

Rediscovery and DNA Sequencing of *Leptanilla tanakai* Baroni Urbani (Hymenoptera: Formicidae) from Yakushima Island, Japan

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Abstract *Leptanilla tanakai* Baroni Urbani, 1977 was rediscovered from Yakushima Island in thirty five years since its original description. DNA sequences have been generated for *L. tanakai* using the six nuclear genes with a total of 5.8 kb. The sequence divergence between the other related species is analyzed.

The ant genus *Leptanilla* is known from Old World, including Europe, Africa to Asia, and Australia (Baroni Urbani, 1977). Workers of the genus are small subterranean ants, and rarely collected in the fields. Whereas the males are sometimes collected separately by light traps or Malaise traps. Such non-nest series collections can lead to a parallel taxonomy, then confusing the species identification (Bolton, 1990; Ward and Sumnicht, 2012). Ogata *et al.* (1995) described the males of two unidentified *Leptanilla* species from Kyushu and the Ryukyus, but the names unknown. As Ward and Sumnicht (2012) suggested, DNA sequence data provide information to link the separated collections, especially in the rare ant group. In



Fig. 1. *Leptanilla tanakai* Baroni Urbani. Lateral view of a worker collected from Onoaida Trail, Yakushima Island.

the present study, we sequence the workers only, but the DNA sequence data can contribute to the correct identification when the male are collected in the future.

Leptanilla tanakai is described by Baroni Urbani (1977) based on the worker specimens from Yakushima Island (30°20'N, 130°31'E), which is located in the northern part of the Nansei Islands, Japan. No additional record has ever been reported from except the original type locality for more than 30 years. During our field surveys in 2012, we collected 74 workers of *L. tanakai* (Fig. 1) from Onoaida Trail (200 m alt) of Yakushima Island. They were foraging in the soil under rotten woods. Here we report the rediscovery of the species and generate DNA sequencing for taxonomic convenience and molecular phylogeny in the future. Up to present, six described species of *Leptanilla* are known from Japan (Japanese Ant Database Group, 2003; Terayama, 2013), but the molecular data has not been sequenced in any other Japanese species. This is the first and comprehensive report of DNA sequencing in Japanese *Leptanilla* species.

Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Maryland, USA). A destructive technique (entire ant pulverized) was used because the nest series are available (collection number: SH12-Yak-12).

We sequenced fragments of six nuclear genes: 18S ribosomal DNA (18S, 1851 bp), 28S rDNA (28S, 1798 bp), wingless (Wg, 412 bp), abdominal-A (AbdA, 600 bp), elongation factor 1-alpha F2 copy (EF1αF2, 517 bp) and ultrabithorax (Ubx, 630 bp). Six new sequences were generated for this study (Table 1), and the remainder were taken from Ward and Downie (2005), Ward

Table 1. List of species included in the analysis, with DDBJ accession numbers.

Species	Gene					
	18S	28S	Wg	AbdA	EF1αF2	Ubx
<i>Leptanilla tanakai</i>	KF724952	KF724953	AB860300	AB857244	AB856539	AB856540
<i>Leptanilla</i> GR01a	EF012871	EF012999	EF013707	JN967846	JN967829	JN967809
<i>Leptanilla</i> GR01b	JN967870	JN967862	JN967854	JN967847	JN967830	JN967810
<i>Leptanilla</i> GR02a	JN967871	JN967863	JN967855	EF013127	EF013431	JN967811
<i>Leptanilla</i> GR02b	JN967872	JN967864	JN967856	JN967848	JN967831	JN967812
<i>Leptanilla</i> GR02c	JN967873	JN967865	JN967857	JN967849	JN967832	JN967813
<i>Leptanilla</i> GR03a	JN967874	JN967866	JN967858	JN967850	JN967833	JN967814
<i>Leptanilla</i> GR03b	JN967875	JN967867	JN967859	JN967851	JN967834	JN967815
<i>Leptanilla</i> GR03c	JN967876	JN967868	JN967860	JN967852	JN967835	JN967816
<i>Leptanilla</i> TH01	JN967869	JN967861	JN967853	JN967845	JN967828	JN967808
<i>Leptanilla</i> ZA01	AY867436	AY867452	AY867421	AY867468	EF013432	JN967807
<i>Protanilla</i> JP01	EF012925	EF013053	EF013761	EF013181	EF013499	JN967806

Table 2. Percent nuclear sequence divergence (uncorrected) between *Leptanilla tanakai* and other species.

Species	Nuclear gene	<i>L. GR01a</i>	<i>L. GR01b</i>	<i>L. GR02a</i>	<i>L. GR02b</i>	<i>L. GR02c</i>	<i>L. GR03a</i>	<i>L. GR03b</i>	<i>L. GR03c</i>	<i>L. TH01</i>	<i>L. ZA01</i>	<i>P. JP01</i>
<i>Leptanilla tanakai</i>	18S	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.9
<i>Leptanilla tanakai</i>	28S	2.2	2.2	1.7	1.7	2.3	2.4	2.3	3.4	3.1	5.5	
<i>Leptanilla tanakai</i>	Wg	5.5	5.5	5.5	5.5	5.9	5.9	5.9	11.9	5.9	17.8	
<i>Leptanilla tanakai</i>	AbdA	25.3	25.3	25.9	25.9	25.3	25.3	25.3	28.4	25.4	28.7	
<i>Leptanilla tanakai</i>	EF1aF2	5.8	5.8	6.8	6.8	6.4	6.4	6.4	8.7	8.1	15.5	
<i>Leptanilla tanakai</i>	Ubx	3.3	3.3	3.4	3.4	2.8	2.8	2.8	9	3.1	9.7	

and Sumnicht (2012). Primers, amplification and sequencing procedures followed Ward and Downie (2005), Brady *et al.* (2006), and Ward *et al.* (2010). The sequence data were deposited at DNA Data Base of Japan, DDBJ.

Reactions were carried out at 10 °C volumes in a PCR Thermal Cycler MP (TaKaRa Bio Inc.) under the following conditions: first 40 cycles of 95°C for 30 s, annealing at 50–58°C for 30 s, and 72°C for 90 s, then 1 cycle of 95°C for 1 min, and finally 72 °C for 3 min. PCR products were visualized on a 1% agarose E-Gel 96-well system (Invitrogen), and then purified with 1.0 μl of ExoSAP-IT (GE Healthcare Life Sciences). All products were sequenced in both directions using BigDye Terminator v3.1 (Applied Biosystems) on an ABI 3100 Avant DNA Sequencer (Applied Biosystems) at the Faculty of Science, Kyushu University, Fukuoka. Contigs were made using Vector NTI Advance TM ver. 11 (Invitrogen Corp.) and subsequently aligned by eye. Genetic distances were estimated using the p-distances with MEGA 5 (Tamura *et al.*, 2011). The hypervariable region of 28S gene were removed prior to the analysis.

The genetic divergences were shown in Table 2. The largest interspecific genetic distances among *Leptanilla* species were manifested by the genes 28S (0.034), Wg (0.119), AbdA (0.284), EF1aF2 (0.087), Ubx (0.09) in *L. tanakai* vs. *L. TH01*. Although no phylogenetic analysis was carried out in this study, *Leptanilla tanakai* was supposed to be closer to *L. GR01*, *L. GR02*, and *L. GR03* than to *L. TH01* and *L. ZA01*.

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