe $(GTG)_5$ (MWG-Biotech AG, Ebersberg, Germany). Resulting banding patterns were visualized by a chemiluminescence de-

Table 1. Genetic similarities ($\bar{S}_{xy}$) within field collection sites (1–17) and within laboratory colonies raised from artificially paired alates (A–E)

Field	$\bar{S}_{xy}$	SD	n	N
1	0.61	0.11	8	28
2	0.67	0.11	7	21
3	0.60	0.13	8	28
4	0.66	0.10	8	28
5	0.63	0.11	9	36
6	0.58	0.08	6	15
7	0.62	0.10	7	21
8	0.60	0.09	28	378
9	0.69	0.09	7	21
10	0.61	0.11	8	28
11	0.67	0.10	13	78
12	0.66	0.12	20	190
13	0.68	0.11	14	91
14	0.63	0.11	15	105
15	0.72	0.10	16	120
16	0.62	0.11	18	153
17	0.65	0.08	8	28
Mean $\bar{S}_{xy}$	0.64			
Laboratory				
A: outbred	0.65	0.11	7	21
B: inbred	0.79	0.08	13	78
C: inbred	0.78	0.11	11	55
D: inbred	0.81	0.08	11	55
E: inbred	0.77	0.11	12	66

Field samples 1–16 were collected on the island of Oahu, and collection 17 came from a separate population on the island of Hawaii (see Fig. 1). Laboratory colony A originates from a pair of unrelated alates, and B–D are the offspring of sibling pairings and therefore inbred.
SD = standard deviation of $\bar{S}_{xy}$; n = number of individuals; N = number of pairwise comparisons.

tection system (Roche) and fragments were scored between 6 and 23 kb. The mean number of bands evaluated per individual was 11.0 (SD = 1.8, n = 17 field-collection sites) and, consequently, the number of loci approximated 6.8 (Lynch 1990).

In addition to commercially available DNA molecular weight standards (Roche), the banding pattern derived from human blood was used to homologize bands across gels, as a positive control and an additional outgroup (Husseneder 1998).

Genetic similarity between individuals was characterized by the pairwise bandsharing probability $S_{xy} = 2\,n_{xy}/(n_x + n_y)$, where n_{xy} is the number of shared bands found in the individuals x and y; n_x and n_y are the total number of bands evaluated for each individual (Lynch 1991). Mean genetic similarities of termites within the same site and between different collection sites ($\bar{S}_{xy}$) were calculated by the mean of all possible pairwise combinations (SD = standard deviation; N = number of pairwise combinations; n = number of collection sites or individuals). Because $\bar{S}_{xy}$ is limited to a range between 0 and 1, the standard deviation of values near the boundaries may be distorted. However, most of the genetic similarities do not approach the boundaries and standard deviations thus generally allow a reliable estimation of the variability around $\bar{S}_{xy}$. Genetic similarities ($\bar{S}_{xy}$) were translated into relatedness (r) based on the genetic background similarity (S_B) according to Lynch (1991): $r = (\bar{S}_{xy\text{-}SB})/(1\text{-}S_B)$. Including the mean number of scored bands per individual, the distributions of the expected genetic similarities for particular degrees of relatedness could be calculated (Lynch 1991).

Statistical evaluations were performed using SPSS 6.1 for Windows, SPSS GmbH and NTSYS-PC 1.70, Exeter Software (Rohlf 1990).

Results and Discussion

Assessment of the Methodology. Genetic similarities between individual workers did not differ from genetic similarities between individual soldiers collected from the same site. Similarly, banding patterns derived from pools of ten workers did not differ from patterns of pooled soldiers. Unlike earlier results obtained with allozyme electrophoresis (Wang and Grace 2000b), identical patterns were obtained when comparing fingerprints of pooled workers without guts to extracts from whole workers containing the various bacterial and protozoan gut symbionts (Lai et al. 1983, Mannesmann and Piechowski 1989). Thus, neither caste membership nor symbiont DNA biases the fingerprints. Genetic similarities of termites from the five campus locations collected at the end of the experimental phase did not differ significantly from the major collection in November 1998 ($Z = -0.58$, $P > 0.20$, two-tailed Wilcoxon matched pairs test) and were therefore included in the evaluation.

Mutations were recognized by the occurrence of single bands, i.e., bands that are not shared with any colony mate, in large samples (>20) of individual profiles from each of the five campus collection sites. The mutation rate is 0.007 per band and generation. Because the evaluated fragment length is 6–23 kb, this mutation rate equals 10^{-3} to 3×10^{-4} mutations per kilobase and generation. This lies in the range described by Jeffreys et al. (1985, 1991), Jarman and Wells (1989), and Wong et al. (1986). However, this is a minimum estimate because some mutations may be latent due to limited resolution, or small fragments produced by new restriction sites may fall outside of the scored kilobase range.

Genetic Background Similarity of the Population. The genetic background similarity is the basic genetic similarity shared by unrelated individuals due to historical common ancestry, such as species membership. The genetic background in our study is represented by the mean genetic similarity between 11 collection sites on Oahu separated by >3 km distance and therefore exceeding the likely flight capacity of swarming alates (Fig. 1). The genetic background similarity for the population of *C. formosanus* on Oahu is 0.21 (SD = 0.13, N = 3,005). This result is similar to the genetic background (0.25) in a natural population of an African rhinotermitid *Schedorhinotermes lamanianus* Sjöstedt (Husseneder 1998). Considering the limited number of introductions proposed for *C. formosanus* on the Hawaiian Islands and the hypothesis of genetic bottlenecks (Strong and Grace 1993, Broughton and Grace 1994), this is surprising. However, it is possible that the use of a limited number of alloenzyme loci (Wang and Grace 1995, 2000a) and restriction site polymorphisms without sequencing mitochondrial DNA (Broughton and Grace 1994) may have masked existing variation.

The genetic background consists of the following two components: (1) the genetic similarity due to historical common ancestry, e.g., common species membership, and (2) an error due to limited resolution power and comigration of bands. To estimate the influence of both factors on the genetic background, we compared DNA fingerprints from the *C. formosanus* population on Oahu to the fingerprint patterns of human blood and of termites from a separate population on the island of Hawaii. The average genetic similarity derived from a comparison of termite DNA and human DNA is only 0.01 (SD = 0.01, N = 121). This demonstrates that the comigration error is negligible. The genetic similarity between termites from the Oahu collection sites and termites from the island of Hawaii is 0.02 (SD = 0.01, N = 580). Thus, the background similarity due to common ancestry of separate populations is low. Consequently, use of this method is restricted to investigations within populations because very few bands are shared between populations.

Genetic Similarities within Collection Sites. The mean genetic similarities between termites within each of the 17 field collection sites lie in the range of 0.58–0.72 (mean 0.64, SD = 0.04; Table 1). This is obviously higher than the genetic background similarity, 0.21, between collection sites (Fig. 2a). Due to this difference, genetic similarities derived from multilocus fingerprinting are promising tools for analyzing

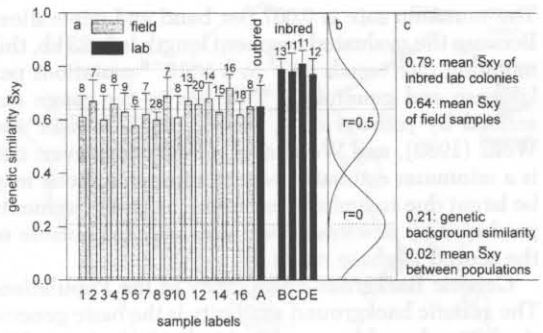

Fig. 2. Genetic similarities within collection sites (a), in comparison to the expected distribution of genetic similarities for particular degrees of relatedness (b), and the complete scale of genetic similarities of *C. formosanus* (c). Number of individuals and the 95% CL are given above columns; for number of pairings see Table 1.

the detailed genetic structure within populations and for colony identification (Husseneder et al. 1998).

The genetic similarity within collection sites is dependent on the degree of inbreeding within *C. formosanus* colonies and populations. Although the degree of inbreeding varies between colonies, there are three lines of evidence that overall indicate rather moderate inbreeding. First, using the genetic background as a reference, the mean genetic similarities of termites within collection sites (0.64) fall into the expected distribution for the genetic similarities of full siblings descended from unrelated parents (Fig. 2 a and b). The average relatedness within collection sites derived from the genetic similarity is 0.54 (Lynch 1991). Second, experiments were conducted to compare the field data to genetic similarities among termites in artificially outbred and inbred laboratory colonies. Experimental pairing of alates from locations 20 km apart, and therefore without recent common ancestry, yields a genetic similarity among the offspring in the magnitude of the genetic similarity within the field collection sites: 0.65 (SD = 0.11, $N = 21$). However, offspring of sibling pairs have a significantly higher genetic similarity than that found within the field samples (0.79; SD = 0.02, $Z = -3.05$, $P < 0.001$, one-tailed Mann-Whitney U test; Table 1; Fig. 2c). Third, the mean genetic similarities within collection sites are similar to those within carton nests observed in a natural rhinotermitid population of *S. lamanianus* based on a comparable background similarity of 0.25 (Kaib et al. 1996, Husseneder 1998). This species has previously shown low inbreeding based on unrelated colony founders and only one or a few inbreeding generations of replacement reproductives (Husseneder et al. 1999). The same is probably true for most of the collection sites of *C. formosanus*. Nevertheless, to complement these results, more data concerning the social organization of *C. formosanus*, such as number and relatedness of reproductives, possibility of interconnected satellite nests, or shared collecting sites by different colonies are required.

The majority of genetic studies in termites suggests a considerable degree of inbreeding due to limited dispersal distance and the existence of nymphoid replacement reproductives (e.g., *Reticulitermes* Holmgren species: Reilly 1987; *Zootermopsis* Emerson species: Korman et al. 1991; *Hodotermopsis* Holmgren: Wang et al. 1992; *Incisitermes schwarzi* Banks: Luykx 1993; reviewed in Shellman-Reeve 1997). Especially in introduced species, such as *C. formosanus* in Hawaii, a limited number of introductions and genetic bottlenecks leading to a high degree of inbreeding and a lack of genetic polymorphism have been hypothesized (Strong and Grace 1993, Broughton and Grace 1994, Wang and Grace 1995). However, the considerable genetic variability within the population and the moderate degree of inbreeding found in the current study challenge those previous hypotheses. Further investigations of the detailed population structure of *C. formosanus* in Hawaii, as well as comparisons to genetic variability within and between colonies of introduced populations on the U.S. mainland (Korman and Pashley 1991) and natural populations in China/Taiwan are in progress to solve this apparent paradox.

In this article we defined the entire genetic hierarchy of *C. formosanus* on the island of Oahu, HI, from genetic background similarity of the population to inbred monogamous colonies. On this basis, genetic similarities and differences on various organizational levels within the population can be interpreted. The range of genetic similarities, i.e., the high variance between collection sites compared with the low variance within sites, makes it possible to study the population structure of *C. formosanus*, and its genetic patterns and processes such as the number of introductions and the history of colonization. Furthermore, colonies can be identified by their particular genetic profile and the genetic similarity of their members. By comparing data from different locations and species to the defined scale of the genetic similarity of *C. formosanus* in Hawaii, general conclusions concerning spatial and social organization of termites can be drawn.

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References Cited

Atkinson, L., and E. S. Adams. 1997. The origins and relatedness of multiple reproductives in colonies of the termite *Nasutitermes corniger*. Proc. R. Soc. Lond. B 264: 1131–1136.

Broughton, R. E. 1995. Mitochondrial DNA variation within and among species of termites in the genus *Zootermopsis* (Isoptera: Termopsidae). Ann. Entomol. Soc. Am. 88: 120–128.

Broughton, R. E., and J. K. Grace. 1994. Lack of mitochondrial DNA variation in an introduced population of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiology 24: 121–126.

Clément, J. L. 1981. Enzymatic polymorphism in the European populations of various *Reticulitermes* species (Isoptera), pp. 49–61. *In* P. E. Howse and J. L. Clément [eds.], Biosystematics of social insects. Academic, London.

Husseneder, C. 1998. Populationsgenetik und soziogenetische Organisation der Termite *Schedorhinotermes lamanianus*. Ph.D. dissertation. University of Bayreuth, Bayreuth, Germany. Bayreuther Forum Ökologie 58.

Husseneder, C., R. Brandl, C. Epplen, J. T. Epplen, and M. Kaib. 1998. Variation between and within colonies in the termite: morphology, genomic DNA, and behaviour. Mol. Ecol. 7: 983–990.

Husseneder, C., R. Brandl, C. Epplen, J. T. Epplen, and M. Kaib. 1999. Within-colony relatedness in a termite species: genetic roads to eusociality? Behaviour 136: 1045–1063.

Jarman, A. P., and R. A. Wells. 1989. Hypervariable minisatellites: recombinators or innocent bystanders? TIG 5: 367–371.

Jeffreys, A. J., M. Turner, and P. Debenham. 1991. The efficiency of multilocus DNA fingerprint probes for individualization and establishment of family relationships, determined from extensive casework. Am. J. Hum. Genet. 48: 824–840.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985. Individual specific 'fingerprints' of human DNA. Nature (Lond.) 316: 76–79.

Jenkins, T. M., C. J. Basten, R. Dean, S. E. Mitchell, S. Kresovich, and B. T. Forschler. 1999a. Matriarchal genetic structure of *Reticulitermes* (Isoptera: Rhinotermitidae) populations. Sociobiology 33: 239–263.

Jenkins, T. M., C. J. Basten, S. Kresovich, and B. T. Forschler. 1999b. Mitochondrial gene sequence questions *Reticulitermes* sp. social structure (Isoptera: Rhinotermitidae). Sociobiology 34: 161–172.

Kaib, M., C. Husseneder, C. Epplen, J. T. Epplen, and R. Brandl. 1996. Kin-biased foraging in a termite. Proc. R. Soc. Lond. B 263: 1527–1532.

Korman, A. K., and D. P. Pashley. 1991. Genetic comparisons among U.S. populations of Formosan subterranean termites. Sociobiology 19: 41–50.

Korman, A. K., D. P. Pashley, M. I. Haverty, and J. P. La Fage. 1991. Allozymic relationships among cuticular hydrocarbon phenotypes of *Zootermopsis* species (Isoptera: Termopsidae). Ann. Entomol. Soc. Am. 84: 1–9.

Lai, P. Y., M. Tamashiro, and J. K. Fujii. 1983. Abundance and distribution of the three species of symbiotic protozoa in the hindgut of *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Proc. Hawaii. Entomol. Soc. 24: 271–276.

Luykx, P. 1993. Turnover in termite colonies: A genetic study of colonies of *Incisitermes schwarzi* headed by replacement reproductives. Insectes Soc. 40: 191–205.

Lynch, M. 1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol. 5: 584–599.

Lynch, M. 1991. Analysis of population genetic structure by DNA fingerprinting, pp. 113–126. *In* T. Burke, A. Dolf, A. J. Jeffreys, and R. Wolff [eds.], DNA fingerprinting: approaches and applications. Birkhäuser, Basel, Switzerland.

Mannesmann, R., and B. Piechowski. 1989. Verteilungsmuster von Gärkammerbakterien einiger Termitenarten. Matr. Organism. (Berlin) 24: 161–178.

Miura, T., K. Maekawa, O. Kitade, T. Abe, and T. Matsumoto. 1998. Phylogenetic relationships among subfamilies in higher termites (Isoptera: Rhinotermitidae) based on mitochondrial COII gene sequences. Ann. Entomol. Soc. Am. 91: 515–523.

Pamilo, P., P. Gertsch, P. Thorén, and P. Seppä. 1997. Molecular population genetics of social insects. Annu. Rev. Ecol. Syst. 28: 1–25.

Reilly, L. M. 1987. Measurements of inbreeding and average relatedness in a termite population. Am. Nat. 130: 339–349.

Rohlf, F. J. 1990. NTSYS-PC, numerical taxonomy and multivariate analysis system. Exeter Software, New York.

Shellman-Reeve, J. S. 1997. The spectrum of eusociality in termites, pp. 52–93. *In* J. C. Choe, and B. J. Crespi [eds.], Social behaviour in insects and arachnids. Cambridge University Press, Cambridge.

Strong, K. L., and J. K. Grace. 1993. Low allozyme variation in Formosan subterranean termite (Isoptera: Rhinotermitidae) colonies in Hawaii. Pan-Pac. Entomol. 69: 51–56.

Tamashiro, M., J. R. Yates, and R. H. Ebesu. 1987. The Formosan subterranean termite in Hawaii: Problems and control. *In* M. Tamashiro and N-Y. Su [eds.], Biology and control of the Formosan subterranean termite. Research Extension Series 083. University of Hawaii, Honolulu.

Thompson, G. J., and P.D.N. Hebert. 1998. Population genetic structure of the Neotropical termite *Nasutitermes nigriceps* (Isoptera: Termitidae). Heredity 80: 48–55.

Wang, J., and J. K. Grace. 1995. Using a genetic marker (MDH-1) to study genetic structure in colonies of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Hawaii Agriculture: Positioning for Growth. Conference Proceedings. CTAHR Univ. Hawaii (Honolulu) 168–169.

Wang, J., and J. K. Grace. 2000a. Genetic relationship of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) populations from the United States and China. Sociobiology 36: 7–19.

Wang, J., and J. K. Grace. 2000b. Allozyme patterns of protozoan symbionts of *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Sociobiology 35: 467–474.

Wang, J., K. Masuko, O. Kitade, and T. Matsumoto. 1992. Alloenzyme variation and genetic differentiation of colonies in the damp wood termite *Hodotermopsis* of Japan and China. Sci. Pap. Coll. Arts Sci. Univ. Tokyo (Biol.) 42: 95–109.

Wong, Z., V. Wilson, A. J. Jeffreys, and S. I. Thein. 1986. Cloning a selected fragment from a human DNA "fingerprint": Isolation of an extremely polymorphic minisatellite. Nucleic Acids Res. 14: 4605–4616.

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