



Digital supplementary material to

CHIU, Y.-C., WANG, C.-C., NAI, Y.-S., PENG, M.-H., HSU, J. & NEOH, K.-B. 2026: Genetics, cuticular hydrocarbon profiles, and bacterial endosymbionts are associated with the nestmate recognition in the invasive African big-headed ant, *Pheidole megacephala*, in Taiwan. – Myrmecological News 36: 59-72.

The content of this digital supplementary material was subject to the same scientific editorial processing as the article it accompanies. However, the authors are responsible for copyediting and layout.

Tab. S1: Locations of 14 *Pheidole megacephala* colonies sampled from forest (F) and urban (U) areas in Taichung, Taiwan.

Colony name	Sampling sites
F1	24°07'17.1"N 120°35'11.7"E
F2	24°09'34.6"N 120°44'45.8"E
F3	24°10'27.8"N 120°33'58.0"E
F4	24°12'27.4"N 120°35'22.0"E
F5	24°15'22.6"N 120°45'17.5"E
F6	24°12'23.1"N 120°46'18.2"E
U1	24°03'34.8"N 120°39'40.6"E
U2	24°06'04.1"N 120°42'22.6"E
U3	24°10'03.7"N 120°36'48.0" E
U4	24°07'14.8"N 120°40'29.7"E
U5	24°02'36.2"N 120°41'47.5"E
U6	24°10'23.8"N 120°43'32.9"E
U7	24°10'07.9"N 120°41'46.1"E
U8	24°15'25.5"N 120°43'50.9"E

Tab. S2: Composition of cuticular hydrocarbons in *Pheidole megacephala*

Peak #	Name	Abb	ECL	Diagnostic MS ion
1	<i>n</i> -Pentacosane	<i>n</i> -C25	24.96	352
2	9-; 11-; 13- Methylpentacosane	9-; 11-; 13- MeC25	25.32	140/141, 252/253; 168/169, 224/225; 196/197, 351 (M-15)
3	7- Methylpentacosane	7- MeC25	25.39	112/113, 280/281, 351 (M-15)
4	5- Methylpentacosane	5- MeC25	25.50	84/85, 308/309, 351 (M-15)
5	3- Methylpentacosane	3- MeC25	25.75	308/309, 336/337, 351 (M-15)
6	<i>n</i> -Hexacosane	<i>n</i> -C26	26.02	366
7	<i>n</i> -Heptacosane	<i>n</i> -C27	27.04	380
8	9-; 11-; 13- Methylheptacosane	9-; 11-; 13- MeC27	27.36	140/141, 280/281; 168/169, 252/253; 196/197, 224/225, 379 (M-15)
9	7- Methylheptacosane	7- MeC27	27.46	112/113, 308/309, 379 (M-15)
10	5- Methylheptacosane	5- MeC27	27.52	84/85, 336/337, 379 (M-15)
11	3- Methylheptacosane	3- MeC27	27.77	336/337, 364/365, 379 (M-15)
12	<i>n</i> -Octacosane	<i>n</i> -C28	28.07	394
13	3,7-; 3,9- Dimethylheptacosane	3,7-; 3,9- diMeC27	28.13	125, 154/155, 280/281, 378/379; 126/127, 308/309, 378/379, 393 (M-15)

14	12-; 13-; 14- Methyl octacosane	12-; 13-; 14- MeC28	28.37	182/183, 252/253; 196/197, 238/239; 210/211, 224/225, 393 (M-15)
15	6-; 7- Methyl octacosane	6-; 7- MeC28	28.43	98/99, 337/338; 112/113, 322/323, 393 (M-15)
16	Nonacosene	C29:1	28.78	406
17	Nonacosene	C29:1	28.83	406
18	<i>n</i> -Nonacosane	<i>n</i> -C29	29.05	408
19	9-; 11-; 13-; 15- Methyl nonacosane	9-; 11-; 13-; 15- MeC29	29.36	140/141, 308/309; 168/169, 280/281; 196/197, 252/253; 224/225, 407 (M-15)
20	7- Methyl nonacosane	7- MeC29	29.46	112/113, 337/338, 407 (M-15)
21	5- Methyl nonacosane	5- MeC29	29.54	84/85, 364/365, 407 (M-15)
22	3- Methyl nonacosane;	3- MeC29	29.74	393, 407 (M-15)
23	5,13-; 5,15- Dimethyl nonacosane	5,13-; 5,15- diMeC29	29.83	84/85, 210/211, 252/253, 378/379; 84/85, 181, 224/225, 238/239, 378/379, 421 (M-15)
24	3,9-; 3,15- Dimethyl nonacosane	3,9-; 3,15- diMeC29	30.04	125, 154/155, 308/309, 406/407; 209, 224/225, 238/239, 406/407, 421 (M-15)
25	13- ; 14-; 15- Methyl triacontane	13-; 14-; 15- MeC30	30.31	196/197, 266/267; 210/211, 252/253; 224/228, 238/239, 421 (M-15)
26	3,7,13- ; 3,9,13- Trimethyl nonacosane	3,7,13- ; 3,9,13- triMeC29	30.36	126/127, 224/225, 252/253, 350/351, 420/421; 154/155, 224/225, 252/253, 322/323, 420/421, 435 (M-15)
27	Underivatized cholesterol	Cholesterol	30.50	107, 145, 213, 275, 386
28	Hentriacontene	C31:1	30.77	434

29	4,14-; 4,16- Dimethyltriacontane	4,14-; 4,16- diMeC30	30.87	181, 224/225, 252/253, 378/379, 407; 196/197, 209, 378/379, 407, 435 (M-15)
30	<i>n</i> -Hentriacontane	<i>n</i> -C31	30.98	436
31	9-; 13-; 15- Methylhentriacontane	9-; 13-; 15- MeC31	31.30	140/141, 336/337; 196/197, 280/281; 224/225, 252/253, 435 (M-15)
32	7- Methylhentriacontane	7- MeC31	31.38	112/113, 364/365, 435 (M-15)
33	5- Methylhentriacontane	5- MeC31	31.48	84/85, 392/393, 435 (M-15)
34	9,15- Dimethylhentriacontane 11,15-; 11,17- Dimethylhentriacontane	9,15- diMeC31 11,15-; 11,17- diMeC31	31.55	140/141, 238/239, 252/253, 350/351; 168/169, 238/239, 252/253, 322/323; 168/169, 196/197, 266/267, 322/323, 449 (M-15)
35	7,13-; 7,15- Dimethylhentriacontane	7,13-; 7,15- diMeC31	31.71	112/113, 210/211, 280/281, 378/379; 112/113, 238/239, 252/253, 378/379, 449 (M-15)
36	5,15-; 5,17- Dimethylhentriacontane	5,15-; 5,17- diMeC31	31.77	84/85, 181, 238/239, 252/253, 407; 84/85, 224/225, 266/267, 407, 449 (M-15)
37	3,9-; 3,13-; 3,15-; 3,17- Dimethylhentriacontane	3,9-; 3,13-; 3,15-; 3,17- diMeC31	31.99	125, 154/155, 336/337, 435; 210/211, 280/281, 435; 209, 238/239, 252/253, 435; 224/225, 266/267, 435, 449 (M-15)
38	12-; 13-; 14-; 15-; 16- Methyldotriacontane	12-; 13-; 14-; 15-; 16- MeC32	32.23	182/183, 308/309; 196/197, 294/295; 210/211, 280/281; 224/225, 266/267; 238/239, 252/253, 435 (M-15)
39	3,7,15- Trimethylhentriacontane	3,7,15- triMeC31	32.29	126/127, 252/253, 378/379, 449, 463 (M-15)

40	10,16-; 12,16- Dimethyldotriacotane	10,16-; 12,16- diMeC32	32.50	154/155, 252/253, 350/351; 182/183, 252/253, 322/323, 463 (M-15)
41	8,14-; 8,16- Dimethyldotriacotane	8,14-; 8,16- diMeC32	32.57	126/127, 224/225, 280/281, 378/379; 126/127, 252/253, 378/379, 463 (M-15)
42	6,12-; 6,14-; 6,16- Dimethyldotriacotane	6,12-; 6,14-; 6,16- diMeC32	32.65	98/99, 196/197, 308/309, 407; 98/99, 224/225, 280/281, 407; 98/99, 252/253, 407, 463 (M-15)
43	4,14-; 4,16- Dimethyldotriacotane	4,14-; 4,16- diMeC32	32.77	70/71, 224/225, 280/281, 435; 70/71, 209, 252/253, 435, 463 (M-15)
44	13-; 15-; 17- Methyltrtriacontane	13-; 15-; 17- MeC33	33.20	196/197, 308/309; 224/225, 280/281; 252/253, 463 (M-15)
45	13,17- Dimethyltrtriacontane	13,17- diMeC33	33.43	196/197, 252/253, 266/267, 323, 477 (M-15)
46	7,13-; 7,15-; 7,17- Dimethyltrtriacontane	7,13-; 7,15-; 7,17- diMeC33	33.60	112/113, 210/221, 308/309, 407; 112/113, 238/239, 280/281, 407; 112/113, 252/253, 226/267, 407, 477 (M-15)
47	5,13-; 5,15-; 5,17- Dimethyltrtriacontane	5,13-; 5,15-; 5,17- diMeC33	33.69	84/85, 210/211, 308/309, 435; 84/85, 238/239, 280/281, 435; 84/85, 209, 252/253, 266/267, 435, 477 (M-15)
48	7,13,15- Trimethyltrtriacontane	7,13,15- triMeC33	33.81	112/113, 210/211, 252/253, 280/281, 322/323, 421, 491 (M-15)
49	3,15-; 3,17- Dimethyltrtriacontane	3,15-; 3,17- diMeC33	33.90	209, 238/239, 280/281, 463; 237, 252/253, 266/267, 463, 477 (M-15)
50	5,9,17- Trimethyltrtriacontane	5,9,17- triMeC33	33.93	154/155, 252/253, 280/281, 378/379, 449, 491 (M-15)

51	Unknown	Unknown	34.15	
52	3,7,13-; 3,7,15- Trimethyltrtriacontane	3,7,13-; 3,7,15- triMeC33	34.21	98/99, 126/127, 224/225, 308/309, 406/407, 448/449, 477; 98/99, 126/127, 252/253, 280/281, 406/407, 448/449, 477, 491 (M-15)
53	6,12-; 6,18- Dimethyltetraatriacontane	6,12-; 6,18- diMeC34	34.59	98/99, 125, 196/197, 336/337, 435; 98/99, 209, 252/253, 280/281, 491 (M-15)
54	4,14-; 4,18- Dimethyltetraatriacontane	4,14-; 4,18- diMeC34	34.72	181, 224/225, 308/309, 463; 237, 252/253, 280/281, 463, 491 (M-15)
55	15-; 17- Methylpentatriacontane	15-; 17- MeC35	35.13	224/225, 308/309; 224/225, 280/281, 491 (M-15)
56	Unknown	Unknown	35.47	
57	7,13-; 7,15-; 7,17- Dimethylpentatriacontane	7,13-; 7,15-; 7,17- diMeC35	35.55	112/113, 125, 210/211, 336/337, 435; 112/113, 153, 238/239, 308/309, 435; 112/113, 266/267, 280/281, 435, 505 (M-15)
58	5,13-; 5,15-; 5,17- Dimethylpentatriacontane	5,13-; 5,15-; 5,17- diMeC35	35.65	84/85, 153, 210/211, 336/337, 463; 84/85, 181, 238/239, 308/309, 463; 84/85, 238/239, 266/267, 280/281, 463, 505 (M-15)
59	7,13,17-; 7,15,19- Trimethylpentatriacontane	7,13,17-; 7,15,19- triMeC35	35.76	112/113, 210/211, 280/281, 350/351, 449; 112/113, 238/239, 252/253, 308/309, 322/323, 449, 519 (M-15)
60	5,9,15-; 5,9,17- Trimethylpentatriacontane	5,9,15-; 5,9,17- triMeC35	35.89	154/155, 252/253, 308/309, 406/407, 477; 154/155, 280/281, 406/407, 477, 519 (M-15)

61	15-; 16-; 17- Methylheptatriacontane	15-; 16-; 17- MeC37	37.15	224/225, 336/337, 252/253, 308/309; 280/281, 519 (M-15)
62	Unknown	Unknown	37.43	
63	7,13-; 7,15- Dimethylheptatriacontane	7,13-; 7,15- diMeC37	37.55	112/113, 125, 210/211, 364/365, 463; 112/113, 153, 238/239, 336/337, 463, 533 (M-15)
64	5,13-; 5,15-; 5,17- Dimethylheptatriacontane	5,13-; 5,15-; 5,17- diMeC37	37.67	84/85, 210/211, 364/365, 491; 84/85, 181, 238/239, 336/337, 491; 84/85, 266/267, 308/309, 491, 533 (M-15)
65	Unknown	Unknown	37.82	
66	5,9,15-; 5,9,17- Trimethylheptatriacontane	5,9,15-; 5,9,17- triMeC37	37.93	84/85, 154/155, 252/253, 336/337, 434/435, 505; 84/85, 154/155, 280/281, 306/307, 434/435, 505, 547 (M-15)

Tab. S3: Genetic diversity in 14 *Pheidole megacephala* colonies assessed using 8 microsatellite loci in Taichung, Taiwan

Locus	F1 (n=8)				F2 (n=8)				F3 (n=8)			
	N _A	Ar	H _E	H _O	N _A	Ar	H _E	H _O	N _A	Ar	H _E	H _O
<i>Pmeg-06</i>	3.000	2.595	0.594	0.750	3.000	2.361	0.555	0.625	3.000	2.260	0.461	0.500
<i>Pmeg-07</i>	3.000	2.595	0.594	0.500	2.000	1.974	0.469	0.750	3.000	2.571	0.539	0.625
<i>Pmeg-09</i>	2.000	1.786	0.305	0.375	2.000	1.942	0.430	0.125	2.000	1.974	0.469	0.500
<i>Pmeg-10</i>	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000
<i>Pmeg-11</i>	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	3.000	2.509	0.531	0.375
<i>Pmeg-12</i>	2.000	1.993	0.500	1.000	2.000	2.000	0.500	1.000	5.000	3.499	0.711	0.875
<i>Pmeg-14</i>	2.000	1.974	0.469	0.500	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000
<i>Pmeg-15</i>	3.000	2.317	0.508	0.750	2.000	1.786	0.305	0.375	2.000	1.375	0.117	0.125
Mean	2.125	1.908	0.371	0.484	2.625	2.035	0.361	0.281	2.250	1.880	0.321	0.375
Locus	F4 (n=8)				F5 (n=8)				F6 (n=8)			
	N _A	Ar	H _E	H _O	N _A	Ar	H _E	H _O	N _A	Ar	H _E	H _O
<i>Pmeg-06</i>	3.000	2.348	0.539	0.625	5.000	3.000	0.570	0.625	2.000	1.625	0.219	0.250
<i>Pmeg-07</i>	3.000	2.361	0.555	0.250	3.000	2.749	0.633	0.625	3.000	2.604	0.602	0.750
<i>Pmeg-09</i>	2.000	1.885	0.375	0.250	2.000	1.625	0.219	0.250	2.000	1.885	0.375	0.250
<i>Pmeg-10</i>	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	3.000	1.750	0.227	0.250
<i>Pmeg-11</i>	2.000	1.786	0.305	0.125	2.000	1.942	0.430	0.375	1.000	1.000	0.000	0.000
<i>Pmeg-12</i>	5.000	3.773	0.766	0.625	2.000	1.993	0.500	0.750	4.000	3.182	0.680	1.000
<i>Pmeg-14</i>	2.000	1.375	0.117	0.125	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000

<i>Pmeg-15</i>	3.000	1.750	0.227	0.250	2.000	1.375	0.117	0.125	2.000	1.786	0.305	0.375
Mean	1.625	1.528	0.239	0.321	1.750	1.345	0.118	0.141	2.375	1.901	0.325	0.344
	U1 (n=8)				U2 (n=8)				U3 (n=8)			
Locus	N_A	Ar	H_E	H_O	N_A	Ar	H_E	H_O	N_A	Ar	H_E	H_O
<i>Pmeg-06</i>	2.000	1.942	0.430	0.375	3.000	2.000	0.320	0.375	2.000	1.786	0.305	0.375
<i>Pmeg-07</i>	1.000	1.000	0.000	0.000	3.000	2.816	0.648	0.625	4.000	2.536	0.484	0.625
<i>Pmeg-09</i>	2.000	1.786	0.305	0.375	2.000	1.786	0.305	0.375	2.000	1.786	0.305	0.375
<i>Pmeg-10</i>	1.000	1.000	0.000	0.000	2.000	1.375	0.117	0.125	1.000	1.000	0.000	0.000
<i>Pmeg-11</i>	1.000	1.000	0.000	0.000	2.000	1.942	0.430	0.375	2.000	1.989	0.492	0.625
<i>Pmeg-12</i>	2.000	2.000	0.500	1.000	3.000	2.749	0.633	1.000	4.000	3.285	0.695	0.875
<i>Pmeg-14</i>	2.000	1.989	0.492	0.625	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000
<i>Pmeg-15</i>	2.000	1.375	0.117	0.125	2.000	1.375	0.117	0.125	2.000	1.375	0.117	0.125
Mean	1.625	1.512	0.231	0.313	2.250	1.880	0.321	0.375	2.250	1.845	0.300	0.375
	U4 (n=8)				U5 (n=8)				U6 (n=8)			
Locus	N_A	Ar	H_E	H_O	N_A	Ar	H_E	H_O	N_A	Ar	H_E	H_O
<i>Pmeg-06</i>	2.000	1.974	0.469	0.750	3.000	1.750	0.227	0.250	2.000	1.375	0.117	0.125
<i>Pmeg-07</i>	2.000	1.429	0.133	0.143	3.000	2.816	0.648	0.500	2.000	1.625	0.219	0.250
<i>Pmeg-09</i>	2.000	1.974	0.469	0.250	2.000	1.885	0.375	0.500	1.000	1.000	0.000	0.000
<i>Pmeg-10</i>	1.000	1.000	0.000	0.000	2.000	1.375	0.117	0.125	1.000	1.000	0.000	0.000
<i>Pmeg-11</i>	1.000	1.000	0.000	0.000	2.000	1.885	0.375	0.500	1.000	1.000	0.000	0.000
<i>Pmeg-12</i>	2.000	2.000	0.500	1.000	3.000	2.749	0.633	0.625	2.000	1.993	0.500	1.000

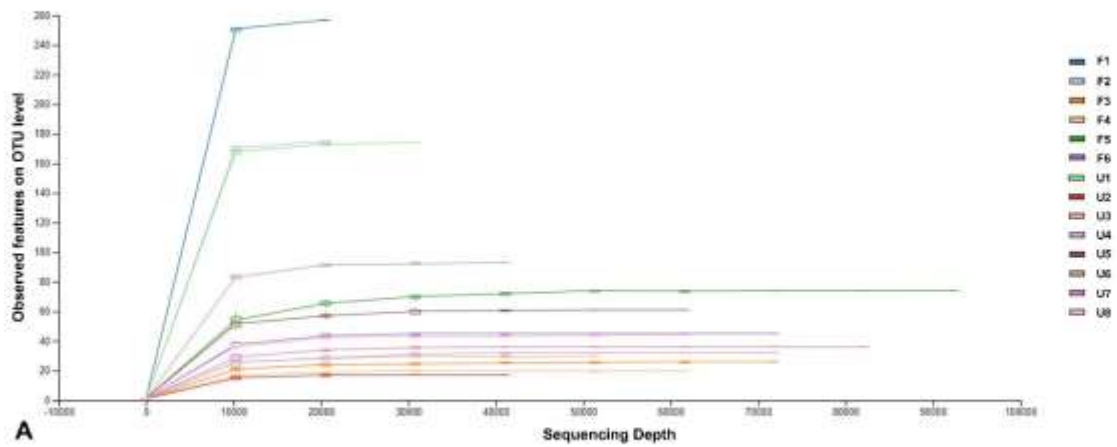
<i>Pmeg-14</i>	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000
<i>Pmeg-15</i>	2.000	1.846	0.337	0.426	3.000	1.750	0.227	0.250	1.000	1.000	0.000	0.000
Mean	1.625	1.528	0.239	0.321	2.375	1.901	0.325	0.344	1.375	1.249	0.105	0.172

Locus	U7 (n=8)				U8 (n=8)			
	N _A	Ar	H _E	H _O	N _A	Ar	H _E	H _O
<i>Pmeg-06</i>	2.000	1.885	0.375	0.500	2.000	1.974	0.469	0.250
<i>Pmeg-07</i>	3.000	1.750	0.227	0.250	2.000	1.989	0.492	0.625
<i>Pmeg-09</i>	1.000	1.000	0.000	0.000	2.000	1.786	0.305	0.375
<i>Pmeg-10</i>	3.000	1.750	0.227	0.250	3.000	2.595	0.594	0.625
<i>Pmeg-11</i>	1.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000
<i>Pmeg-12</i>	1.000	1.000	0.000	0.000	3.000	2.161	0.398	0.500
<i>Pmeg-14</i>	1.000	1.000	0.000	0.000	2.000	1.786	0.305	0.375
<i>Pmeg-15</i>	2.000	1.375	0.117	0.125	3.000	1.750	0.227	0.250
Mean	1.750	1.345	0.118	0.141	2.250	1.880	0.349	0.375
Overall mean								
	N _A	Ar	H _E	H _O				
Mean	2.071	1.745	0.283	0.330				
SE	0.035	0.023	0.008	0.008				

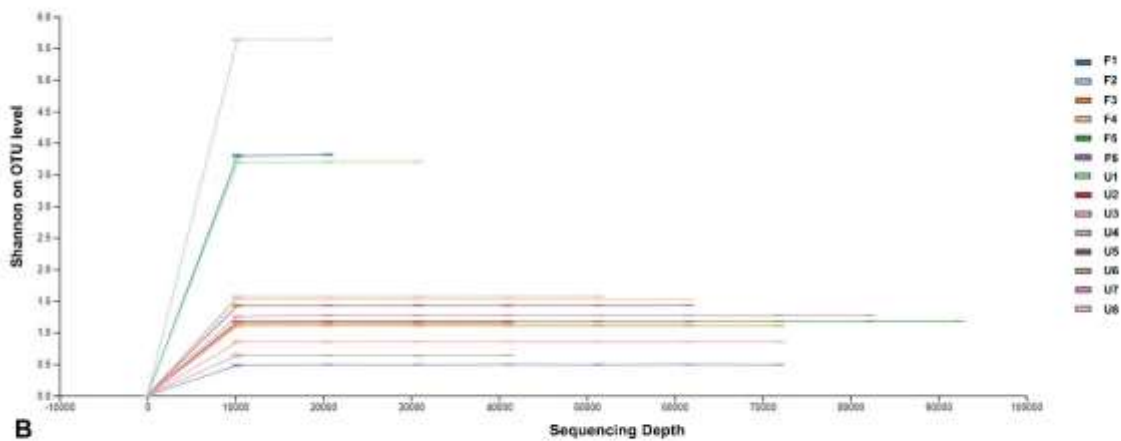
Abbreviations: Ar, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; n, total number of colonies; N_A, number of alleles.

Tab. S4: Summary of observed bacterial OTUs and Shannon index of the bacterial endosymbionts of *Pheidole megacephala* collected from forest (F) and urban (U) areas in Taichung, Taiwan

Sample ID	OTUs	Shannon
F1	120	2.99
F2	79	4.33
F3	25	1.11
F4	10	1.51
F5	64	1.17
F6	21	0.46
U1	74	2.31
U2	10	1.16
U3	13	0.84
U4	16	1.14
U5	12	1.36
U6	33	0.46
U7	20	1.26
U8	27	1.12



1



2

3 Fig. S1. Rarefaction curves for gut bacterial communities in *Pheidole megacephala*. (a)
 4 Observed features and (b) Shannon index. Each line represents a colony collected from forest
 5 (F) and urban (U) areas. Rarefaction plots were produced using QIIME 2 (<https://qiime2.org/>).

6

7

8