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Chemotaxonomic Analysis of the Venom Composition within the Ant Genus *Strumigenys* (Hymenoptera: Formicidae) in Taiwan

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Abstract

In Taiwan, the ant genus *Strumigenys* is represented by 13 species, nine of which being endemic to this island. Classic morphological taxonomy can be complex and may lead to equivoque identification within this group. To clarify subtle species assignments, we investigated the venom composition of five *Strumigenys* species, using SPME extraction and GC/MS analyses, and searched for a suitable chemical marker. Our results indicate that three out of the five species tested showed enough specificity in their chemical profiles to allow clear differentiation. However, the two remaining species could not be distinguished from each other on the basis of their venom composition. We further assessed the phylogenetic relationships between the five species, analyzing both morphological and chemical characters. Our clusters revealed congruency between some species associations and suggested that the analysis of venom composition may apply, at least partially, to *Strumigenys* chemosystematics. However, important discrepancies also appeared, signifying that selective pressures for chemical diversification have operated differentially during the speciation and dispersal processes within this genus in Taiwan.

Introduction

The myrmicine ant tribe Dacetini includes 9 genera and about 872 species largely distributed in the tropical and subtropical areas (Bolton, 2000). Dacetine ants are characterized by their pyriform head, their prey-seizure shaped mandibles and a singular spongiform tissue in the petiolar area (Bolton, 1999, 2000). Within the tribe, *Strumigenys* and *Pyramica* are the most speciose genera, containing 90% of total species (Bolton, 2000). In Taiwan, all the 27 species of dacetine ants belong to these two genera, but *Strumigenys* ants received comparatively more attention by authors, especially in regard to taxonomy and phylogeny (Lin & Wu, 1998, 2001; Hung et al., 2004). Thirteen species of *Strumigenys* are present across the island, nine of which being endemic (Lin & Wu, 1998, 2001). Like all Dacetini species, they are predators capturing small arthropods, mostly collembolans, by means of their specialized snap jaws (Brown & Wilson, 1959; Masuko, 1984). Colonies nest in rotten wood and under rocks of original or secondary forest litter. Personal observations (C.-C. Lin) indicate that they hardly contain more than 200 individuals in laboratory and that some species may be polygynous. However, because of their minute size and cryptic habits, field collections proved hazardous and some species are described from one specimen only (Lin, 1998; Lin & Wu, 2001). Consequently, their biology and social organization remain poorly investigated.

In addition to these impediments, many Taiwanese *Strumigenys* species can display a great morphological resemblance, that can only be solved by subtle differences in the pilosity or other characters solely observed under electron microscope (Lin, 1998, and see hereafter). This makes their identification complex even for specialists and strongly hampers the conduct of sound experimental studies. In ants, chemical analyses of the diverse exocrine secretions can be a valuable addition to the use of morphological characters to help for

accurate species assignments. For instance, diagnostic suites of compounds have been identified in the secretions of the mandibular, postpharyngeal and Dufour glands within the formicine ant genus *Cataglyphis* (Keegans et al., 1992; Hefetz & Lenoir, 1992; Dahbi et al., 1996, 2008) and their specificity allowed the assessment of phylogenetic relationships that were in agreement with morphological data. In a same way, venom chemical composition also revealed its diagnostic power within the fire ants complex. Similar *Solenopsis* species and their hybrids can be accurately identified through their characteristic amounts of various 2,6-dialkyl piperidine (Brand et al., 1973a,b; Vander Meer et al., 1985; Vander Meer and Lofgren, 1990; Dall'Aglio-Holvorcem et al., 2009) and $\Delta^{1,6}$-piperideine alkaloids (Chen et al., 2010). More generally, an important diversification of both the function and chemistry of the venom has occurred in ants, which provides interesting material for chemotaxonomic analyses (Hefetz, 1993).

As part of our ongoing taxonomic investigation of the genus *Strumigenys* in Taiwan (Lin, 1993, 1998; Lin & Wu, 2001), the composition of worker venom was investigated through GC/MS analyses in order to identify specific chemical markers for five species. We also assessed their phylogenetic relationships using classical taxonomy and finally compared the results obtained from both morphological and chemical analyses to evaluate the usefulness of our method for *Strumigenys* chemosystematics.

Materials and methods

Ant collection and laboratory conditions

Colonies of *Strumigenys* ants belonging to five species (*S. chuchihensis*, *S. formosensis*, *S. liukueiensis*, *S. minutula* and *S. solifontis*, respectively) were collected at four different localities in Taiwan (Table 1, Fig 1) by hand searching. Each colony contained about 50~150 workers, together with some developing brood and at least one queen. In the laboratory, they were reared in plastered nest, covered by a red plastic plate and kept at 25°C, 60% RH and a 13/11 LD photoperiod. They were fed every three days with collembolans, and water was provided *ad libitum*.

Table 1. Summary of the respective dates and locales of collections for the five *Strumigenys* species (*cf.* Fig 1 for details of Taiwan geography).

Species	Locale	Collection time	Colonies
S. chuchihensis	1	2009/2011	1/ 1
S. minutula	2	2009	5
S. formosensis	2	2009	6
S. liukueiensis	3	2008/2011	1/ 3
S. solifontis	4	2009	3

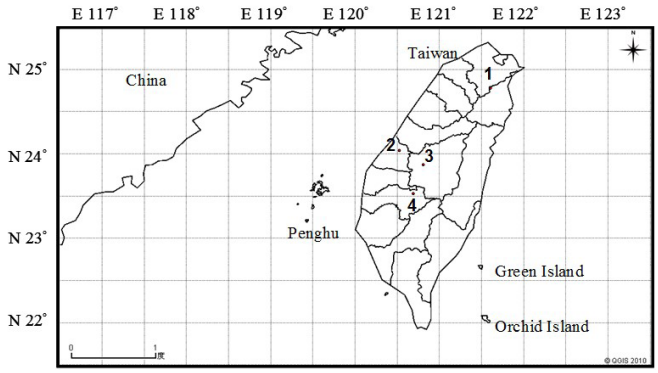

Fig 1. Collecting sites of *Strumigenys* ants. 1: Fushan, Ilan County; 2: Pakuashan, Changhua City; 3: Chichi, Nantou County; 4: Shuisheliao, Chiai County.

Morphological characters state definition

The characters state definition and polarities used in this study largely followed prevailing theories of evolutionary change within the tribe Dacetini (Brown & Wilson, 1959; Baroni Urbani & de Andrade, 1994; Bolton, 1999, 2000). The characters and character state codes used in the cladistic analysis are defined in Table 2. Characters include both those with simple binary state and multistate characters. All multistate characters were treated as unordered. If more than one state was present for a given taxon, the character was recorded as '0 & 1' (meaning that the taxon had state 0 and 1). These characters were not weighted in the analysis.

Chemical analysis

Solid-Phase-Microextraction (SPME). When applied to physically tiny ants, the chemotaxonomic techniques traditionally used to analyze their cuticular hydrocarbons or exocrine glands secretions require the dissection or sacrifice of dozens of workers, which may hamper any further laboratory experiments on the small and rare colonies of *Strumigenys*. Instead, we sampled the venom secreted by workers that were kept alive, using a 7 μm layer polydimethylsiloxane (PDMS) fiber (Supelco, Sigma-Aldrich, St Louis, MO, USA). Ants were initially immobilized on a special device made of foam (Fig 2). When their abdomen is stimulated, *Strumigenys* workers protrude their sting and droplets of venom stand out. The SPME fiber was carefully thrust into the droplets to collect the venom. For each sample, we pooled the venom from three different

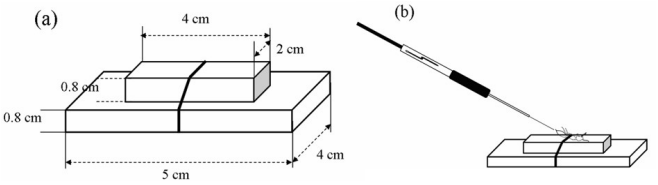

Fig 2. Device used for ant immobilization (a) before SPME venom extraction (b).

Table 2 Morphological characters and their states used for the phylogenetic analysis of the genus *Strumigenys*. (Q: queen, W: worker).

1.	Number of teeth or denticles of apical fork of mandible (W, Q): (0) 4; (1) 3; (2) 6.
2.	Preapical teeth of mandible (W, Q): (0) spiniform; (1) reduced.
3.	Mandible shape (W, Q): (0) hook like; (1) sickle like.
4.	Anterior clypeal margin (W, Q): (0) transverse; (1) deeply concave medially.
5.	Long flagellate hair on margin of antennal acrobe (W, Q): (0) absent; (1) present.
6.	Flagellate hair on genae (W, Q): (0) absent; (1) present.
7.	A row of erect hairs on occiput (W, Q): (0) absent; (1) present.
8.	Pilosity type of carnium (W, Q): (0) spatulate ; (1) erect; (2) flagellate; (3) spoon-shaped
9.	Hair on pronotal humeli (W): (0) absent; (1) flagellate; (2) erect; (3) columnar.
10.	Lateral marginal hair on mesonotum (W): (0) absent; (1) flagellate; (2) erect; (3) columnar.
11.	Flocculus hairs on pronotum (W): (0) absent; (1) sparse; (2) numerous.
12.	Sculpture on mesopleuron (W): (0) absent; (1) present.
13.	Sculpture on propodeum (W): (0) smooth; (1) microreticulate; (2) lacunous.
14.	Propodeal teeth (W, Q): (0) well developed; (1) sub-spongiform ; (2) spongiform.
15.	Pilosity type on pedicel (W, Q): (0) spatulate ; (1) erect; (2) flagellate; (3) spoon-shaped
16.	Density of hairs on fourth abdominal tergum (W, Q): (0) sparse; (1) numerous.
17.	Mandiblular index, MI (Q, W): (0) MI > 40; (1) MI < 40.
18.	Number of ommatidia (W): (0) > 10; (1) 6 ~ 10; (2) < 6.
19.	Ocellus (Q): (0) large; (1) small.
20.	Mesonotum (Q): (0) convex; (1) even.
21.	Pilosity type on tergum of gaster (W, Q): (0) erect; (1) flagellate; (2) columnar; (3) spoon-shaped.
22.	Sculpture on lateral surfaces of pronotum (W): (0) smooth; (1) sculpture on part; (2) sculpture presnet on all.
23.	Total body length, TL (W): (0) > 3 mm; (1) 2 mm ~ 3 mm; (2) < 2 mm.

individuals. The SPME fiber was then immediately desorbed in the injection port of a gas chromatograph. Three samples were performed for each colony.

GC/MS analyzes. Venom composition was analyzed on a Varian GC 3800 gas chromatograph coupled with Varian Saturn 2200 turbo mass (operating at 70eV, former Varian Inc. now Agilent, Palo Alto, Ca, USA). The GC/MS was fitted with a VF-5ms 5% phenyl 95% dimethylpolysiloxane capillary column (30 m x 0.25 mm). Helium (purity 99.995%) was used as the carrier gas at 1.0 ml/min flow rate. The SPME fiber was directly inserted into the injection port of gas chromatograph which was set at 250^{0}C in split/splitless mode. The venom samples were run using the following temperature program from 120^{0}C (5 min. initial hold) to 180°C at 10°C min^{-1}(3 min. intermediate hold), then to 240^{0}C at 10°Cmin^{-1} (10 min. second intermediate hold), and finally to 280^{0}C at 5^{0}Cmin^{-1} (6 min. final hold). The standard of the series n-alkane (C_{10}~C_{22}) was used to determine retention indexes (RI) (Kováts, 1965). Our objective in this study was to compare the chemical profiles of the five *Strumigenys* species, not to provide absolute identifications of all of the different compounds that were found.

Statistical analysis

All total ion chromatograms were processed through the MS Workstation 6.9 (Varian Inc.). Differences in the chemical profiles were assessed using principle component analysis (PCA) for all peaks integrated. Morphological and chemical patterns of the different species were further analyzed on separate clusters obtained from the respective coefficient of correlation matrices (Single-link method, 1-Pearson r). For the analysis, each chromatogram was divided into three sections while assigning a value to each peak according to its relative area (RA = target peak area / total peak area): peaks ranging from 0 to 10%, 10-50%, and >50% were assigned values of 1-3, respectively (modified from Dahbi et al., 1996). All statistics were performed with Statistica 7 (StatSoft Inc., Tulsa, OK, USA).

Results

Morphological study

The status of the characters for the various *Strumigenys* species and the ensuing cluster analysis (Fig 3) revealed three assemblies of species. A first clade includes the two species *S. liukueiensis* and *S. solifontis*. A second clade is composed of the two species *S. chuchihensis* and *S. minutula*. Finally, placed at the root of the clustering, *S .formosensis* appears distinct from the other four species. Our results match those obtained by Lin (1998), who, analyzing a total 12 Taiwanese and 5 Japanese *Strumigenys* ants, also classified these five species in three different groups, but in a slightly different way: the two species *S. solifontis* and *S. liukueiensis* were identically

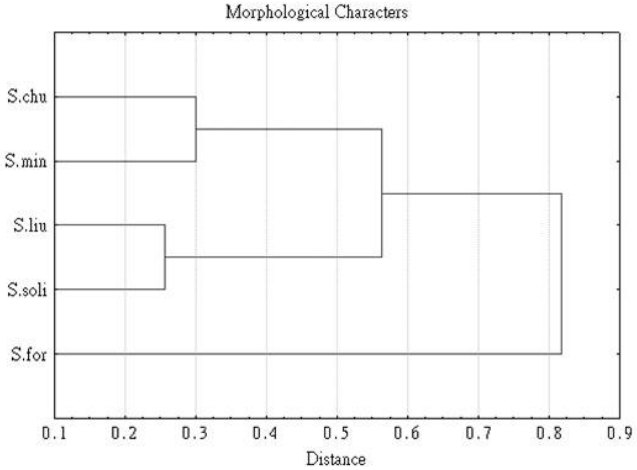

Fig 3. Cluster analysis based on the morphological characters. (Single link, 1-Pearson r). (S.for: *S. formosensis*; S.min: *S. minutula*; S.chu: *S. chuchihensis*; S.liu: *S. liukueiensis*; S.soli: *S. solifontis*).

clustered in a same *solifontis* group, confirming their close phylogenetic relation, whereas *S. chuchihensis* was included in their sister *lewisi* group. However, *S. formosensis* and *S. minutula* were both integrated in a distinct "*tropical"* group of *Strumigenys* ants. Later, in their description of the newly-discovered *S. chuchihensis*, Lin & Wu (2001) finally considered this species closely related to those of the *godeffroyi* group, which includes, among others, *S. minutula*, *S. solifontis* and *S. luikueiensis* while *S. formosensis* was included in the *mayri* sister group of *Strumigenys* species (Bolton, 2000).

Chemical study

For the five *Strumigenys* species, a total of 16 constituents were detected in our chemical analysis of the workers venom composition (Fig 4). According to their respective retention index and spectrum (Table 3), we suggest that these compounds may be terpenes (and more precisely sesquiterpenes for most of them) but this will need further analyses to be clearly confirmed. Nevertheless, they were present in sufficient quantities in our samples to allow for a comparison between species. Despite important similarities as for the number and identity of constituents, as well as for the presence of a major peak (RA>50%) in all chemical profiles, some qualitative and quantitative differences between species were nonetheless revealed. First, none of each 16 compounds were commonly present in all venoms. In particular, the identity of the major constituents varies across the different profiles. The

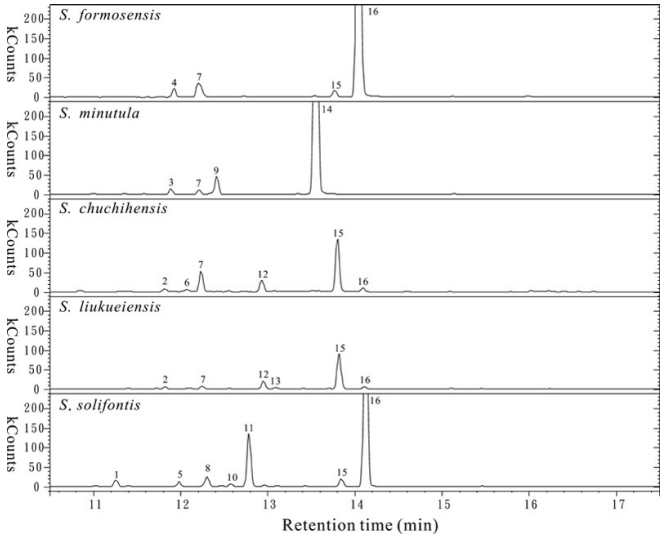

Fig 4. Gas chromatograms of venom revealing 16 different compounds across the five *Strumigenys* species.

same is true concerning their respective abundance, ranging from 53.60% to 93.61% respectively (Table 3). The PCA illustrates the relative specificity of the various venom compositions (Fig 5). Three species present clearly distinct profiles. Venom in *S. minutula* is mainly characterized by its major constituent (peak 14, absent in all other profiles), while *S. formosensis* and *S. solifontis* venoms share the same major compounds (peak 16) but in different proportions (Table 3). These two species are further separated on the basis of their secondary constituents (peaks 7 and 11, respectively). The PCA analysis also shows that the chemical composition of the workers venom appears relatively constant for each of these three species. In comparison, the venoms of the two remaining species, *S. liukueiensis* and *S. chuchihensis*, show a distinct composition, but our chemical analysis was insufficient to distinguish them from each other. They both share the same major compound (peak 15), in similar proportions, and present several minor constituents in common.

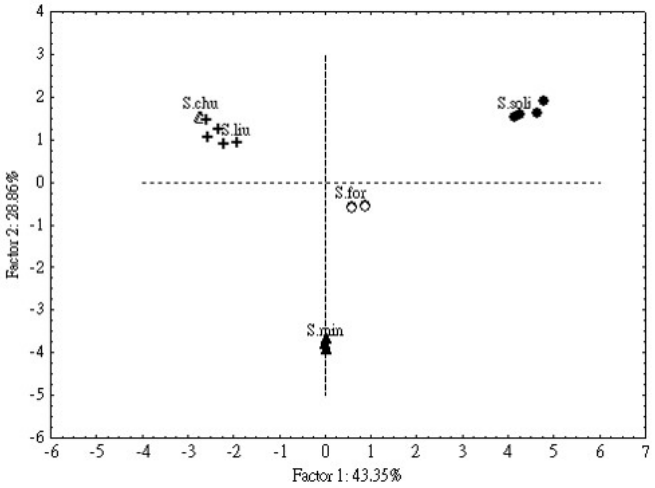

Fig 5. PCA analysis based on the peaks' relative areas (RAs) (◯: *S. formosensis*; ●: *S. solifontis*; ▲: *S. minutula*; △: *S. chuchihensis*; +: *S. liukueiensis*).

The result of the cluster analysis using the assigned values for all integrated peaks is shown in Fig 6. As in the morphological analysis (Fig 3), three clades were revealed, but important discrepancies in their respective composition appeared. *S. formosensis* was clustered with *S. solifontis*, while *S. chuchihensis* and *S. liukueiensis* were associated, *S. minutula* being clearly distinguished from the other four species.

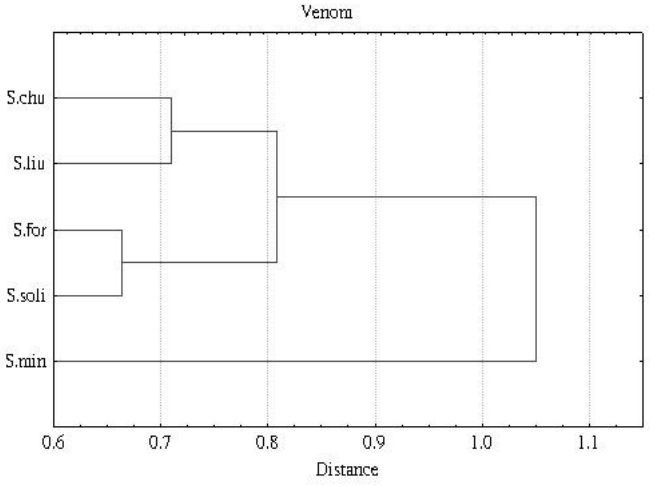

Fig 6. Cluster analysis based on the chemical characters. (Single link, 1-Pearson r). (S.for: *S. formosensis*; S.min: *S. minutula*; S.chu: *S. chuchihensis*; S.liu: *S. liukueiensis*; S.soli: *S. solifontis*).

Table 3 Results of the GC/MS analysis for the five *Strumigenys species*. *(Peak no*: Peak number, in reference to Fig. 4; Rt: Retention time; RA ± S.D. = mean relative area (%) ± S.D.; RI: Retention index).

	Peak no.	Rt	RA (%) ± S.D.	values	RI	Spectrum
S. formosensis	4	11.92	1.46 ± 0.22	1	1430	41 (40), 55 (100), 67 (19), 79 (38), 91 (37), 107 (87), 121 (26), 133(29), 148 (49), 161 (22), 175 (4), 189 (14), 217 (12), 246 (3)
	7	12.22	4.03 ± 0.34	1	1448	41 (42), 55 (100), 67 (37), 79 (50), 91 (24), 107 (37), 119 (17), 133(19), 147 (25), 161 (31), 175 (5), 189 (12), 217 (9), 246 (6)
	15	13.77	1.37 ± 0.07	1	1534	41 (45), 55 (83), 67 (26), 79 (38), 91 (47), 107 (100), 119 (47), 135 (23), 147 (31), 161 (13), 175 (5), 189 (15), 217 (15), 246 (4)
	16	14.04	92.12 ± 0.19	3	1547	41 (38), 55 (65), 67 (21), 79 (32), 91 (33), 107 (100), 121 (47), 135 (23), 147 (21), 163 (17), 175 (4), 189 (17), 217 (15), 246 (5)
S. minutula	3	11.88	1.07 ± 0.14	1	1427	41 (50), 55 (31), 69 (57), 79 (91), 91 (60), 107 (64), 119 (42), 137 (37), 147 (25), 161 (11), 175 (2), 189 (7), 203 (13), 217 (2)
	7	12.20	0.75 ± 0.17	1	1447	41 (30), 55 (100), 67 (34), 79 (42), 91 (20), 107 (31), 119 (12), 133(15), 147 (22), 161 (23), 175 (2), 189 (10), 217 (11), 246 (5)
	9	12.41	3.84 ± 0.36	1	1459	41 (52), 55 (100), 67 (26), 79 (58), 93 (70), 107 (43), 119 (23), 133 (23), 151 (32), 161 (4), 175 (8), 203 (13), 217 (1), 232 (2)
	14	13.55	93.61 ± 0.77	3	1523	41 (56), 55 (100), 67 (44), 79 (76), 91 (49), 107 (70), 119 (43), 133 (26), 147 (26), 151 (35), 163 (15), 175 (6), 189 (10), 217 (21), 246 (4)
S. chuchihensis	2	11.81	3.23 ± 0.34	1	1423	41 (71), 55 (64), 69 (32), 79 (37), 93 (57), 107 (100), 121 (93), 135 (42), 150 (36), 163 (37), 175 (7), 189 (31), 217 (8), 233 (8), 246 (7)
	6	12.07	2.78 ± 0.06	1	1438	41 (78), 55 (49), 69 (35), 79 (44), 91 (68), 107 (100), 119 (80), 135 (15), 147 (37), 161 (9), 175 (5), 189 (5), 203 (10), 217 (4), 232 (4)
	7	12.23	18.45 ± 1.29	2	1449	41 (31), 55 (100), 67 (37), 79 (50), 91 (25), 107 (34), 119 (15), 133(22), 147 (30), 161 (30), 175 (4), 189 (13), 217 (10), 246 (6)
	12	12.93	12.49 ± 1.05	2	1491	41 (45), 55 (47), 69 (18), 79 (20), 91 (44), 107 (86), 121 (93), 135 (36), 147 (9), 163 (100), 175 (2), 189 (14), 217 (8), 231 (2), 246 (4)
	15	13.80	53.60 ± 2.21	3	1536	41 (43), 55 (64), 67 (23), 79 (30), 91 (45), 107 (100), 119 (66), 133 (17), 147 (36), 163 (13), 175 (3), 189 (12), 217 (13), 232 (1), 246 (3)
	16	14.09	3.97 ± 0.41	1	1550	41 (48), 55 (94), 67 (28), 79 (40), 91 (49), 107 (100), 121 (38), 135 (23), 147 (24), 163 (14), 175 (4), 189 (11), 217 (11), 246 (1)
S. liukueiensis	2	11.83	3.84 ± 0.52	1	1424	41 (45), 55 (52), 69 (23), 79 (31), 91 (53), 107 (100), 121 (82), 135 (38), 150 (32), 163 (40), 176 (3), 189 (30), 217 (10), 233 (15), 247 (5)
	7	12.25	3.18 ± 1.57	1	1450	41 (28), 55 (100), 67 (34), 79 (41), 95 (24), 107 (30), 119 (9), 133(19), 147 (19), 161 (24), 173 (4), 189 (9), 217 (10), 246 (4)
	12	12.95	14.32 ± 0.41	2	1493	41 (44), 55 (51), 69 (19), 79 (24), 91 (45), 107 (95), 121 (87), 135 (35), 147 (9), 163 (100), 175 (2), 189 (16), 217 (7), 231 (2), 246 (4)
	13	13.09	4.76 ± 1.60	1	1501	41 (60), 57 (100), 71 (69), 85 (45), 91 (12), 97 (10)
	15	13.82	61.52 ± 2.02	3	1537	41 (39), 55 (68), 67 (18), 79 (30), 91 (44), 107 (100), 119 (60), 133 (14), 147 (35), 163 (11), 175 (3), 189 (10), 217 (11), 231 (1), 246 (2)
	16	14.11	4.16 ± 0.31	1	1551	41 (50), 55 (93), 67 (36), 79 (56), 91 (66), 107 (100), 121 (42), 135 (24), 147 (19), 163 (14), 175 (3), 189 (10), 217 (12), 246 (4)
S. solifontis	1	11.25	1.71 ± 0.43	1	1387	41 (74), 55 (28), 69 (39), 79 (46), 91 (66), 107 (100), 121 (40), 133 (44), 147 (15), 161 (6), 175 (13), 205 (6)
	5	11.98	1.43 ± 0.06	1	1433	41 (38), 55 (100), 67 (21), 79 (38), 91 (36), 107 (87), 121 (29), 133 (27), 148 (39), 161 (19), 175 (5), 189 (8), 217 (10)
	8	12.30	2.80 ± 0.67	1	1453	41 (76), 55 (25), 69 (33), 79 (36), 91 (55), 107 (100), 121 (37), 136 (15), 147 (22), 163 (10), 175 (4), 189 (10), 203 (12), 217 (3), 232 (4)
	10	12.57	0.93 ± 0.01	1	1469	41 (53), 55 (82), 67 (30), 79 (37), 91 (58), 107 (100), 121 (40), 133 (68), 149 (9), 161 (6), 175 (11), 189 (4), 203 (4), 217 (3), 232 (4)
	11	12.78	15.25 ± 1.99	2	1482	41 (39), 55 (65), 67 (17), 79 (34), 91 (54), 107 (100), 121 (38), 133 (37), 149 (11), 161 (5), 175 (10), 189 (1), 203 (5), 217 (2), 232 (4)
	15	13.84	2.59 ± 0.21	1	1538	41 (44), 55 (68), 67 (23), 79 (29), 91 (46), 107 (100), 119 (63), 133 (15), 147 (35), 163 (12), 175 (3), 189 (11), 217 (15), 246 (3)
	16	14.12	72.17 ± 2.11	3	1551	41 (42), 55 (70), 67 (24), 79 (39), 91 (41), 107 (100), 121 (42), 135 (24), 147 (18), 163 (14), 175 (3), 189 (14), 217 (13), 246 (5)

Discussion

Taiwan relative geographic isolation and mountainous relief led to the development of an important endemism within its ant fauna (Forel, 1912; Terayama & Kubota, 1989; Lin, 1993, 1998). For instance, among Dacetine ants, nine out of 13 species of *Strumigenys* are only found on this island or surrounding islets. The present study aimed at providing a suitable chemical marker to elucidate subtle species assignments within this genus.

For this purpose, we analyzed the venom composition of *Strumigenys* workers that were maintained alive during the extraction process. We directly sampled droplets oozing from their extruded stings, which, as a result, contained a mixture of poison and Dufour glands secretions. The GC/MS analysis suggested that most of the compounds revealed are terpenes, which are frequent constituents of ant exocrine secretions, often present in Dufour gland (Francke & Schulz, 2010; Morgan, 2008). The functions of Dufour secretions are diverse and only fragmentarily understood but when experiments have been conducted, they revealed to act as semiochemicals rather than noxious secretions used for predation or colony defense (Billen & Morgan, 1998; Morgan, 2008). In some species, they can be the medium for the trail pheromone (Ritter et al., 1977; Vander Meer et al., 1981, 1988; Alvarez et al., 1987), or used to mark a territory or delimit a home range (Attygalle et al., 1983a,b). In others, the Dufour gland can be the source of alarm and/or propaganda substances (Daloze et al., 1991; Ruano et al., 2005). Conversely to noxious secretions, these communicative chemicals are usually species-specific in their composition, hence subjected to selective pressures to diversify (Hefetz, 1993).

Interestingly, our chemical analysis revealed that the venom of *Strumigenys* workers offers diagnostic suites of constituents, at least for some species. Three out of the five species used in this study (*S. minutula, S. formosensis* and *S. solifontis*) could be clearly separated in the PCA analysis. They showed sufficient differences, both in the identity and relative abundance of their main compounds, to permit unambiguous species assignment. However, the remaining two species (*S. chuchihensis* and *S. liukueiensis*) presented similar chemical profiles and could not be distinguished on the basis of their venom composition. Alternately, chemical convergence in exocrine secretions might occur when species share identical ecological conditions. More likely however, similar venom composition may reflect a lack of selective pressure for chemical diversification between sister species (Hefetz, 1993; Dahbi et al., 1996).

In an attempt to clarify the situation, we assessed the phylogenetic relationships among the five *Strumigenys* species. Results of our morphological analysis are in agreement with those of earlier cladistic studies (Lin, 1993, 1998; Lin & Wu, 2001; Bolton, 1999, 2000), confirming the phylogenetic proximity between *S. chuchihensis*, *S. liukueiensis*, *S. minutula* and *S. solifontis*. We thus support the view that these four species may belong to the same "*godeffroyi*" group, whereas *S. formosensis* appears rather different and should reasonably be considered as member of the distinct "*mayri*" group. These species associations are further supported by genetic analysis. Hung et al. (2004) compared the size of internal transcribed spacer 2 (ITS2) rDNA sequences to investigate the phylogenetic relationships within *Strumigenys* ants in Taiwan and Japan. Their results indicated that *S. chuchihensis*, *S. minutula* and *S. solifontis* belonged to a same clade, while *S. formosensis* was included into a distinct, monophyletic group, together with other species endemic to Taiwan or surrounding islets. Importantly, for this study *S. chuchihensis* and *S. liukueiensis* were sampled in distant locales, respectively in the northern and central part of Taiwan (Fig 1). Previous collections concur to suggest that both endemic species occur in distinct areas and, altogether, evoke an allopatric speciation process between these two sister species (Lin, 1993, 1998). Were this hypothesis to be confirmed by additional biogeographic studies, the absence of ecological competition could imply a lack of any selective pressure to diversify their Dufour gland secretions. The great similarity in their chemical profiles might therefore reflect the ancestral composition within the "*godeffroyi*" group. This suggestion should be further tested on other semiochemicals, in particular cuticular hydrocarbons which are involved in nestmate recognition.

A significant discrepancy between our two clusters concerns the chemical separation of *S. minutula* from the three other species of the "*godeffroyi*" group. In the same time *S. formosensis*, though morphologically distinct, became closely associated with *S. solifontis*, and, at a lesser degree, with the two remaining "*godeffroyi*" species. This important shift in species arrangements strongly suggests that ecological constraints have shaped the composition of Dufour secretions in these *Strumigenys* ants during their dispersal in Taiwan (Hefetz, 1993; Dahbi et al., 1996). Indeed, conversely to *S. chuchihensis* and *S. liukueiensis*, the three other species show a broader geographic repartition and can occur sympatrically on Taiwan Main Island (Lin, 1998). Given their overlapping distributions and similar ecological niches, these closely related species have to maintain distinct exocrine compositions to preserve strict behavioral barriers, as illustrated by the PCA analysis of the venom profiles. Nevertheless, our understanding of the biogeographic history of *Strumigenys* ants in Taiwan is too fragmented to allow deducing the ancestral venom composition from these five chemical profiles, and further inferring the way they diversified. For instance, three species used in the present study are endemic to Taiwan but belong to separate clades (*S. chuchihensis* and *S. liukueiensis* on one hand and *S. formosensis* on the other hand) and consequently do not share direct common ancestor. This provides interesting material to investigate the origin, dispersal, speciation and extinction processes within this genus in Taiwan. From then on, it will be possible to speculate about the putative ancestral Dufour composition and clarify the implications of our results in *Strumigenys* chemosystematics.

Finally, chemical analyses of workers venom secretions revealed to be a complementary but limited addition to the use of morphological characters for *Strumigenys* taxonomy in Taiwan. More experiments are needed to explore and compare the usefulness of other glandular secretions as phylogenetic indicators.

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