



## Identification of microsatellite markers for a worldwide distributed, highly invasive ant species *Tapinoma melanocephalum* (Hymenoptera: Formicidae)

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**Abstract.** *Tapinoma melanocephalum* is a worldwide distributed, highly invasive ant species. It lives in close association with human societies and its distribution is human-mediated in large measure. The geographical origin of this ant species is unknown, but its introduction in areas previously devoided of its presence can represent a threat to the native biota, act as an agricultural pest or as a pathogen vector. To investigate the genetic structure and phylogeography of this species we identified 12 new polymorphic microsatellite markers, and in addition, we tested and selected 12 ant-universal microsatellites polymorphic in *T. melanocephalum*. We genotyped 30 individuals from several islands of Micronesia and Papua-New Guinea. All 24 loci exhibited strong homozygosity excess (45–100%, mean = 86%), while the number of alleles per locus reached usual values (2–18, mean = 6.5), resulting in levels of expected heterozygosity much higher than observed. Based on several robust tests, we were able to exclude artefacts such as null alleles and allelic dropout as a possible cause of the observed pattern. Homozygosity excess might be a consequence of founder effect, bottleneck and/or inbreeding. As our sample population was composed of individuals from several distinct localities, the Wahlund effect might have contributed to the increased homozygosity as well. Despite the provisionally observed deviation from the Hardy-Weinberg equilibrium, the newly developed microsatellites will provide an effective tool for future genetic investigations of population structure as well as for the phylogeographic study of *T. melanocephalum*.

### INTRODUCTION

*Tapinoma melanocephalum* (Fabricius, 1793), also known as the “ghost ant”, is one of the most widely distributed ant species on Earth. It is ubiquitous throughout tropical and subtropical areas and is also present in temperate zones, where it is confined to indoor environments. It has been documented at more than 1500 sites across the globe with the highest latitude records from Finland, Scotland, Manitoba, and Quebec in the northern hemisphere and from New Zealand in the southern hemisphere (Wetterer, 2009). This species is one of the most common ants associated with humans and is often restricted to disturbed habitats and human-made environments. Indoor nests are usually found within the structures of buildings such as cracks and wall voids, and outdoor nests in flowerpots or under objects on the ground (Choe et al., 2009).

*T. melanocephalum* has been so widely distributed by commerce that it is difficult to determine its exact geographical origin (Wen, 2007). Although it has been debated, the current hypothesis is that its natural range is located in tropical Asia or in the Indo-Pacific (Wetterer, 2009) where it is also most abundant. Clearly,

a comprehensive phylogeographic study is needed to identify the region where the species originated.

Morphologically, *T. melanocephalum* can be characterized by a length of 1.5–2 mm, a laterally compressed mesonotum broadening anteriorly (Collingwood, 1979) and by a distinctly bicoloured body, with the head and thorax brown to dark brown while the abdomen, legs, and antennae are yellow or whitish. However, variability in the coloration pattern and other characters has been documented and several regional forms are recognized (e.g. *Tapinoma melanocephalum malesianum*), most of them comprehensively summarized by Wetterer (2009). In Asia and the Indo-Pacific, *T. melanocephalum* can also be confused with several similar species, in particular *Tapinoma minutum* or *Tapinoma indicum*. Furthermore, there are reports of species with intermediate characters between these taxa (Clouse, 2007) and their exact species status remains to be determined by a phylogenetic analysis. Consequently, positive identification of *Tapinoma melanocephalum* in the Indo-Pacific can be difficult.

Surprisingly, the social structure of *T. melanocephalum* has not been studied in close detail. The species was reported to be po-

lygynous with individual nests containing hundreds to thousands of workers (Harada, 1990), but more specific information about the numbers of queens in a colony is absent. The species is also considered unicolonial and polydomous. It reproduces by colony budding and does not exhibit aggressiveness among colonies co-existing in the same area (Smith, 1965; Bustos & Cherix, 1998). However, detailed information about occurrence and population-level variability of these traits is not available. These features of colony organization are typical of many invasive ant species (Tsutsui & Suarez, 2003) and are most likely the underlying reasons behind the unprecedented biological success of this species. The combination of polygyny and polydomy allows for fast and frequent relocations of the colonies and allows the species to occupy temporary habitats (Passera, 1994; Appel et al., 2004).

Despite being virtually omnipresent, *T. melanocephalum* has not received as much attention as other invasive ants, e.g. *Solenopsis invicta* or *Linemiptema humile*. This is likely due to its rather non-aggressive nature, lack of sting and because it does not cause as obvious disturbances to affected environment as some of the other introduced ants.

On the other hand, several studies show that it can be a serious pest. The species is known to dominate some subtropical and tropical agricultural systems where it tends phloem feeding Hemiptera. This leads to plant damage and to an increase of plant pathogens, including viral and fungal infections (Venkataramaiah & Rehman, 1989). In Papua New Guinea, *T. melanocephalum* was one of the only ant species whose number increased greatly in the canopy and understory of primary and secondary forests following a focused eradication of ant assemblages (Klimes et al., 2012).

In indoor habitats, this species has been found abundantly in hospitals in Brazil, where it has the high potential of acting as an agent in the spread of pathogens. Moreira et al. (2005) found multiple types of bacteria associated with *T. melanocephalum* workers, some of them with antibiotic resistance. This suggests that its presence may have, in some cases, serious medical and epidemiological consequences.

Considering how common *T. melanocephalum* is, our lack of knowledge on its social structure and life history is surprising. While most of the literature has focused on practical tasks related to its eradication, studies on its genetic structure and population history are missing. Here, we present the first study investigating and identifying microsatellite markers for this species in order to provide an insight into the genetic structure of *T. melanocephalum* populations. This will establish a baseline which will facilitate future genetic studies on the social structure and phylogeography of *T. melanocephalum*. Indeed, a better knowledge of the genetic relationships between and among the colonies and populations of *T. melanocephalum* will contribute to a more efficient management of this pest. Furthermore, the species-specific markers will allow detailed studies of the species' population genetic and phylogeographic history. This can allow us to determine the region of origin of *T. melanocephalum* and to compare its dispersal routes with the patterns of human movement and/or with trajectories of trade and commerce.

Despite the development and increasing availability of next-generation sequencing (NGS) (Eklom & Galindo, 2011), classical genetic markers such as mitochondrial DNA and microsatellites remain irreplaceable tools for most molecular ecologists. Their sequencing and assessment are user friendly, easy to perform, cheap, and comparable with constantly growing number of analysed organisms. Microsatellites also have several important advantages in comparison to NGS. For example, with the use of genomic data, Butler et al. (2014) developed microsatellite markers that are both conserved and applicable among such large

taxonomic group as ants, yet polymorphic within species. Their study highlights possible trend in future utilization of microsatellites for researchers investigating other taxonomic groups. Mesak et al. (2014) compared the performance of microsatellites with RAD-seq SNP methods to characterize clonal patterns in a killifish (*Kryptolebias marmoratus*). They concluded that next-generation RAD-seq technology may have significant constraints in revealing the true genetic pattern compared to classical microsatellites (i.e. phylogenetic noise, issues when lacking a reference genome). Finally, a study by Schlick-Steiner et al. (2014) compared the characteristics of microsatellites to two NGS approaches and found microsatellite-based population genetics to require a smaller amount of DNA, exhibited fewer issues caused by DNA contaminants and were time-efficient (among other advantages). Furthermore, they pointed out that non-model organisms do not benefit as much from NGS as model organisms do due to a lack of background information and financial resources.

## MATERIALS AND METHODS

Samples (exclusively female workers) were collected between 2008 and 2014 at several locations in Papua New Guinea (PNG) and the Federate States of Micronesia (Table 1). Specimens were determined preliminarily in the field and their identification later confirmed under stereoscope by comparisons with specimens deposited at Harvard Museum of Comparative Zoology (MCZ) and using online identification resources at [www.antkeys.org](http://www.antkeys.org). To confirm correct species identification and the species limits, a 601 bp fragment of *cytochrome c oxidase 1 (COI)* subunit was sequenced from each individual and the haplotypes were compared with 41 additional *COI* sequences of four other *Tapinoma* species using Neighbour-Joining and Bayesian phylogenetic reconstruction (Lebrasseur, 2014).

Genomic DNA was extracted from specimens using Genomic DNA Mini Kit Tissue (Geneaid Biotech Ltd., New Taipei City, Taiwan). The concentration of isolated DNA was measured using NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). The DNA of 12 individuals was pooled together and sent to Genoscreen (Lille, France) for the Geno Sat<sup>®</sup> service, which includes microsatellite-enriched library preparation and sequencing by 454 Genome Sequencer FLX Titanium (454, Roche Applied Science; for more details see Malausa et al., 2011). The following eight probes were used to enrich the total DNA in these motifs: (TG)<sub>10</sub>, (TC)<sub>10</sub>, (AAC)<sub>8</sub>, (AAG)<sub>8</sub>, (AGG)<sub>8</sub>, (ACG)<sub>8</sub>, (ACAT)<sub>6</sub>, (ACTC)<sub>6</sub>. GsFLX libraries were performed on PCR products. The company processed our samples with three others on an eighteen-sample run, with each sample individualized by the use of a Tag sequence. Concentration of each library was determined by fluorometry in order to get a minimum quantity of 1.46E + 8 mol/μl.

**Table 1.** Information about localities where the samples were collected.

Area	Locality	GPS	N
Micronesia	Chuuk	7°27'N, 151°53'E	1
Micronesia	Yap	9°31'N, 138°07'E	2
Micronesia	Pohnpei	6°58'N, 158°12'E	9
PNG	Wanang	5°13'S, 145°05'E	3
PNG	Port Moresby	9°14'S, 147°16'E	1
PNG	Weam	8°39'S, 141°08'E	2
PNG	Daru	9°52'S, 143°13'E	2
PNG	Baitabag	5°09'S, 145°47'E	4
PNG	Nagada	5°15'S, 145°79'E	4
PNG	Lepa Island	5°11'S, 145°50'E	1
PNG	Sinu Island	5°08'S, 145°49'E	1

The obtained primers were analysed for all primer secondary structures including hairpins, self-dimers and cross-dimers in primer pairs, using the on-line application NetPrimer (<http://www.premierbiosoft.com/netprimer/>). These secondary structures should be avoided if possible, because they could reduce amplification success. Primers were also checked for the presence of G or C bases within the last five bases from the 3' end of primer (GC clamp), which helps to promote specific binding at the 3' end due to the stronger bonding of G and C bases. In general, we followed the PCR primer design guidelines reviewed at [http://www.premierbiosoft.com/tech\\_notes/PCR\\_Primer\\_Design.html](http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html). Based on the NetPrimer analysis, we selected the 20 most suitable primer pairs for further testing. Initially, we performed monoplex PCR on 16 individuals with fluorescently labelled primers, followed by fragment analysis on the automated sequencer ABI 3730XL (Applied Biosystems, Foster City, California, USA). One PCR reaction consisted of 4 µl of Multiplex PCR Master-Mix (Qiagen, Hilden, Germany), 0.2 µM of each primer, 20 ng of template DNA and 3.6 µl of PCR water. For PCR conditions, we followed the manufacturer's protocol, with the annealing temperature of 54°C. Allelic patterns were scored using the software Genemapper 3.7 (Applied Biosystems).

Out of the tested loci, we selected those which were constantly amplified successfully and which provided clearly scorable and polymorphic PCR products. In the next step we pooled these loci into multiplex panels and used them to genotype all 30 individuals under the same PCR conditions as for the monoplex PCR. Basic parameters of loci, such as number of alleles, observed and expected heterozygosities, and Hardy-Weinberg equilibrium were calculated using the software GenAlEx (Peakall & Smouse, 2006). Exact tests for linkage disequilibrium were performed using the software Genepop 4.0 (Rousset, 2008). In order to obtain detailed information on population-genetic patterns and also

for possible comparisons, we analysed the same 30 individuals using recently developed microsatellite markers, which should be universal among ants (Butler et al., 2014). We tested 23 of these loci for polymorphism in *T. melanocephalum* and calculated the same population-genetic parameters using polymorphic loci. To get the comparison of population-genetic patterns, we analysed six other ant species using the set of universal microsatellites which were found polymorphic in *T. melanocephalum*. Finally, to get the information about genetic variability within a single population, we genotyped 20 individuals of *T. melanocephalum* sampled at Wanang village, Papua-New Guinea, using the 12 newly developed loci. In *T. melanocephalum*, PCR, fragment analysis and genotyping were performed twice independently for all 30 individuals and all loci to assess the possible occurrence of allelic dropout (Gagneux et al., 1997).

## RESULTS

The concentration of isolated DNA ranged from 0.5 to 15 ng/µl. The library quantification resulted in  $5.24E + 09$  mol/µl, a sufficient amount for performing emulsion PCR. Emulsion PCR results of the pool which contained our samples showed 88% beads recovery and 10% enrichment. In total, we obtained 31584 sequences (average length = 316 bp), of which 8090 contained microsatellite motifs and the software analysis (provided by GenoScreen) resulted in 450 bioinformatically validated pairs of primers. Out of the 20 loci tested, 12 were amplified consistently, were clearly scorable and polymorphic. We pooled these loci into 3 multiplex panels and the obtained genotypes were used to calculate the basic parameters of the loci (Table 2). The number of alleles per locus ranged from 2 to 18 (mean = 6.6) and the average expected and observed heterozygosities were 0.645 and 0.144, respectively. None of the loci were in Hardy-Weinberg equilibrium (HWE). The tests for linkage disequilibrium resulted in signifi-

**Table 2.** Characterization of 12 newly developed microsatellite markers in *Tapinoma melanocephalum*. Locus code, GenBank accession number, forward (F) and reverse (R) primer sequences, repeat motif, size range of alleles, number of alleles (Na), observed (*Ho*) and expected (*He*) heterozygosities based on analysis of 30 individuals.

Locus	Primer sequences (5'–3')	Repeat motif	Size range (bp)	Na	<i>Ho</i>	<i>He</i>	Multiplex
TM_2	F: GAAGGATGTCGAAATCGCAG	AC	88–136	18	0,367	0,916	1
KX197932	R: ACAATGTCCGAGAAGCAAGC						
TM_3	F: TGCAATGTAACAGTTTGAGGC	CT	141–150	5	0,033	0,626	1
KX197933	R: GGGAATAATTACCGTGTTGG						
TM_6	F: GAGCAAGTGAAGAAAGGAGG	AGG	87–90	2	0,033	0,206	1
KX197934	R: GTAGTGCGAAGACAATGGCA						
TM_9	F: ATACTTCCATGCACCAAGG	ACA	197–221	5	0,467	0,600	1
KX197935	R: GCGTCGTTGTCTTCTTCCTC						
TM_10	F: TCATCCGTTTGATGAAGTGC	CT	87–97	5	0,067	0,663	1
KX197936	R: CTAGGAGGAAGCAGTGGAGC						
TM_5	F: AGAGCGTCACCCGATGTC	TC	184–216	12	0,300	0,772	2
KX197937	R: TGTCATTTTCTTGCACTTGTGA						
TM_13	F: CTTCTGATGCAGCTAGGGGA	TC	160–166	4	0,133	0,610	2
KX197938	R: ATTTCTTCGCGTCTGATTCG						
TM_16	F: AGCCAAGAGTTTCGTTCTCG	TCG	217–238	7	0,067	0,642	2
KX197939	R: TCTCTTTACGACGGTTCGCT						
TM_17	F: AATCCTTCTATTCCCACCG	AC	150–154	3	0,033	0,485	3
KX197940	R: TCACCTTACTCAAGTCCAGGG						
TM_18	F: ATTCTCCGTAATCACGCTCG	TC	105–109	3	0,000	0,631	3
KX197941	R: CGTGGTGGAAAGGATATACG						
TM_19	F: AATGTGATCGTGATGAGCCA	CA	217–238	9	0,200	0,806	3
KX197942	R: GGTTCCTAGAACTTTGCGACG						
TM_20	F: TTAGCTGAATTTTCATTGAACG	AT	287–301	6	0,033	0,777	3
KX197943	R: CTCGCTGGCCTGTTCTCCTC						
Mean				6,6	0,144	0,645	

**Table 3.** Parameters of universal microsatellite loci polymorphic in *Tapinoma melanocephalum*. Locus code, size range of alleles, number of alleles (Na), observed (*Ho*) and expected (*He*) heterozygosities based on analysis of 30 individuals.

Locus	Size range (bp)	Na	<i>Ho</i>	<i>He</i>
Ant859	190–203	5	0,037	0,629
Ant7680	274–278	2	0,037	0,036
Ant5035	325–358	9	0,222	0,621
Ant10878	300–399	4	0,032	0,232
Ant7249	339–347	3	0,074	0,201
Ant1343	187–205	3	0,000	0,271
Ant4155	192–219	10	0,556	0,822
Ant2794	256–323	8	0,074	0,527
Ant1368	326–330	5	0,074	0,722
Ant9218	341–379	7	0,370	0,783
Ant3648	332–372	11	0,037	0,864
Ant8424	200–248	10	0,185	0,835
Mean		6,4	0,142	0,545

cant values in 166 out of 276 pairs of loci. Out of the 23 universal microsatellites, 12 were polymorphic in *T. melanocephalum* and were analysed under the same conditions as the de novo developed loci. The number of alleles per locus ranged from 2 to 11 (mean = 6.4) and the average expected and observed heterozygosities were 0.545 and 0.142 respectively. None of the loci were in HWE (Table 3). Population genetic parameters of six other ant species obtained with the use of the same 12 universal microsatellites are summarized in Table 4. Values of observed (*Ho*) and expected (*He*) heterozygosities within these six species ranged from 0.23 to 0.63 (mean = 0.46) and from 0.24 to 0.68 (mean = 0.53), respectively, and the mean number of alleles ranged from 2.1 to 4.9 (mean = 3.8). Genetic variability parameters of the single population of *T. melanocephalum* from Wanang village (PNG) are given in Table 5. The number of alleles per locus ranged from 1 to 3 (mean = 1.66) and the average expected and observed heterozygosities were 0.147 and 0.008, respectively. No differences in genotypes were observed comparing the two independent PCRs and fragment analyses.

**DISCUSSION**

We detected enormously high level of homozygosity in the newly developed microsatellite loci, ranging between 53 and 100% among individual loci and with a mean over all loci of

**Table 5.** Genetic variability among 20 individuals of *T. melanocephalum* within a single population (Wanang, PNG) and the 12 newly developed microsatellite loci. Number of alleles (Na), observed (*Ho*) and expected (*He*) heterozygosity.

Locus	Na	<i>Ho</i>	<i>He</i>
TM_2	2	0,000	0,375
TM_3	1	0,000	0,000
TM_5	1	0,000	0,000
TM_6	1	0,000	0,000
TM_9	1	0,000	0,000
TM_10	2	0,000	0,255
TM_13	2	0,050	0,289
TM_16	2	0,000	0,095
TM_17	1	0,000	0,000
TM_18	3	0,050	0,096
TM_19	1	0,000	0,000
TM_20	3	0,000	0,654
Mean	1,66	0,008	0,147

85.6%. Simultaneously, we detected average levels of genetic diversity, with the number of alleles per locus ranging from 2 to 18 and a mean over all loci of 6.6. Several biological processes might lead to such pattern, in particular founder effect, bottleneck and/or inbreeding (Frankham et al., 2008). These phenomena are expected in *T. melanocephalum* based on what has been described about its life history with the strong ability to disperse over large distances and establishing new colonies with only a few individuals (Wetterer, 2009). The genetic variability detected within the single population from Wanang village (PNG) was extremely low, both in terms of number of alleles per locus and observed heterozygosity. This finding strongly supports the conclusion about the presence of population-genetic phenomena mentioned above in *T. melanocephalum*.

The presence of colony budding, polydomy and lack of aggressiveness among physically separate colonies are suggestive of a decreased genetic variability or high levels of relatedness among individuals. These are often a consequence of within-nest mating, limited dispersal of males and/or females or parthenogenetic reproduction (Pearcy et al., 2006) and have been documented in multiple ant species (Tronetti et al., 2005; Thurin & Aron, 2009; Kureck et al., 2012).

To support this interpretation, we performed a comparative analysis of microsatellite genotypic patterns using universal mi-

**Table 4.** Parameters of 12 universal microsatellite loci, which were polymorphic in *Tapinoma melanocephalum* used for analyses of six other ant species (4 individuals per species). Number of alleles (Na), observed (*Ho*) and expected (*He*) heterozygosities. \*Mean values were calculated based on polymorphic loci only.

Locus	<i>Anonychomyrma</i>			<i>Camponotus</i>			<i>Odontomachus</i>			<i>Pseudolasius</i>			<i>Nylanderia</i>			<i>Pholidris</i>		
	Na	<i>Ho</i>	<i>He</i>	Na	<i>Ho</i>	<i>He</i>	Na	<i>Ho</i>	<i>He</i>	Na	<i>Ho</i>	<i>He</i>	Na	<i>Ho</i>	<i>He</i>	Na	<i>Ho</i>	<i>He</i>
Ant859	4	0,500	0,688	2	0,000	0,375	2	0,000	0,375	3	1,000	0,625	2	0,000	0,500	1	0,000	0,000
Ant7680	7	1,000	0,844	1	0,000	0,000	3	0,500	0,594	1	0,000	0,000	2	0,000	0,375	7	1,000	0,844
Ant5035	6	0,500	0,813	5	0,750	0,688	5	1,000	0,750	2	0,000	0,500	6	1,000	0,781	1	0,000	0,000
Ant10878	3	0,250	0,406	6	0,750	0,781	5	0,750	0,750	6	0,500	0,813	1	0,000	0,000	3	0,250	0,531
Ant7249	2	0,250	0,219	2	0,000	0,375	3	0,500	0,406	2	0,500	0,375	2	0,250	0,469	2	0,250	0,219
Ant1343	7	1,000	0,844	1	0,000	0,000	1	0,000	0,000	5	1,000	0,750	2	0,250	0,219	7	0,750	0,844
Ant4155	3	0,000	0,625	4	0,500	0,719	1	0,000	0,000	5	1,000	0,781	7	1,000	0,844	7	0,750	0,844
Ant2794	6	1,000	0,813	4	0,500	0,563	1	0,000	0,000	5	1,000	0,781	6	0,500	0,813	8	1,000	0,875
Ant1368	7	1,000	0,844	4	0,750	0,656	1	0,000	0,000	6	0,750	0,813	4	0,500	0,688	6	0,750	0,813
Ant9218	6	0,750	0,813	5	0,750	0,750	1	0,000	0,000	2	0,000	0,500	4	0,250	0,656	5	0,500	0,750
Ant3648	3	0,500	0,531	5	0,750	0,688	1	0,000	0,000	3	0,667	0,667	4	0,250	0,656	7	0,750	0,844
Ant8424	5	0,750	0,688	5	0,250	0,781	1	0,000	0,000	3	0,250	0,594	5	0,500	0,750	4	0,500	0,688
Mean*	4,9	0,625	0,677	4,2	0,500	0,638	4,6	0,550	0,575	3,82	0,606	0,650	4	0,410	0,610	5,6	6,500	0,730

crossatellite loci developed by Butler et al. (2014). We tested 23 universal microsatellites for polymorphism in *T. melanocephalum* and found 12 polymorphic loci (Table 3). The same 30 individuals were genotyped using these universal loci. We detected similar patterns to those of our newly developed loci – high levels of homozygosity (mean over all loci = 86%) and common levels of allelic variability (mean number of alleles per locus = 6.4). To show that such parameters are specific for *T. melanocephalum*, we used the same 12 universal microsatellites to assess genetic diversity in six other Indo-Pacific ant species/genera (*Anonychomyrma scrutator*, *Camponotus maculatus*, *Odontomachus similimus*, *Pseudolasius australis*, *Nylanderia vaga*, *Philidris cordata*), analysing four individuals per species. In all six species the levels of observed and expected heterozygosities were balanced and no homozygosity excess was observed (Table 4).

The populations of *T. melanocephalum* from PNG and Micronesia exhibited deviations from Hardy-Weinberg equilibrium in all analysed loci. We performed several tests to prove that this observation was not an artefact but represented genuine population-genetic pattern. As we aimed to detect the allelic diversity within the region, we composed the dataset of individuals from three different islands of Micronesia and eight different (and geographically distinct) areas of PNG. Such combination may and obviously did result in the Wahlund effect, e.g. homozygosity excess caused by several genetically distinct units grouped and considered as a single sampling unit (Selkoe & Toonen, 2006). Tests for linkage disequilibrium resulted in significant values in 60% of loci pairs, however this is clearly an artefact caused by the homozygosity excess and Hardy-Weinberg disequilibrium (Sabbati & Risch, 2002; Slatkin, 2008).

Genotypes based on two independent PCRs were identical in all 30 individuals and 24 loci, so the occurrence of allelic dropout can be considered absent or negligible. This conclusion is also supported by the sufficient concentration of isolated DNA and by our success in amplifying 601 bp fragment of mtDNA.

The last possible artefact responsible for these observed patterns would be null alleles, but we can also reject this option as we did not detect any individual that would fail to amplify any allele at just one or several loci, while the rest of the loci would amplify normally (Selkoe & Toonen, 2006) – in this study, we observed homozygote excess in all 24 analysed loci and at least one allele was amplified in all individuals and all loci.

## CONCLUSION

In this paper, we described 12 newly developed polymorphic microsatellite markers for *Tapinoma melanocephalum*, a widespread invasive ant species. Recognition of whole-area population structure of this ant species might contribute to reveal the most important colonization pathways of this ubiquitous pest species and means of dispersal. Moreover, reconstructions of its population genetic structure would provide an interesting comparison to the migration patterns and dispersal history of *Homo sapiens*, a worldwide distributed primate species, being reasonably suspected of playing an important role in the ghost ant's dispersal. Besides the newly developed loci, we have tested recently published ant-universal microsatellite markers. In total, we reported 24 microsatellite markers useful for population-genetic investigations of the target species. Within a sample of *T. melanocephalum* populations from Papua-New Guinea and Micronesia, we have detected high levels of homozygosity. This finding was confirmed by a comparison of genetic diversity parameters within six other Indo-Pacific ant species using the same universal loci. Despite the deviation from the Hardy-Weinberg equilibrium in the sampled populations composed of individuals from numerous distinct localities, the newly developed microsatellites provide an effective

tool for future investigations of genetic population structure as well as for phylogeographic analyses of *T. melanocephalum*. Moreover, microsatellite analysis can also help clarify the taxonomy and species delimitation within genus *Tapinoma*, which remains until today partially unclear, especially in the Indo-Pacific region where several sister species co-occur sympatrically.

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