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Redescription of *Proformica nasuta* (Nylander, 1856) (Hymenoptera, Formicidae) using an integrative approach

Christophe GALKOWSKI [1], Claude LEBAS [2], Philippe WEGNEZ [3],
Alain LENOIR [4] & Rumsaïs BLATRIX [5,*]

[1,2,3,4,5] AntArea (www.antarea.fr), Association for the Study and
Mapping of Ants from Metropolitan France.
[1] 104 Route de Mounic, 33160 Saint-Aubin-de-Medoc, France.
[2] 2 Impasse del Ribas, 66680 Canohès, France.
[3] Walbru (www.fourmiswalbru.com), Belgian Association for the Inventory of Ant Species in
Wallonia and Brussels, and Rue de la Grotte 23, 4651 Herve, Belgium.
[4] IRBI, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261,
Université François Rabelais, Faculté des Sciences, Parc Grandmont, 37200 Tours, France.
[5] CEFE UMR 5175, CNRS – Université de Montpellier – Université Paul Valéry
Montpellier – EPHE, 1919 Route de Mende, 34293 Montpellier Cedex 5, France.

[*] Corresponding author: rumsais.blatrix@cefe.cnrs.fr
[1] E-mail: chris.gal@wanadoo.fr
[2] E-mail: cllebas@free.fr
[3] E-mail: wegnez.phil@gmail.com
[4] E-mail: alain.lenoir@univ-tours.fr

[1] urn:lsid:zoobank.org:author:C480F514-D567-4787-AF55-2BE00FB79706
[2] urn:lsid:zoobank.org:author:99DB1C68-3E40-48A7-A665-8BDAF6D8D1C3
[3] urn:lsid:zoobank.org:author:44194EA4-001C-4CE0-9567-F9977E103B40
[4] urn:lsid:zoobank.org:author:0B03EB0F-06EB-413C-A91E-B57CBB22C623
[5] urn:lsid:zoobank.org:author:AFF6B4B9-C054-43A2-8095-84F0FB7B6EE9

Abstract. The taxonomy of the Palaearctic ant genus *Proformica* Ruzsky, 1902 is confused and in need of revision. The type specimen for *P. nasuta* (Nylander, 1856), the type species of the genus, was from Beaucaire, southern France, and is presumably lost. Based on extensive sampling of *Proformica* nests in southern France, including the type locality, we show that the concept of *P. nasuta* has been erroneous for more than a century. We integrate information from the morphology of workers and sexual castes, DNA markers, and cuticular hydrocarbons to re-define species in southern France. This allowed us to provide a new, accurate description of *P. nasuta* and designate a neotype, as well as reference individuals for all castes. In addition, we propose a name, *P. longipilosa* sp. nov., for a species that since the end of the 19th century has mistakenly been included in *P. nasuta*.

Keywords. Ants, *Proformica longipilosa* sp. nov., Western Palaearctic, molecular markers, cuticular hydrocarbons.

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Introduction

The ant genus *Proformica* Ruzsky, 1902 is composed of 25 species (Bolton 2014) restricted to dry and open environments such as steppes, mountain meadows and Mediterranean seashores (Agosti 1994). It is endemic to the Palaearctic region, with a disjunct distribution. A first area extends from eastern Europe to eastern Asia and contains most of the species, and a second area, much more limited in species number and distribution, occurs at the southwestern tip of Europe (Portugal, Spain and southern France). This distribution is somewhat reminiscent of that of the meadow and steppe vipers, the *Vipera ursinii* species complex, which is composed of taxa restricted to steppe-like ecosystems. Asia and Europe show distinct viper taxa that diverged in the early Pliocene, about 4 Mya (Zinenko *et al.* 2015). The genus *Proformica* may have experienced the same biogeographic history as these vipers and several other organisms inhabiting steppe-like environments (Ruano *et al.* 2011; Sanllorente *et al.* 2015). Only one taxon, *P. nasuta* (Nylander, 1856), is reported to occur in both Asian and western European areas.

The taxonomy of the genus *Proformica* is complicated and in need of revision. The situation is particularly complex in the eastern area, with currently 23 species reported. In Western Europe, two distinct zoogeographical areas can be distinguished, the Iberian Peninsula and southern France, which are separated by a barrier formed by the Pyrenees mountain range. Three described species are currently recorded for the Iberian Peninsula (Collingwood 1976), but at least six forms are recognized by ant taxonomists (Xavier Espadaler, Barcelona, pers. comm.) and substantial morphological variation within each form makes species delimitation difficult. In contrast, only one described species, *P. nasuta*, has been recorded for southern France (*P. ferreri* Bondroit, 1918 may also be present in the French part of the Pyrenees).

Proformica nasuta is the type species for the genus *Proformica* and was described from Beaucaire, France. The concept of this species is unclear. For instance, variation in the number of erect hairs on the mesosoma, a character commonly used in the taxonomy of *Proformica*, has been interpreted either as mere intraspecific variation (Espadaler & Cagniant 1987), or as an indication that the name *P. nasuta* actually covers two taxa (Santschi 1925; Collingwood & Yarrow 1969). Populations of species of *Proformica* are small, inconspicuous and patchily distributed, and the species are often considered rare. As a consequence, the genus is poorly represented in institutional collections and most taxonomic work is based on few specimens, rendering the accurate perception of intra-specific variation difficult. Moreover, the type specimen of *P. nasuta* has not been located. Having not been found in the most likely candidate collections and not explicitly referred to in the literature, it is presumably lost. As *P. nasuta* is the oldest name in the genus, designation of a neotype and a precise redefinition of this taxon are indispensable before further taxonomic work on this genus can be undertaken. For this purpose, we analysed a sample, unprecedented in its size and geographic extent, of *Proformica* nests in southern France using an integrative taxonomy approach based on morphological data from workers and sexuals, DNA sequences and cuticular hydrocarbons. Southern France was the best location for this investigation as it encompasses the type locality for *P. nasuta* and harbours no other known *Proformica* species. Combining the results of these different characters can increase our ability to provide valid decisions about species delimitations (Schlick-Steiner *et al.* 2010). Although some of these kinds of data are less relevant than others for the descriptive taxonomy of a particular species group, incongruences between results based on different kinds of data can provide information on the biology of the group studied and insights into ongoing ecological and evolutionary processes.

Material and methods

Sampling

We sampled 110 nests of *Proformica* at 22 localities from across southern France (Fig. 1), including four nests at the type locality of *P. nasuta* (Beaucaire) and seven nests less than 10 km from that site. Collection details for each nest are provided in Appendix 1. We give here a more detailed description of the collection localities. Fourteen sites (Aurons, Beaucaire, Bonnieux, Collias, Plaine de la Crau, Grospierres, Jonquières, Montpellier, Orange, Pompignan, Sauteyrargues, Sisteron, Tarascon, Vinsobre) were found in lowland Mediterranean garrigue or sun-exposed grassland. Nests were usually found in areas where soil had been heavily trampled. Four sites (Grand Luberon, Montagne de Lure, Sainte-Baume, Sumène) were on rocky summits of medium-sized calcareous mountains with little vegetation cover. Finally, four sites (Plateau de Calern, Plateau de Caussols, Gréolières, Mont Ventoux) were on mountain slopes or plateaus in the foothills of the Alps, also bearing little vegetation cover.

Morphological investigation

Most of the morphological characters used were introduced by Seifert (2007).

Eight morphological characters were measured on 321 worker individuals originating from 97 nests collected at 21 localities (Appendix 1). Colonies are monodomous, so each nest represents one colony. Mean values were calculated for worker individuals collected from each nest (range 1–8, mean 3.3 workers per nest).

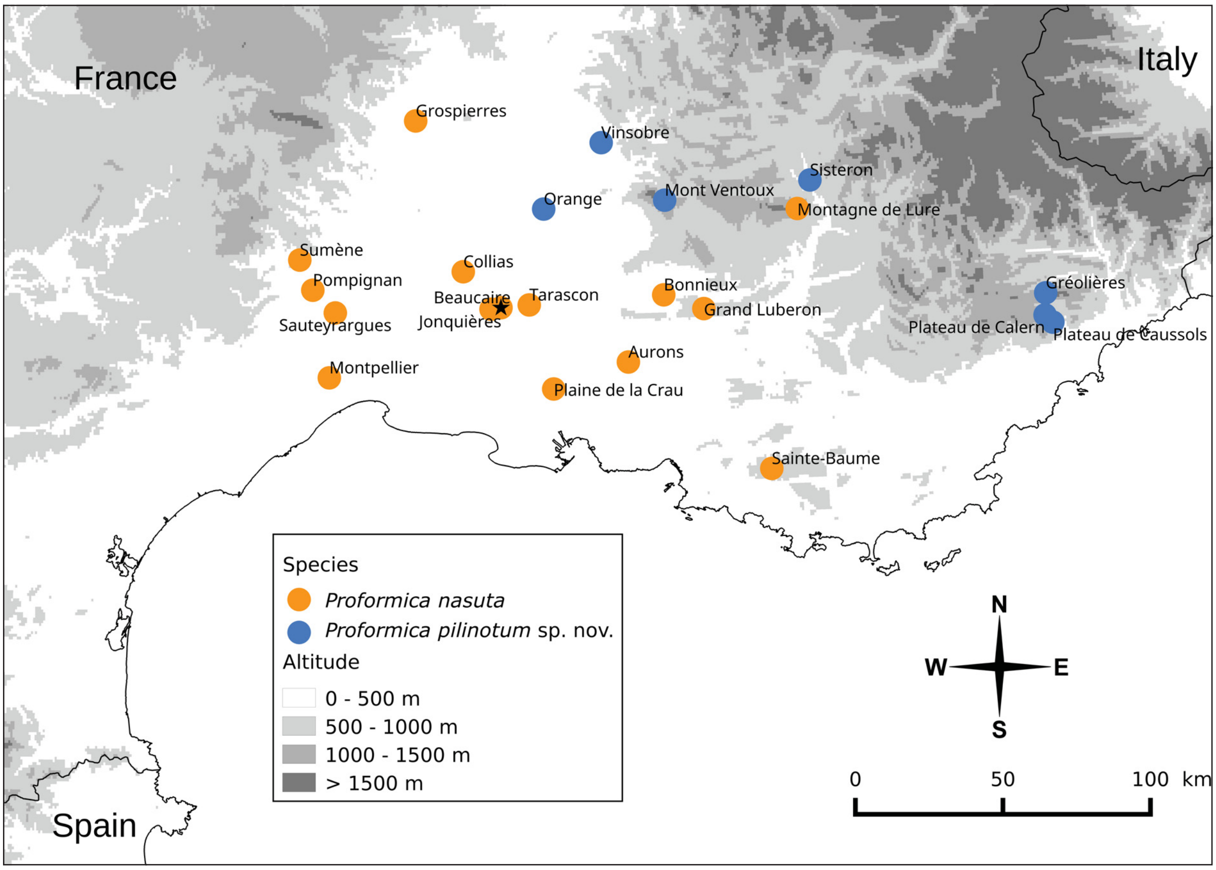

Fig. 1. Distribution of the 22 localities where *Proformica* nest samples were collected. The star indicates the type locality of *Proformica nasuta* (Nylander, 1865) (i.e., Beaucaire).

Morphometric characters measured:

CW	=	Maximum cephalic width (including eyes) (in µm)
GHL	=	Length of the longest erect seta on the gaster (in µm)
nCH	=	Unilateral number of erect setae on the vertex
nCU	=	Unilateral number of erect setae on the underside of the head
nG	=	Unilateral number of erect setae on the gaster
nSc	=	Unilateral number of erect setae on the petiole
nTx	=	Unilateral number of erect setae on the mesosoma
PDG	=	Pubescence distance on dorsum of the first gaster tergite, computed as L/N, where N is the number of pubescent (appressed) hairs crossing a transverse line of length L; here we used a line of length 340 µm

Ten queens from six localities (Beaucaire, Orange, Sainte-Baume, Pompignan, Tarascon, Mont Ventoux) were measured for 14 characters:

ML	=	Mesosoma length (in µm)
MW	=	Mesosoma width (in µm)
nMes	=	Unilateral number of erect setae on the scutum and scutellum, or on the mesonotum if scutum and scutellum were not discernible
nPn	=	Unilateral number of erect setae on the pronotum
nPP	=	Unilateral number of erect setae on the propodeum
ScW	=	Width of petiole scale (in µm)
SL	=	Scape length (in µm)

CW, nCH, nCU, nSc, nG, PDG and GHL: see worker morphology.

Measurements of workers and queens are given below (Appendices 2–3).

We collected a total of 11 males from three localities (Plaine de la Crau, Pompignan and Tarascon) and examined males from Sainte-Baume that had been collected by F. Bernard (MNHN) in July 1974. Males were only described qualitatively.

Acronyms of depositories:

AT	=	Alberto Tinaut Collection, University of Granada, Granada, Spain
IRSNB-BC	=	Bondroit Collection, Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium (contact: Wouter Dekoninck)
LB	=	Personal collection of Lech Borowiec, University of Wroclaw, Wroclaw, Poland
MCSN-EC	=	Emery Collection, Museo Civico di Storia Naturale, Genova, Italy (contact: Roberto Poggi)
MCZL-KC	=	Kutter Collection, Musée Cantonal de Zoologie, Lausanne, Switzerland (contact: Anne Freitag)
MHNG	=	Muséum d'Histoire Naturelle de Genève, Geneva, Switzerland (contact: Bernhard Merz)
MHNG-FC	=	Forel Collection, Muséum d'Histoire Naturelle de Genève, Geneva, Switzerland (contact: Bernhard Merz)
MNHN	=	Muséum national d'Histoire naturelle, Paris, France
MNHN-BAC	=	Collections from Bernard, André and others, Muséum national d'Histoire naturelle, Paris, France (contact: Agniële Touret-Alby and Quentin Rome)
NMB-SC	=	Santschi Collection, Naturhistorisches Museum, Basel, Switzerland (contact: Isabelle Zürcher-Pfander)

SMNH	=	Senckenberg Museum of Natural History, Görlitz, Germany
XE	=	Personal collection of Xavier Espadaler (Autonomous University of Barcelona, Barcelona, Spain)
ZISP	=	Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia

Molecular phylogenetic analysis

DNA was extracted from 1–2 individuals from each of 98 nests distributed in 20 localities (Appendix 1), using the REDExtract-N-Amp PCR Kit (Sigma–Aldrich, St. Louis, MO). Amplification by polymerase chain reaction (PCR) was performed using either the Goldstar Red DNA Polymerase (Eurogentec), the Qiagen Multiplex kit (Qiagen, Venlo, Netherlands) or the Sigma REDExtract-N-Amp PCR Ready Mix (Sigma–Aldrich). Conditions for PCR first followed manufacturer's instructions, and were then adjusted if amplification failed. Sanger sequencing of PCR products was performed at the Genoscope (Evry, France).

We amplified four DNA markers, two mitochondrial, two nuclear: (i) COI (~600 bp), coding for part of the cytochrome c oxydase subunit 1, (ii) Cytb (~700 bp), corresponding to the end of the sequence coding for NADH dehydrogenase subunit 6 and part of cytochrome b, (iii) 28S (~600 bp), coding for part of the large ribosomal subunit, and (iv) LW Rh (~550 bp), coding for part of the long-wavelength rhodopsin. COI was amplified for 45 *Proformica* individuals (GenBank accession numbers: KU749600–KU749637 and KU749641–KU749654) using two sets of primers covering the same region: either LepF1 (5'-ATTCAACCAATCATAAAGATAT-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAA-3') (Hebert *et al.* 2004), or CI13 (5'-ATAATTTTTTTATAGTTATACC-3') and CI14 (5'-ATTTCTTTTTTT-CCTCTTTC-3') (Hasegawa *et al.* 2002). For some individuals we used the two different primer pairs and compared the sequences obtained for the same individual. For each of four individuals, two highly divergent copies of COI were sequenced. To detect sequences that might come from accidental amplification of numts (copies of mitochondrial DNA transferred into the nuclear genome), we searched for the presence of premature stop codons in the amino-acid sequences. Three sequences (belonging to two individuals) had one premature stop codon. Based on the distribution of these sequences and the divergent copies from the same individual in the COI phylogeny, we identified a clade of putative numts. We amplified the Cytb marker using primers Cytb-FeF (5'-CAGTTTAATTTCTAATGAACAAAC-3') and Cytb-FeR (5'-GGATCTCTAAAAATATATGGG-3') (Liautard & Keller 2001) for a subset of *Proformica* individuals, and we used these sequences to design internal primers more specific to *Proformica* in order to increase amplification success: cytbPf (5'-CCTTTTAATAATRTYACTATTGC-3') and cytbPr (5'- TATAARTTTCTATTAATTCCAAG-3'). In total we amplified the Cytb marker in 103 individuals of *Proformica* (GenBank accession numbers: KU749655–KU749737 and KU749739–KU749758). The 28S marker was amplified for 31 individuals of *Proformica* (GenBank accession numbers: KU749759–KU749783 and KU749788–KU749793) using primers D2B (5'-GTCGGGTTGCTTGAGAGTGC-3') (Saux *et al.* 2004) and D2R (5'-TTGGTCCGTGTTTCAAGACGGG-3') (Belshaw & Quicke 1997). The LW Rh marker was amplified for 31 individuals of *Proformica* (GenBank accession numbers: KU749794–KU749818 and KU749823–KU749828) using primers LR143F (5'-GACAAAGTKCCACCRGARATGCT-3') and LR639ER (5'-YTTACCGRTTCCATCCRAACA-3') (Ward & Downie 2005). DNA sequences were aligned with MUSCLE (Edgar 2004). Alignments were inspected visually and edited manually using MEGA5 (Tamura *et al.* 2011) when they could be improved. Alignment of the intergenic region in the Cytb marker was ambiguous, and thus removed from the analysis.

As outgroup we used species for which we obtained new sequences (Appendix 1): *Bajcaridris theryi* (Santschi, 1936) (GenBank accession numbers, 28S: KU749786, LW Rh: KU749821), *Cataglyphis cursor* (Fonscolombe, 1846) (GenBank accession numbers, COI: KU749638 and KU749640, Cytb: KU749738, 28S: KU749787, LW Rh: KU749822), *Cataglyphis mauritanica* (Emery, 1906) (GenBank accession numbers, COI: KU749639, 28S: KU749785, LW Rh: KU749820), *Formica cunicularia* Latreille, 1798 (GenBank accession numbers, 28S: KU749784, LW Rh: KU749819), and species for which sequences were retrieved from GenBank (Hasegawa *et al.* 2002; Goropashnaya *et al.* 2004, 2007, 2012; Ward & Downie 2005; Moreau *et al.* 2006): *Cataglyphis iberica* (Emery, 1906) (COI: DQ353343), *Formica cunicularia* (COI: AB010926), *Formica exsecta* Nylander, 1846 (COI: AB010927, Cytb: JX170868), *Formica pratensis* Retzius, 1783 (Cytb: AY584233), *Formica rufibarbis* Fabricius, 1793 (Cytb: JX170889), *Formica truncorum* Fabricius, 1804 (COI: AB010929), *Formica uralensis* Ruzsky, 1895 (Cytb: JX170879), *Formica wheeleri* Creighton, 1935 (28S: DQ353556, LW Rh: DQ353149).

A partition scheme was defined with PartitionFinder (Lanfear *et al.* 2012) for each phylogenetic analysis, using the Bayesian Information Criteria for nucleotide substitution model selection. Prior data blocks were defined by marker and codon position. Three separate phylogenetic reconstructions were performed using both maximum likelihood and Bayesian inference algorithms: one for COI (to highlight the position of the clade of putative numts), one for Cytb (which includes the largest number of individuals), and one for the concatenated nuclear markers (28S + LW Rh) (because nuclear and mitochondrial markers might tell different stories).

Maximum likelihood phylogenies were constructed with RAxML (Stamatakis *et al.* 2008) on the web server at vital IT, Switzerland (http://embnet.vital-it.ch/raxml-bb/), using the GAMMA model of rate heterogeneity. Node support was estimated by generating 100 trees by bootstrapping. Bayesian inference phylogenies were constructed with MrBayes 3.2 (Ronquist *et al.* 2012). For the COI phylogeny we used the substitution models SYM+G, F81 and GTR+G for the first, second and third codon position, respectively. For Cytb we used HKY+G, HKY+I and GTR+I+G for the first, second and third codon position respectively. For the concatenated nuclear genes we used K80 for the first codon position of LW Rh, and K80+I for 28S and the second and third codon positions of LW Rh. Each analysis consisted of two runs of four Markov chains run for 10 million generations. Parameters were unlinked for all partitions. A standard deviation of split frequencies of less than 0.01 between two independent runs was reached after less than 2.4 million generations. A burn-in fraction of the first 25 % of the trees was discarded.

Cuticular hydrocarbons

Colonies from nine localities (Bonnieux, Plaine de la Crau, Montpellier, Grand Luberon, Montagne de Lure, Sainte-Baume, Pompignan, Sisteron, Mont Ventoux) were used for analysis of cuticular hydrocarbons. Using forceps, we gathered three to five workers from each colony and put them into glass vials containing 1 ml of hexane. The containers were stored in a freezer at -20°C until chemical analysis. For chemical analysis, the ants were retrieved from the vials and the solvent evaporated. The extract was re-dissolved in 10 µl of hexane. Two µl of each extract were injected into a Perkin-Meyer GC-MS functioning at 70eV with a source temperature of 230°C and equipped with a ZB-5HT column (30 ml × 0.25 mm ID × 0.252 µm df; 5% phenyl- 95% dimethylpolysiloxane). The temperature program was 2 min at 150°C, and then 5°C/min until 320°C, and a 5 min hold at 320°C (total 41 min). Substances were identified using standard alkanes, library data and Kovats retention indices. For the comparisons, we calculated the percentage of each hydrocarbon from the total hydrocarbon content in each ant sample. The data were analysed using Principal Component Analysis. We chose not to transform the data since transformation introduces additional background noise into the data when numerous zero values are present; these have to be replaced to make transformation possible when comparing species. Indeed, reanalysis of the data after transformation (following the procedure of Reyment 1989) gave similar results, but with slightly less efficient separation of groups (Oppelt *et al.* 2008). Analyses were made with the Statistica software.

We also performed chromatograms of cuticular hydrocarbons for two species used as outgroups: *Proformica longiseta* Collingwood, 1978 from Sierra Nevada (Spain) and *Cataglyphis cursor* from Aix-en-Provence (France). Lists of cuticular hydrocarbons known for these species have been published in Errard *et al.* (2006) and Nowbahari *et al.* (1990), respectively, but without quantification.

Nest census and queen reproductive status

Six nests were excavated in July 2011 and the ants counted. Six queens from two nests were dissected to assess their reproductive status. In addition, one apterous queen was obtained by rearing pupae from Sainte-Baume and was dissected to confirm its queen status. Several workers of various sizes were also dissected.

Results

Morphology and altitudinal distribution

Two groups of nests were separated by combining two morphological characters, GHL and PDG for the workers, and GHL and nG for the queens (Fig. 2). The two groups were distinct for both characters, independently of CW, a proxy for size (Fig. 2). One of the groups, coloured in orange in the figures and hereafter denominated as the orange taxon, encompasses the type locality of *Proformica nasuta* (Fig. 1). The other group is coloured in blue in the figures and is hereafter denominated as the blue taxon. Workers of the orange group present denser pubescence, shorter erect hairs on the gaster and fewer erect hairs (or none) on the mesosoma (PDG < 29, GHL < 77 µm, nest means, nTx × 2 ± SD = 9.5 ± 10.4) than those of the blue group (PDG > 33, GHL > 85 µm, nest means, nTx × 2 ± SD = 46.8 ± 16.7).

Further, queens of the blue taxon were all winged or showed wing sclerites, while all queens of the orange taxon were ergatoid. We did not find males of the blue taxon in the field and we could not locate specimens in museum collections. Males of the orange taxon have dense and long hairs on the head, mesosoma and the anterior face of the first gaster segment.

For both the orange taxon and the blue taxon, altitudinal distribution of the nests was bimodal (Fig. 3). Most nests of the orange taxon were found below 200 m, but those from Sainte-Baume, Sumène, Grand Luberon and Montagne de Lure were found above 800 m, at the tops of medium-sized mountains. In contrast, most nests of the blue taxon were found above 1000 m on plateaus and mountains, but those from Orange, Sisteron and Vinsobre were found lower, below 600 m. Interestingly, within each of these taxa, GHL and PDG are highest for workers from the mountain localities (except for Sumène) (Fig. 2).

Molecular phylogenetic analysis

Maximum likelihood and Bayesian inference produced very similar phylogenies, so we chose to present only Bayesian inference phylogenies. The clade of putative numts in the COI phylogeny is delimited in red in Fig. 4A. The two mitochondrial markers yielded similar topologies (Fig. 4A–B), showing two main clades which corresponded approximately to the two taxa defined in the morphological analysis. Mismatch between morphotypes and clades was observed for some specimens. The nuclear markers showed very little variation. As a consequence, the resulting tree is poorly resolved (Fig. 4C).

Cuticular hydrocarbons

Identification of compounds and examples of chromatograms are included below (Appendices 4–5). Chromatograms showed very different profiles. Most hydrocarbons of the blue taxon are shorter (< C28) than those of the orange taxon (> C28). Two forms can be distinguished in the orange taxon: the lowland localities, in which individuals have the longest hydrocarbons (> C31), and the mountain localities (Montagne de Lure and Grand Luberon), where the hydrocarbons are of an intermediate length

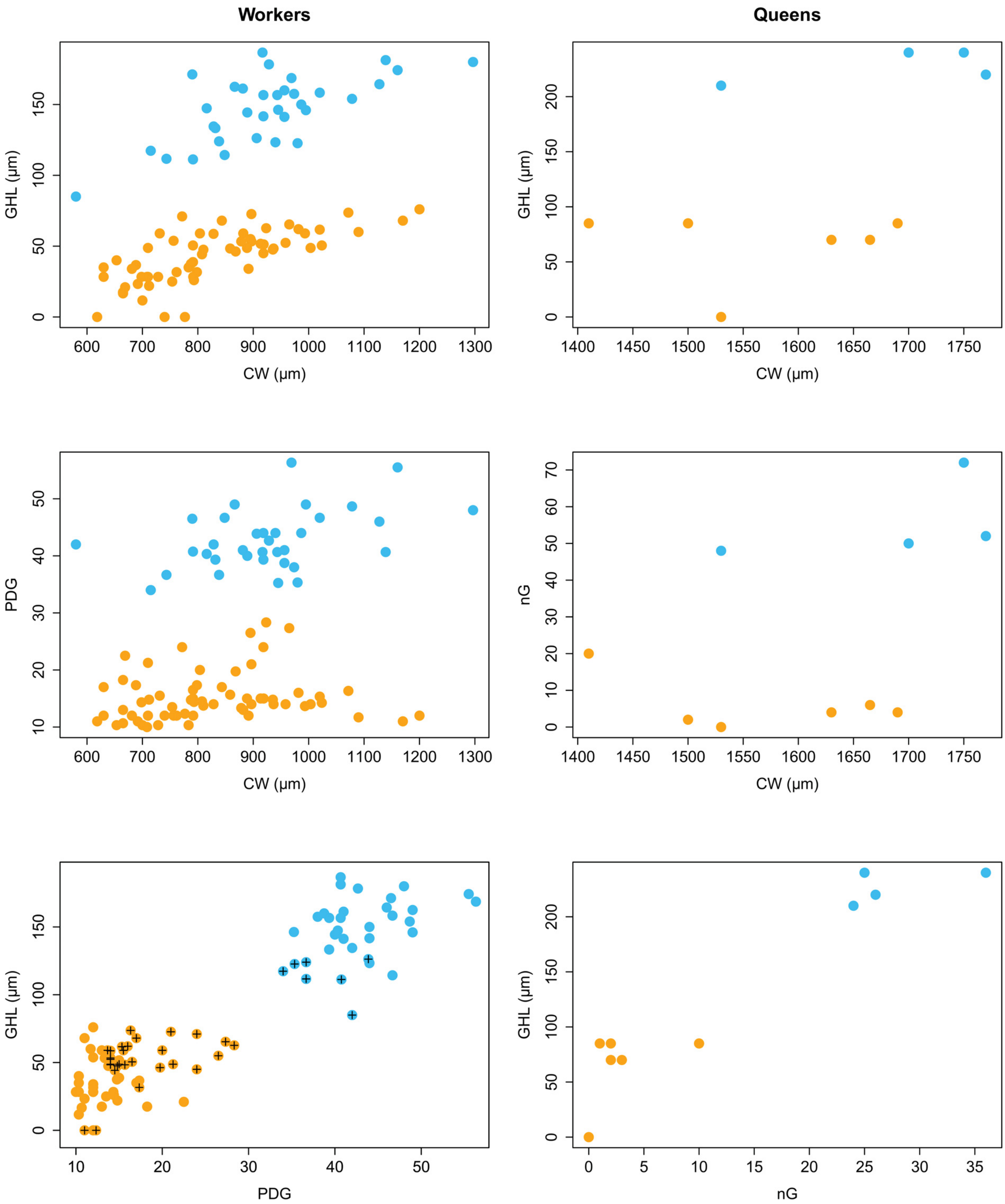

Fig. 2. Distribution of morphological characters of *Proformica* workers (left) and queens (right) collected in southern France. Top and middle graphics represent the regression of gaster hair length (GHL), pubescence distance on the gaster (PDG) and unilateral number of hairs on the gaster (nG) against cephalic width (CW), a proxy for size. The graphics at the bottom represent a combination of two morphological characters that highlights two distinct groups of nests. These two groups are coloured in blue and orange respectively. The dots with a black cross correspond to nests from mountain and lowland localities for the orange and blue groups, respectively.

(Appendix 5). The PCA distinguishes the two outgroups, *Proformica longiseta* and *Cataglyphis cursor*, from the *Proformica* samples from southern France (Fig. 5). The blue and orange taxa are segregated along the first axis of the PCA. In addition, the strongest differentiation occurs within the orange taxon, between a group formed by the two mountain localities (Montagne de Lure and Grand Luberon) and the others.

Queen reproductive status, nest census

Excavation of nests of the two taxa revealed the same general structure: the entrance opens directly at the ground surface, sometimes under a small stone; a vertical gallery of 10–20 cm leads to a small chamber where males can be found when present; then, the gallery goes down obliquely and reaches a final chamber, about 50 cm below ground level, where queens are present. Secondary galleries, lateral (perpendicular) to the principal one, may be present and lead to chambers. The content of nests is presented in Table 1. Repletes, i.e. workers with inflated gaster serving as stores of liquid food, were

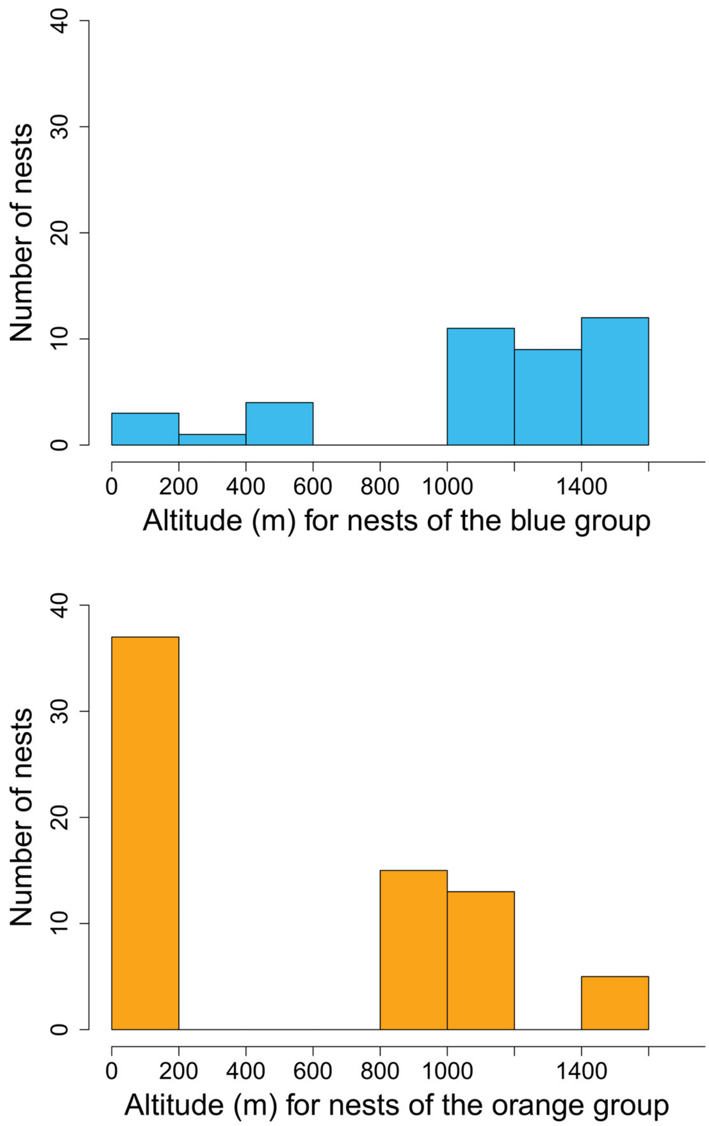

Fig. 3. Altitudinal distribution of 110 nests of *Proformica* from southern France belonging to two morphological groups.

found in colonies of both taxa. Colonies had one to many queens that appeared to be actively reproducing (mated, with numerous mature oocytes and yellow bodies) (Table 2). Workers, even the largest, always had fewer than 3 ovarioles per ovary and never had a spermatheca. In contrast, apterous and winged queens had a spermatheca and many more ovarioles per ovary (~ 10).

Fig. 4. Bayesian consensus trees of COI (**A**), Cytb (**B**) and concatenated sequences of 28S and LW Rh (**C**) for *Proformica* workers from southern France and outgroups. Labels are composed of the locality name, the colony code (figure), the code of the individual (w1 for worker 1, w2 for worker 2) and, for COI, the primer pair used (Ci for CI13 and CI14, Lep for LepF1 and LepR1). Sequences where a stop codon was detected are labelled with a red "STOP". Sequences with an asterisk specify individuals for which another sequence was obtained and fitted outside the clade of putative numts. Colours match the groups defined in the morphological analysis. Posterior probabilities are given for major nodes. Accession numbers are indicated for sequences retrieved from GenBank.

Taxonomy

Class Hexapoda Blainville, 1816
Order Hymenoptera Linnaeus, 1758
Superfamily Vespoidea Latreille, 1802
Family Formicidae Latreille, 1809
Subfamily Formicinae Latreille, 1809

Proformica Ruzsky, 1902

Nylander (1856) described *P. nasuta*, the type species of the genus, from Beaucaire. Our analyses assigned workers from the type locality and from two other localities within a radius of 10 km (Jonquières and Tarascon) to the orange taxon. They lack erect hairs on the mesosoma, agreeing with the description of *Proformica nasuta* by Nylander as "nuda". Although the type is presumably lost (as it could not be found in the following collections: Nylander (Helsinki) (Radchenko 2007), Forel (Geneva), Emery (Genoa), Bondroit (Brussels) and Santschi (Basel)), we are confident that the nest samples we collected in Beaucaire and in the surrounding area correspond to the species described by Nylander. Below we provide a redescription of *P. nasuta* (the orange taxon), and the description of a new species, *P. longipilosa* sp. nov. (the blue taxon).

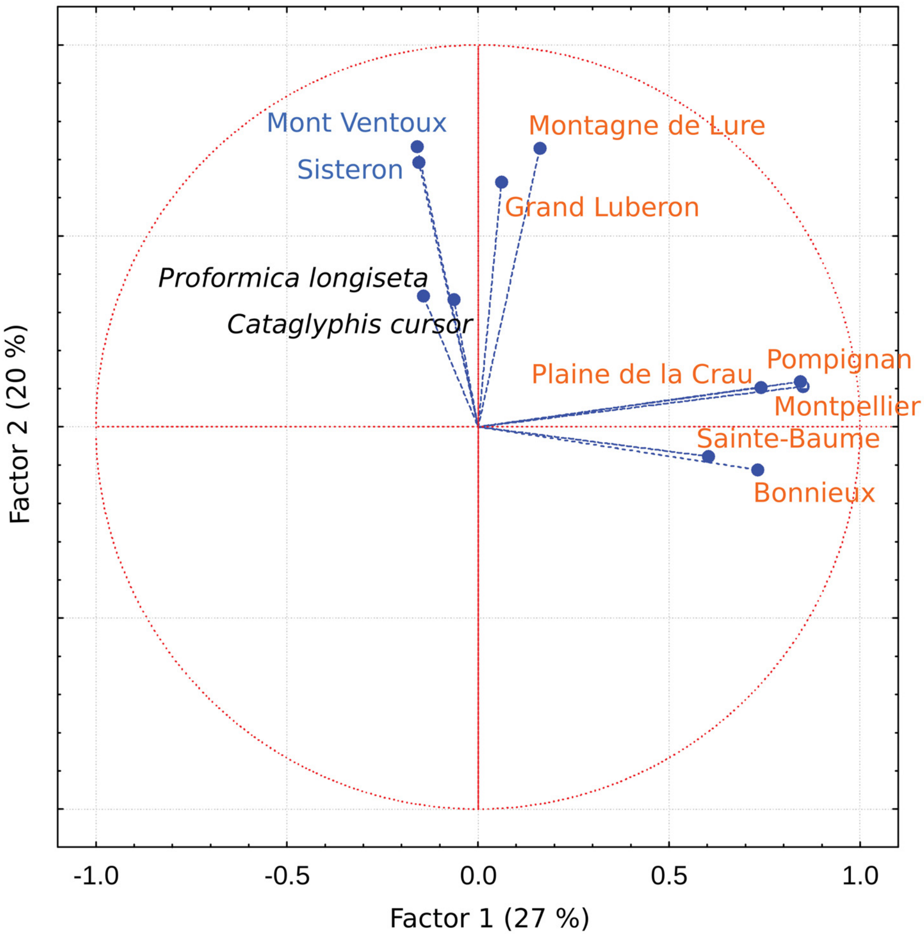

Fig. 5. Principal Component Analysis of *Proformica* localities based on relative proportions of cuticular hydrocarbons identified by gas chromatography and mass spectrometry. Colours match the groups defined in the morphological analysis.

Table 1. Content of excavated nests of *Proformica* Ruzsky, 1902. * = a large part of the nest could not be collected. The numbers of individuals are underestimates.

Colony	Taxon colour code	Species	No. of queens	Queen status	No. of males	No. of repletes	No. of other workers	Total no. of workers
Beaucaire 1	orange	*P. nasuta*	2	apterous	0	11	65	76
Pompignan 10	orange	*P. nasuta*	16	apterous	0	87	515	602
Tarascon 1	orange	*P. nasuta*	1	apterous	5	17	121	138
Orange 1	blue	*P. longipilosa*	7	wing sclerites	0	9	226	235
Mont Ventoux 10*	blue	*P. longipilosa*	1	wing sclerites	0	4	339	343
Mont Ventoux 14*	blue	*P. longipilosa*	5	wing sclerites	0	79	91	170

Table 2. Ovary status of seven queens of *Proformica* Ruzsky, 1902. * = queen emerged after rearing pupae.

Colony	Queen type	Taxon colour code	Species	Spermatheca	Mature oocytes	Yellow bodies	No. of ovarioles per ovary
Pompignan 10	apterous	orange	*P. nasuta*	full	many	many	~10
Pompignan 10	apterous	orange	*P. nasuta*	full	many	many	~10
Pompignan 10	apterous	orange	*P. nasuta*	full	many	many	~10
Sainte-Baume*	apterous	orange	*P. nasuta*	empty	absent	absent	~10
Orange 1	dealate	blue	*P. longipilosa*	full	many	many	> 15
Orange 1	dealate	blue	*P. longipilosa*	full	many	many	> 15
Orange 1	dealate	blue	*P. longipilosa*	full	many	many	> 15

Redescription of *P. nasuta* (Nylander, 1856) and designation of the neotype

As the type specimen of *P. nasuta* is presumably lost, we propose fixation of a neotype from a nest sample collected in Beaucaire, France, *terra typica* of the species, and matching Nylander's concept of *P. nasuta*. The original description (Nylander 1856: 66) is based on a small worker (" *Long. 3 – 3.5 mm* ") with elongated head (" *... facies producta antice visa subrectangularis...* "). This feature is found exclusively in minor workers. Therefore, a minor worker was selected from Beaucaire, France (colony Beaucaire 1) and designated as the neotype. The neotype is deposited as MNHN-1598 with the labels "FRA, N43.83544 E4.61828, Beaucaire, 9 juillet 2011, leg. R. Blatrix & C. Lebas" and "Néotype *Proformica nasuta* (Nylander, 1856), des. Galkowski, Lebas, Wegnez, Lenoir & Blatrix, 2016". In case of loss or destruction of this specimen, a replacement neotype can be designated from a series of ten other minor workers collected from the same nest and deposited at the MNHN. Other workers from the same nest are deposited at the following collections: AT (no. 15557), LB, SMNH, XE, ZISP and the collections of the authors. A queen from the same nest and a male from colony Tarascon 1 (a few kilometers away from the type locality) are deposited at MNHN.

Proformica nasuta (Nylander, 1856)
Figs 6–8

Formica nasuta Nylander, 1856: 66.
Formica (Proformica) nasuta var. *depilis* Santschi, 1925: 353.

Material examined

Museum material

FRANCE: MHNG: Charleval, Bouches-du-Rhône, leg. E. della Santa, labelled *P. nasuta*, 11 Jul. 1987: 4 workers, 25 Jul. 1988: 3 workers. – NMB-SC: Carrière des Anglais, Vaucluse: 2 workers, types of *Formica* (*Proformica*) *nasuta* var. *depilis*. – MNHN-BAC: Sainte-Baume, ~90 workers (12 pins), 3 ♂♂, one of which labelled "Type *phoenica*" [*Proformica phoenica* is a *nomen nudum*, as it was never described by Bernard. *Proformica* ants at this locality form a mountain isolate which most probably derived from lowland populations of *P. nasuta*; see Discussion]. – IRSNB-BC: 2 ♂♂, without locality.

New material

FRANCE: All in personal collections of CF and RB: Aurons, 4 workers, 43.66° N, 5.15° E, Jun. 2006, leg. V. Bouchet; Beaucaire, 14 workers, 2 queens, 43.8354° N, 4.6187° E, 9 Jul. 2011, leg. R. Blatrix and C. Lebas; Bonnieux, 8 workers, 43.8625° N, 5.3069° E, 1 Oct. 2011, leg. C. Lebas; Collias, 5 workers, 43.9477° N, 4.4623° E, 12 Jun. 2010, leg. R. Blatrix; Plaine de la Crau, Saint-Martin-de-Crau, 18 workers, 1 ♂, 43.5833° N, 4.8333° E, Jun. and Aug. 2011, leg. C. Lebas; Grospierres, 30 workers, 44.4116° N, 4.2713° E, 6 Jul. 2012, leg. T. Colin; Jonquières-Saint-Vincent, 7 workers, 43.8314° N, 4.5765° E, 11 May 2011, leg. R. Blatrix; Montpellier, 3 workers, 43.6292° N, 3.8907° E, 8 Mar. 2012, leg. R. Blatrix; Pompignan, 3 workers, 5 queens, 2 ♂♂, 43.8979° N, 3.8252° E, May 2010 and 10 Jul. 2011, leg. R. Blatrix and P. Wegnez; Sauteyrargues, 4 workers, 43.8275° N, 3.9192° E, 28 May 2011, leg. R. Blatrix; Sumène, 6 workers, 43.9904° N, 3.7714° E, 9 Apr. 2014, leg. R. Blatrix; Tarascon, 12 workers, 1 queen, 3 ♂♂, 43.8421° N, 4.7382° E, 9 Jul. 2011, leg. R. Blatrix and C. Lebas.

Description

Minor worker (n = 89)

For each character extreme values and the mean are given in brackets:

CW 540–900 µm; nCH 0–2 (0.06); nCU 0–3 (0.18); nTx 0–6 (0.57); nSc 0–2 (0.13); nG 0–5 (1); PDG 9–28 (13.2); GHL 35–75 µm (45.2); GHL/CW 0.040–0.102 (0.064).

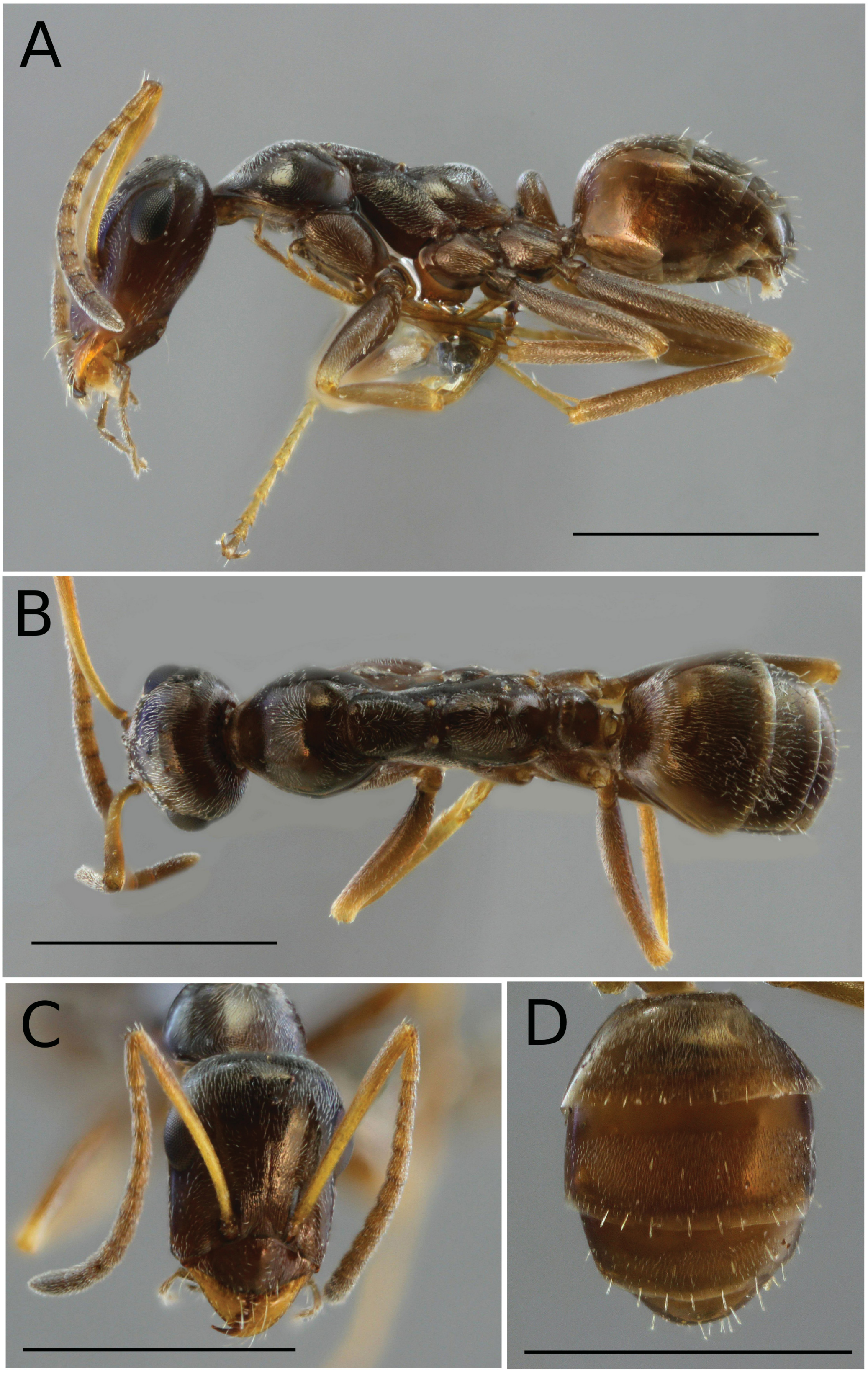

Fig. 6. *Proformica nasuta* Nylander, 1856, neotype, minor worker from colony Beaucaire 1, France. **A**. Lateral view. **B**. Dorsal view. **C**. Head in full face view. **D**. Gaster in dorsal view. Scale bars = 1 mm. Automontage: Claude Lebas.

Media and major workers (n = 25)
CW 900–1240 µm; nCH 0–1 (0.13); nCU 0–4 (1.13); nTx 0–7 (1.6); nSc 0–4 (0.27); nG 0–7 (2.3); PDG 10–18 (14.2); GHL 35–90 µm (60.2); GHL/CW 0.039–0.091 (0.054).

Body uniformly dark brown to black, appendices and mandibles lighter. Erect hairs rare or absent, short when present (GHL/CW < 0.11). Dense pubescence on dorsal surface of first and second gaster tergites (PDG < 29). Profile of mesosoma sinuous. Petiolar scale erect, thick, slightly notched at summit in large workers. Head of minor workers clearly elongate, rectangular (CL/CW > 1.3). Head of media and major workers less elongate (CL/CW 1.1–1.3), a bit shiny toward occiput, faintly sculptured in anterior part. Clypeus finely striate longitudinally. Mandible with five teeth of increasing size from base to apex.

Queen (n = 8)
CW 1500–1690 µm (1600); CL 1530–1660 µm (1590); SL 1230–1270 µm (1250); nCH 0–1 (0.2); nCU 2–6 (3.2); nTx 2–19 (11.8); nSc 0–2 (1.2); nG 0–3 (1.6); PDG 9–14 (11.2); GHL 70–90 µm

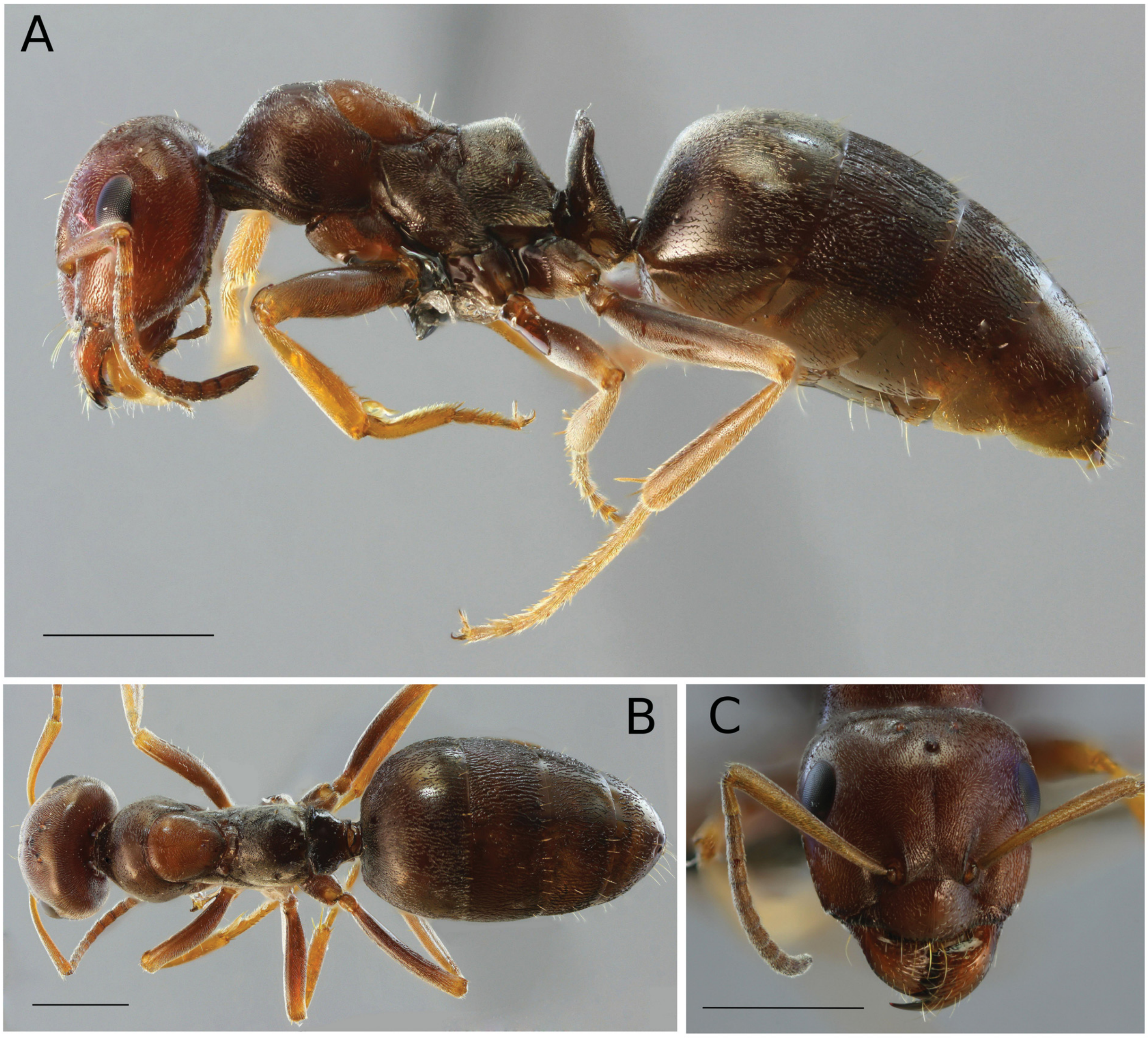

Fig. 7. *Proformica nasuta* Nylander, 1856, queen from colony Beaucaire 1, France. **A**. Lateral view. **B**. Dorsal view. **C**. Head in full face view. Scale bars = 1 mm. Automontage: Claude Lebas.

(77.5); MW 1020–1240 µm (1150); ML 2040–2310 µm (2200); ScW 620–860 µm (710); GHL/CW 0.042–0.056 (0.048).

Only ergatoid queens collected. Body brown with a wide orange spot on mesosoma on some specimens. Few and relatively short erect hairs (nG < 10, GHL < 90 µm). Pubescence very dense on the dorsal

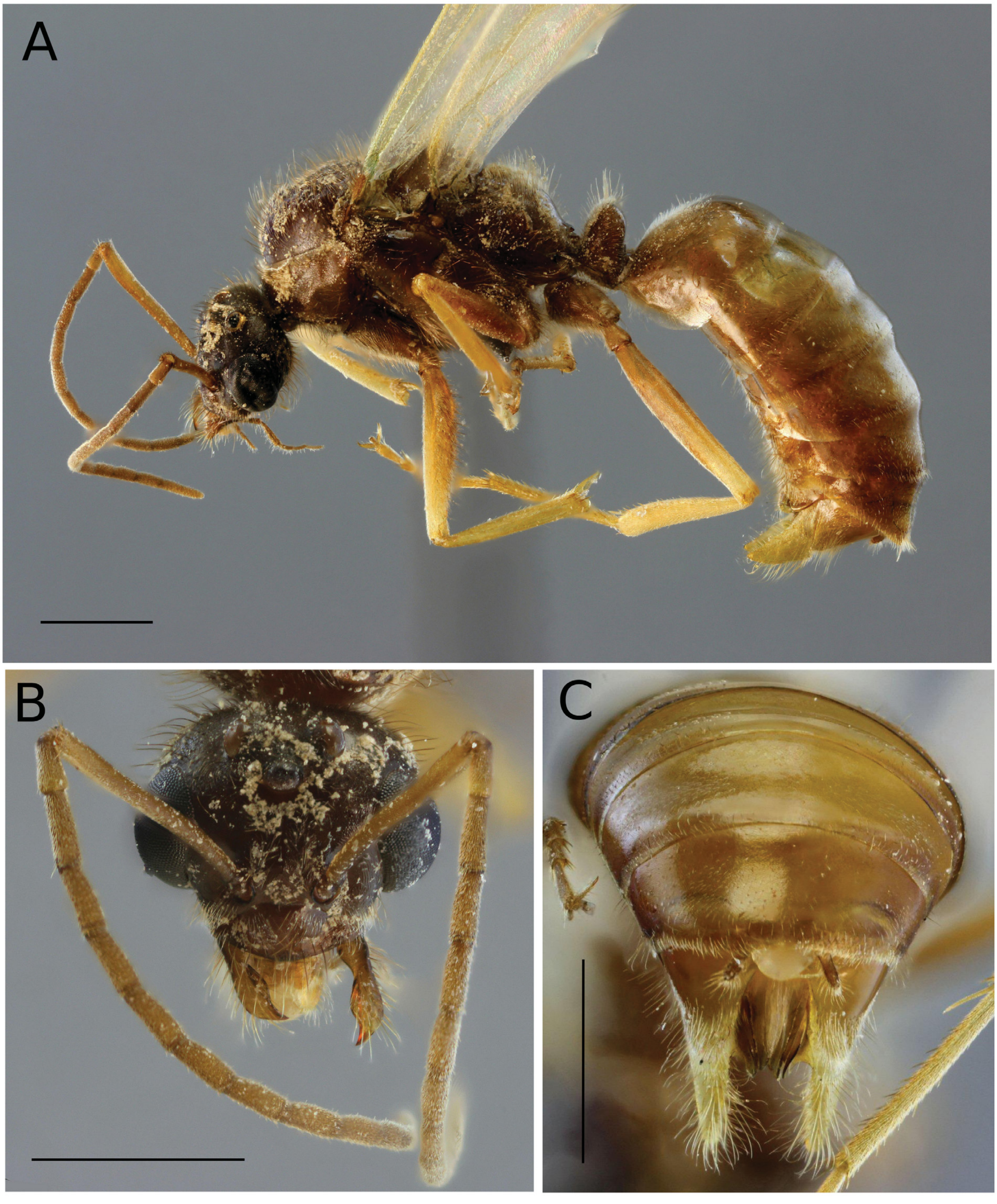

Fig. 8. *Proformica nasuta* Nylander, 1856, male from colony Tarascon 1, France. **A**. Lateral view. **B**. Head in full face view. **C**. Genitalia in dorsal view. Scale bars = 1 mm. Automontage: Claude Lebas.

surface of first and second gaster tergites. Profile of mesosoma similar to that of worker, but mesonotum more domed and propodeum very high. Tegulae absent. Petiolar scale high and wide, distinctly notched at summit. Head as long as wide. Same sculpture as in worker.

Male (n = 6)
CW 1340–1380 µm (1360); CL 1100–1170 µm (1140); SL 1220–1330 µm (1280); MW 1700–1750 µm (1730); ML 2720–2970 µm (2830); ScW 560–590 µm (580).

Head, mesosoma and scale dark brown, appendices and sometimes gaster lighter. Erect hairs numerous and dense on entire head, mesosoma and petiolar scale, becoming rare on gaster, where restricted to anterior face of first tergite, angled. Pubescence almost lacking on dorsal surface of gaster tergites. Eyes and ocelli prominent. Mandibles reduced, without teeth. Many erect black hairs on extensor profile of anterior and median femurs. Maximal length of these hairs shorter than width of femur. Rare erect white hairs on tibias. Wings well developed, yellowish.

Distribution

Southern France (Fig. 1).

Remarks

We have not examined the type specimens of the Asian varieties of this species, *P. nasuta metalica* Kuznetsov-Ugamsky, 1923 and *P. nasuta syrdariana* Santschi, 1928, described from Kazakhstan, nor the type specimen of the taxon *Formica aerea* (Roger, 1859), which was described based on a single minor worker collected in Greece and later synonymized with *P. nasuta* by Emery (1925). Although we decided not to change the status of these eastern taxa until a thorough revision of the eastern *Proformica* is undertaken, we believe it is very unlikely that they will be conspecific with *P. nasuta*.

Proformica longipilosa sp. nov.

urn:lsid:zoobank.org:act:6EF784CD-C8B2-4474-840E-04AFE460C0CE

Figs 9–10

Diagnosis

Workers varying greatly in size, the smallest having a strongly elongated head. Body black. Pubescence sparse and sculpture of tegument weak, giving a shiny aspect. Hairs on mesosoma and gaster very long.

Etymology

The epithet of this species refers to the long erect hairs on the mesosoma and gaster of workers.

Material examined

Holotype
FRANCE: minor worker from colony Mont Ventoux 10 of this study, 44.15261° N, 5.32081° E, alt. 1442 m, 14 Jul. 2011, coll. R. Blatrix (MNHN 1598).

Paratypes
FRANCE: Same collection data (including nest) as holotype: 10 workers, (MNHN 1598); other workers in AT (15556), LB, SMNH, XE and collections of the authors; queen (MNHN 1598).

Museum material
FRANCE: MHNG-FC: 47 specimens, Orange, 14 Sep. 1883. – MCSN-EC: Orange, label with bad handwriting which could be by Forel. – MNHN-BAC: Mont Ventoux, S face, Drôme, 16 Sep. 1978, coll.

Casevitz-Weulersse; 8 workers (2 pins), Gréolières, Alpes-Maritimes; ~30 workers (9 pins), Plateau de Caussols. – MCZL-KC: 5 pins, Caussol, S France, 9 Jun. 1954, leg. Stumper.

New material

FRANCE: All in personal collections of CG and RB: Plateau de Calern, 22 workers, 43.7553° N, 6.9113° E, leg. C. Galkowski, Aug. 2012, leg. A. Touchard; Plateau de Caussols, 23 workers, 43.7319° N, 6.9426° E, leg. C. Galkowski, Jun. 2012, leg. A. Touchard; Gréolières, 14 workers, 43.82° N, 6.92° E, Aug. 2012, leg. A. Touchard; Orange, 13 workers, 2 queens, 44.1344° N, 4.8084° E, 14 Jul. 2011, leg. R. Blatrix; Sisteron, 12 workers, 44.1989° N, 5.9419° E, 17 Jun. 2012, leg. R. Blatrix; Mont Ventoux, 41 workers, 4 queens, 44.1519° N, 5.3226° E, 2009, leg. C. Galkowski, 14 Jul. 2011, leg. R. Blatrix, Aug. 2011, leg. C. Lebas.

Description

Minor worker (n = 72)

CW 540–900 µm; nCH 0–10 (4.5); nCU 0–8 (3.4); nTx 3–23 (12.9); nSc 0–7 (3.4); nG 1–27 (10.1); PDG 34–48 (40.3); GHL 70–170 µm (123.7); GHL/CW 0.124–0.263 (0.171).

Media and major worker (n = 53)

CW 900–1530 µm; nCH 2–15 (8.4); nCU 2–17 (7.1); nTx 20–75 (38.9); nSc 3–22 (7.6); nG 12–43 (24.8); PDG 24–68 (43.7); GHL 140–240 µm (178.9); GHL/CW 0.117–0.186 (0.145).

Body black; only tibiae, scape and mandible brown. All parts of body with long erect hairs (GHL/CW > 0.12). Pubescence on dorsal surface of first gaster tergite sparse in all worker categories (PDG > 24), revealing smooth and shining cuticle. Profile of mesosoma sinuous. Petiolar scale erect, thick, slightly notched at summit in large workers. Head of minor workers clearly elongate, but less than in *P. nasuta* (CL/CW 1.16–1.28). Head of media and major workers even less elongate (CL/CW 1.046–1.19). Clypeus finely striate longitudinally, with faint trace of median carina. Frontal triangle and space between frontal carina also finely striate. Sculpture disappears toward occiput, cuticle becoming smooth and shining, or faintly punctuated in large workers. Scape long, surpassing occipital border.

Queen (n = 6)

CW 1530–1770 µm (1690); CL 1550–1750 µm (1660); SL 1220–1390 µm (1300); nCH 4–13 (8.20); nCU 5–7 (6.20); nTX 62–92 (79); nSc 8–13 (11.2); nG 26–36 (31.5); PDG 12–17 (14.5); GHL 210–240 µm (227); MW 1280–1410 µm (1330); ML 2240–2510 µm (2390); ScW 760–850 µm (820); GHL/CW 0.124–0.141 (0.135).

Color as in worker. Many long and erect hairs on all body parts (nG > 26, GHL > 200 µm). Some erect hairs also on femora and tibiae. Dense pubescence on entire body, masking surface of cuticle. Mesosoma less wide than head. Wing remains indicate winged queens, although wings possibly small given reduced development of scutum and scutellum. Petiolar scale high and wide, deeply notched at summit. Gaster rather small. Head almost as large as long, entirely and finely sculptured, faint riddles of anterior part replaced by puncture on posterior part, giving head an almost dull aspect. Clypeus finely striate longitudinally. Scape surpassing occipital border.

Male

Unknown.

Distribution

Southern France (Fig. 1).

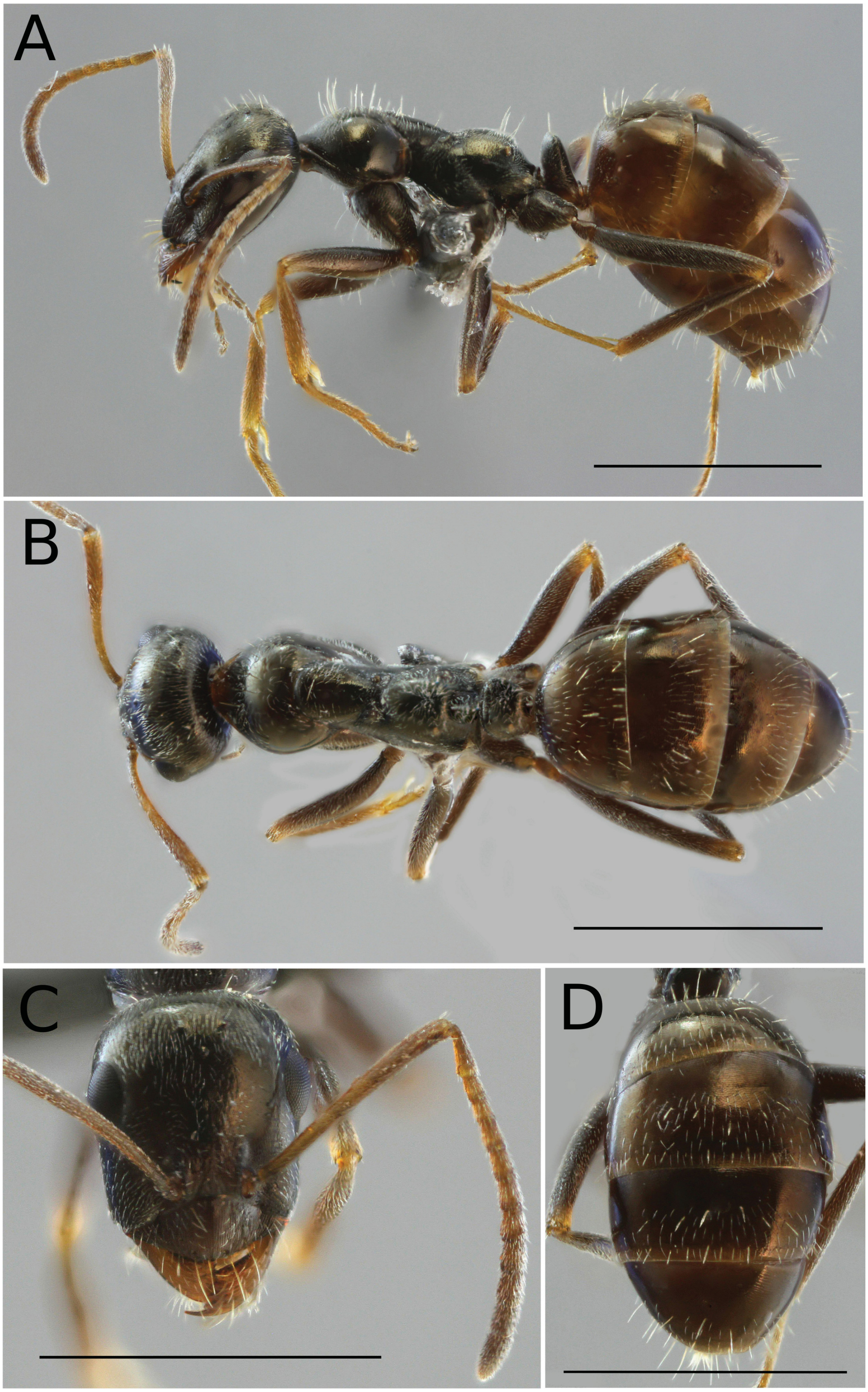

Fig. 9. *Proformica longipilosa* sp. nov., holotype, minor worker from colony Mont Ventoux 10, France. **A**. Lateral view. **B**. Dorsal view. **C**. Head in full face view. **D**. Gaster in dorsal view. Scale bars = 1 mm. Automontage: Claude Lebas.

Remarks

We made direct comparisons between specimens of *P. longipilosa* sp. nov. and *P. longiseta* (A. Tinaut leg.) from Sierra Nevada, Spain, and *P. ferreri* (IRSNB-BC, 2 workers, 1 ♂ (type specimen) from Spain). The latter two species, in addition to *P. nasuta*, were formally described from western Europe. Specimens of *P. longipilosa* sp. nov. are unambiguously distinguished from those of these two species by the combination of the following characters: erect hairs on the body are longer (GHL/CW > 0.12) and the pubescence on the dorsal surface of the first gaster tergite is sparser. This last character is especially discriminant in media and major workers (PDG 24–68, mean = 43.7). In addition, the cuticle is smoother, giving a shinier appearance, in particular on the head.

Discussion

Based on arguments from morphometric analysis, DNA sequences and cuticular hydrocarbons, we show that the populations of *Proformica* in southern France belong to two species, one of which is *P. nasuta*, and the other a new species that we name *P. longipilosa* sp. nov.

Fig. 10. *Proformica longipilosa* sp. nov., queen from colony Mont Ventoux 10, France. **A**. Lateral view. **B**. Dorsal view. **C**. Head in full face view. Scale bars = 1 mm. Automontage: Claude Lebas.

Consequences for the taxonomy of *Proformica*

There has been much confusion in the concept of the taxon *P. nasuta*, in part because the type appears to have been lost a long time ago. None of the taxonomic studies published after the description of the species by Nylander (1856) made reference to the type, which was collected in Beaucaire (France). Forel (1886) described the worker and the queen from specimens collected in Orange (France) and sent samples to many of his colleagues throughout Europe and Russia. We examined the specimens from Orange in the Forel, Emery and Bondroit collections, and we collected new samples from the same locality in 2011. All differ markedly from those of Beaucaire and belong to the new species we describe in this study, *P. longipilosa* sp. nov. All the taxonomic studies after 1886 used the samples from Orange, or descriptions of them, as a reference for *P. nasuta*. These studies described *P. nasuta* as having long erect hairs and sparse pubescence (e.g., Ruzsky 1905; Emery 1909; Wheeler 1913; Bondroit 1918; Santschi 1925; Bernard 1968; Dlussky 1969; Collingwood 1976; Agosti & Collingwood 1987), two characters that are typical of *P. longipilosa* sp. nov. Other specimens from southeastern France (Plateau de Caussols, Tourettes-sur-Loup) have also been used as references for *P. nasuta* (Collingwood 1956; Stumper 1957; Dlussky 1969), but they come from an area that we now recognise as belonging to the range of *P. longipilosa* sp. nov., and are thus likely distinct from *P. nasuta*. This mistake has been perpetuated so that the actual conception of *P. nasuta* refers to *P. longipilosa* sp. nov. A consequence of this is that all reports of *P. nasuta* since 1886, including all those from eastern Europe and Asia, are probably erroneous.

There is a striking similarity between the biogeographic patterns of the genus *Proformica* and those observed for other steppe elements (Ruano *et al.* 2011; Sanllorente *et al.* 2015) such as, for instance, the meadow and steppe vipers (*Vipera ursinii* species complex). Both *Proformica* and the *V. ursinii* complex are known to occupy the same type of habitats and show the same pattern of disjunct distribution, with one area across Asia and one area restricted to western Europe. Moreover, they are both poor dispersers (Sanllorente *et al.* 2015; Ferchaud *et al.* 2011). It is likely that the current distribution and speciation patterns of the two groups have been induced by the same climatic and geographic events. The eastern and western clades of the *Vipera ursinii* species complex diverged about 4 Mya (Zinenko *et al.* 2015), and the taxa from the two geographic areas are completely distinct. In addition, mutation rates and the number of generations per unit time are expected to be higher in insects than in snakes (Martin & Palumbi 1993). We thus expect that the *Proformica* species from western Europe are distinct from those from the East.

Intraspecific variation

Localities for *P. nasuta* can be divided into two subgroups. One is composed of lowland localities that can be close to each other and form an almost continuous distribution in the plain of the Languedoc and the Rhône valley (Montpellier, Sauteyrargues, Pompignan, Grospierres, Collias, Beaucaire, Jonquières, Tarascon, Plaine de la Crau, Aurons and Bonnieux). The other subgroup is composed of localities isolated on the summits of medium-sized mountains (Sumène, Sainte-Baume, Grand Luberon and Montagne de Lure). Beyond ecological differences (mountain vs lowland), the distinction between the two groups is supported by the analysis of morphological characters, DNA and cuticular hydrocarbons. Except for Sumène, individuals from mountain localities have some erect hairs on the mesosoma, whereas hairs are lacking in most lowland individuals. Mountain individuals also have longer hairs and sparser pubescence on the gaster compared to lowland individuals. Interestingly, mitochondrial sequences from Sainte-Baume, Grand Luberon and Montagne de Lure form three monophyletic clades that are highly differentiated from each other, whereas most of the lowland localities show little differentiation. This pattern is consistent with a particularly high degree of isolation of the mountain localities, as already shown for *P. longiseta* in Spain (Sanllorente *et al.* 2015). Cuticular hydrocarbons also differ between lowland and mountain localities. At this stage we are reluctant to consider the mountain localities as a separate species because lowland and mountain localities form a consistent group morphologically and do not form two monophyletic clades in the mitochondrial phylogenies. Instead, we consider that the population of each mountain locality is derived independently from the lowland population. Sanllorente *et al.* (2015) proposed that climate-driven range fluctuation of populations of *P. longiseta* during Pleistocene glaciations induced a strong isolation among populations that are now restricted to mountain tops in southern Spain, because this species is adapted to cold and arid environments. Extant populations would be derived from an ancient, large population, independently from each other. A similar process may explain the divergence we noted between lowland and mountain populations of *P. nasuta*, and among mountain populations, and may have induced local adaptation of mountain populations. The morphological features of the mountain individuals of *P. nasuta* make them more similar to *P. longipilosa* sp. nov., especially where localities of the two species are close to each other (Grand Luberon and Montagne de Lure). These features might be the result either of introgression of *P. longipilosa* sp. nov. into populations of *P. nasuta*, or of morphological convergence in response to a similar environment.

Proformica longipilosa sp. nov. also shows two subgroups. One is composed of populations from localities on plateaus and mountains (Plateau de Calern, Plateau de Caussols, Gréolières and Mont Ventoux) and the other is composed of lower elevation localities (Orange, Sisteron and Vinsobre). Individuals of the lowland localities tend to have shorter hairs and denser pubescence on the gaster than those from high-elevation localities, and thus are morphologically closer to *P. nasuta* than the high-elevation individuals. As proposed for *P. nasuta*, these features could result from either introgression or convergence. Although lowland *P. longipilosa* sp. nov. and mountain *P. nasuta* tend to converge morphologically, they can still be easily distinguished, leaving no doubt regarding their assignment to species. All nests from the three low-elevation localities of *P. longipilosa* sp. nov. had mitochondrial sequences typical of *P. nasuta*. We hypothesise that this incongruence between morphological and molecular characters, specifically for low-elevation localities, results from introgression of mitochondrial DNA from *P. nasuta* to *P. longipilosa* sp. nov. Such introgression would be most likely to occur in low-elevation localities because *P. nasuta* is relatively widespread in the lowlands. Complete or gene-specific introgression of maternal DNA is a well-known phenomenon in insects (Ballard 2000; Chan & Levin 2005; Linnen & Farrell 2007). An isolated event of partial mitochondrial introgression is also suggested in Plateau de Calern, where all nests were morphologically classified as *P. longipilosa* sp. nov., but one had Cytb sequences typical of *P. nasuta*. All other nests in this locality fitted within *P. longipilosa* sp. nov. for both mitochondrial markers. It is worth noting that we could not find any locality with nests of both *P. nasuta* and *P. longipilosa* sp. nov., although the two can be found in similar habitats.

Interpretation of cuticular hydrocarbons

Cuticular hydrocarbons separate the two species without ambiguity. Populations from all localities of *P. nasuta* form a homogeneous clade with relatively little differentiation except for two localities isolated at the summits of mountains (Grand Luberon and Montagne de Lure). *Proformica longipilosa* sp. nov. (Mont Ventoux and Sisteron) appears to be well separated from all other ants studied here, including the outgroups (*P. longiseta* and *Cataglyphis* Förster, 1850), confirming its status as a separate species. This classification is globally consistent with spatial distribution of the localities and with the classification based on morphology and DNA sequences. Localities of *P. nasuta* from the lowlands form continuous populations without important geographical isolation, allowing regular exchange of migrants resulting in little differentiation of cuticular hydrocarbons. However, the mountain localities Grand Luberon and Montagne de Lure are isolated and, probably as a consequence, are divergent for cuticular hydrocarbons. On the mitochondrial tree they also diverge from other localities of *P. nasuta*. Cuticular hydrocarbons in *Proformica* appear to be linked to phylogenetic signature but seem to change rapidly with geographical isolation, even faster than mitochondrial DNA. Geographic variation in cuticular hydrocarbons depends on the taxon. For instance, profiles are very stable for *Formica* ants from Finland to Great Britain (Martin *et al.* 2008) and for *Lasius niger* Linnaeus, 1758 from Denmark to the south of France (Lenoir *et al.* 2009; Lenoir unpubl.). In contrast, rapid spatial changes in hydrocarbons are present in some taxa like *Odontomachus* Latreille, 1804 (Smith *et al.* 2013) and *Cataglyphis* (Dahbi *et al.* 1996). Interestingly, *Rossomyrmex minuchae* Tinaut, 1981, a slave-maker parasite of *Proformica longiseta*, also has different chemotypes in three populations in Sierra Nevada, Spain (Sanllorente *et al.* 2012). It is noteworthy that the genus *Cataglyphis* is phylogenetically, biologically and ecologically very close to *Proformica*. Both have very limited queen dispersal, are specialized on dry habitats and forage on dead invertebrates at the warmest time of the day. It would be worth investigating whether strong divergence in cuticular hydrocarbons within species could be related to one or more of these characteristics.

Conclusions

Although we relied on an integrative taxonomy approach, using several complementary sources of information, we confirm the general view that the taxonomy of the genus *Proformica* is a complex problem. The nuclear markers chosen for use here evolve too slowly and thus lack resolution. Information from mitochondrial genes is blurred by genetic processes such as transposition and introgression and may be biased by queen philopatry. Our results suggest that morphology is a better tool to resolve taxonomy in this genus than either cuticular hydrocarbons or DNA sequences of the genes commonly used for phylogenetic analyses and barcoding, although genetic markers other than those used in this study should also be investigated. However, for the genus *Proformica*, the zoogeographical region of southern France is the least complex in taxonomic terms. Thus, the morphological approach developed in this paper may prove unreliable in other regions, such as the Iberian Peninsula and eastern Europe. A population-genetic approach using tools such as microsatellites or single nucleotide polymorphisms from Next Generation Sequencing (e.g., RADseq) may help disentangle this taxonomic knot.

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Appendices

Appendix 1. List of the nest samples collected for this study, with geographic locality, number of individuals per nest used for morphological analyses and GenBank accession numbers for DNA markers.

colony# species locality municipality country date latitude longitude altitude_m collector morpho_workers morpho_queens COI Cytb 28S LwRh

RB0000530 *Cataglyphis cursor* Riboux (83) France 3Jun.2011 43.32693 5.76608 Rumsais Blatrix KU749638 KU749738

RB0000577 *Formica cunicularia* Estoher (66) France 25Jun.2011 42.574 2.48461 Rumsais Blatrix KU749784 KU749819

RB0000971 *Cataglyphis mauritanica* Morocco 27May2012 33.3818 -5.1324 Philippe Geniez KU749639 KU749785 KU749820

RB0001590 *Bajcaridris theryi* Morocco Jul.2012 Christophe Galkowski KU749786 KU749821

Salon 1 *Cataglyphis cursor* Salon-de-Provence (13) France Jul.2012 43.63705 5.02083 Claude Lebas KU749640 KU749787 KU749822

Aurons 1 *Proformica nasuta* Aurons Aurons (13) France May2006 43.66 5.15 199 V. Bouchet 4

Beaucaire 1 *Proformica nasuta* Beaucaire Beaucaire (30) France 9Jul.2011 43.83544 4.61828 21 Rumsais Blatrix, Claude Lebas 4 2 KU749600 KU749655

Beaucaire 2 *Proformica nasuta* Beaucaire Beaucaire (30) France 9Jul.2011 43.83547 4.61858 19 Rumsais Blatrix, Claude Lebas 3 KU749656

Beaucaire 3 *Proformica nasuta* Beaucaire Beaucaire (30) France 9Jul.2011 43.83533 4.61878 17 Rumsais Blatrix, Claude Lebas 3 KU749601, KU749602 KU749657

Beaucaire 4 *Proformica nasuta* Beaucaire Beaucaire (30) France 9Jul.2011 43.83531 4.61928 15 Rumsais Blatrix, Claude Lebas 4 KU749603, KU749604, KU749605 KU749658

Bonnieux 1 *Proformica nasuta* Bonnieux Bonnieux (84) France 1Oct.2011 43.8625 5.30694 166 Claude Lebas 4 KU749606 KU749659 KU749759 KU749794

Bonnieux 2 *Proformica nasuta* Bonnieux Bonnieux (84) France 1Oct.2011 43.8625 5.30694 166 Claude Lebas 4 KU749660

Plateau de Calern 10 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France 19Aug.2010 43.7514 6.92349 1264 Christophe Galkowski 4

Plateau de Calern 18 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France 19Aug.2010 43.7514 6.92349 1264 Christophe Galkowski 4

Plateau de Calern 2 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France Aug.2012 43.75325 6.9159 1256 Axel Touchard 3 KU749607 KU749661 KU749760 KU749795

Plateau de Calern 3 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France Aug.2012 43.75457 6.91025 1272 Axel Touchard 3 KU749608 KU749662 KU749761 KU749796

Plateau de Calern 4 *Proformica longipilosa* sp. nov. Plateau de Calern Cipières (06) France Aug.2012 43.75678 6.90642 1283 Axel Touchard 3 KU749609 KU749663 KU749762 KU749797

Plateau de Calern 6 *Proformica longipilosa* sp. nov. Plateau de Calern Andon (06) France Aug.2012 43.77595 6.85128 1127 Axel Touchard 3 KU749610 KU749664 KU749763 KU749798

Plateau de Calern 7 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France 19.Aug2010 43.7514 6.92349 1264 Christophe Galkowski 2 KU749611, KU749612 KU749665, KU749666 KU749764 KU749799

Plateau de Calern 8 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France 19.Aug2010 43.7514 6.92349 1264 Christophe Galkowski KU749667

Plateau de Calern 9 *Proformica longipilosa* sp. nov. Plateau de Calern Caussols (06) France 19.Aug2010 43.7514 6.92349 1264 Christophe Galkowski KU749668

Plateau de Caussols 1 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France Jun.2012 43.73504 6.94663 1102 Axel Touchard 3 KU749672

Plateau de Caussols 10 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France 19Aug.2010 43.7331 6.94244 1115 Christophe Galkowski KU749669

Plateau de Caussols 11 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France 19Aug.2010 43.7331 6.94244 1115 Christophe Galkowski KU749613 KU749670 KU749765 KU749800

Plateau de Caussols 19 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France 19Aug.2010 43.7331 6.94244 1115 Christophe Galkowski 3 KU749671

Plateau de Caussols 2 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France Jun.2012 43.73497 6.94676 1100 Axel Touchard 3 KU749673

Plateau de Caussols 3 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France Jun.2012 43.73488 6.94681 1100 Axel Touchard 3 KU749674

Plateau de Caussols 4 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France Jun.2012 43.73493 6.94699 1098 Axel Touchard 2

Plateau de Caussols 5 *Proformica longipilosa* sp. nov. Plateau de Caussols Caussols (06) France Aug.2012 43.7219 6.90923 1124 Axel Touchard 3 KU749614 KU749675 KU749766 KU749801

Plateau de Caussols 6 *Proformica longipilosa* sp. nov. Plateau de Caussols Caussols (06) France Aug.2012 43.72737 6.93098 1139 Axel Touchard 3 KU749615 KU749676 KU749767 KU749802

Plateau de Caussols 7 *Proformica longipilosa* sp. nov. Plateau de Caussols Gourdon (06) France Aug.2012 43.73065 6.97157 1033 Axel Touchard 3 KU749677

Collias 1 *Proformica nasuta* Collias Collias (30) France 12Jun.2010 43.947721 4.462309 162 Rumsais Blatrix 5

Plaine de la Crau 1 *Proformica nasuta* Plaine de la Crau Saint-Martin-de-Crau (13) France Jun.2011 43.58333 4.83333 19 Claude Lebas 5 KU749616 KU749678 KU749768 KU749803

Plaine de la Crau 2 *Proformica nasuta* Plaine de la Crau Saint-Martin-de-Crau (13) France Aug.2011 43.58333 4.83333 19 Claude Lebas 5

Plaine de la Crau 3 *Proformica nasuta* Plaine de la Crau Saint-Martin-de-Crau (13) France Aug.2011 43.58333 4.83333 19 Claude Lebas 5 KU749679

Plaine de la Crau 4 *Proformica nasuta* Plaine de la Crau Saint-Martin-de-Crau (13) France Aug.2011 43.58333 4.83333 19 Claude Lebas 3

Gréolières 7 *Proformica longipilosa* sp. nov. Gréolières Gréolières (06) France Aug.2012 43.82862 6.94738 1409 Axel Touchard 3 KU749617 KU749680 KU749769 KU749804

Gréolières 8 *Proformica longipilosa* sp. nov. Gréolières Gréolières (06) France Aug.2012 43.81688 6.8898 1202 Axel Touchard 3 KU749770 KU749805

Grospierres 1 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749682

Grospierres 10 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749681

Grospierres 2 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749618 KU749683 KU749771 KU749806

Grospierres 3 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749684

Grospierres 4 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749619 KU749685 KU749772 KU749807

Grospierres 5 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749686

Grospierres 6 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749687

Grospierres 7 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749688

Grospierres 8 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 Théotime Colin 3 KU749689

Grospierres 9 *Proformica nasuta* Grospierres Grospierres (07) France 6Jul.2012 44.41163 4.27133 102 ThÃ©otime Colin 3 KU749690

Jonquières 1 *Proformica nasuta* Jonquières Jonquières-Saint-Vincent (30) France 11May2011 43.83144 4.57653 21 Rumsais Blatrix 2 KU749620 KU749691, KU749692

Jonquières 2 *Proformica nasuta* Jonquières Jonquières-Saint-Vincent (30) France 11May2011 43.83144 4.57653 21 Rumsais Blatrix 2 KU749621 KU749693 KU749773 KU749808

Jonquières 3 *Proformica nasuta* Jonquières Jonquières-Saint-Vincent (30) France 11May2011 43.83144 4.57653 21 Rumsais Blatrix 3 KU749622 KU749694, KU749695

Jonquières F *Proformica nasuta* Jonquières Jonquières-Saint-Vincent (30) France 11May2011 43.83144 4.57653 21 Rumsais Blatrix KU749696

Grand Luberon 1 *Proformica nasuta* Grand Luberon Castellet (84) France 2Jun.2012 43.81713 5.4782 1038 Rumsais Blatrix 3 KU749623 KU749697 KU749774 KU749809

Grand Luberon 2 *Proformica nasuta* Grand Luberon Castellet (84) France 2Jun.2012 43.81716 5.47816 1038 Rumsais Blatrix 4 KU749698

Grand Luberon 3 *Proformica nasuta* Grand Luberon Castellet (84) France 2Jun.2012 43.81689 5.47868 1040 Rumsais Blatrix 3 KU749699

Grand Luberon 4 *Proformica nasuta* Grand Luberon Cabrières-d'Aigues (84) France 2Jun.2012 43.81662 5.478 1050 Rumsais Blatrix 3 KU749700

Grand Luberon 5 *Proformica nasuta* Grand Luberon Castellet (84) France 2Jun.2012 43.81677 5.47548 1091 Rumsais Blatrix 3 KU749701

Grand Luberon 6 *Proformica nasuta* Grand Luberon Cabrières-d'Aigues (84) France 2Jun.2012 43.8155 5.47376 1085 Rumsais Blatrix 3 KU749702

Grand Luberon 7 *Proformica nasuta* Grand Luberon Cabrières-d'Aigues (84) France 2Jun.2012 43.81543 5.47168 1086 Rumsais Blatrix 3 KU749703

Grand Luberon 8 *Proformica nasuta* Grand Luberon Castellet (84) France 2Jun.2012 43.81574 5.46942 1091 Rumsais Blatrix 3 KU749704

Grand Luberon 9 *Proformica nasuta* Grand Luberon Castellet (84) France 2Jun.2012 43.81941 5.46737 1047 Rumsais Blatrix 3 KU749624 KU749705 KU749775 KU749810

Montagne de Lure 1 *Proformica nasuta* Montagne de Lure Châteauneuf-Val-Saint-Donat (04) France 16Jun.2012 44.11319 5.88414 1416 Rumsais Blatrix 3 KU749706 KU749776 KU749811

Montagne de Lure 2 *Proformica nasuta* Montagne de Lure Châteauneuf-Val-Saint-Donat (04) France 16Jun.2012 44.11317 5.88399 1417 Rumsais Blatrix 3 KU749707

Montagne de Lure 3 *Proformica nasuta* Montagne de Lure Châteauneuf-Val-Saint-Donat (04) France 16Jun.2012 44.11314 5.88378 1419 Rumsais Blatrix 3 KU749708

Montagne de Lure 4 *Proformica nasuta* Montagne de Lure Châteauneuf-Val-Saint-Donat (04) France 16Jun.2012 44.11312 5.8837 1419 Rumsais Blatrix 3 KU749709

Montagne de Lure 5 *Proformica nasuta* Montagne de Lure Châteauneuf-Val-Saint-Donat (04) France 16Jun.2012 44.1131 5.88352 1419 Rumsais Blatrix 3 KU749710 KU749777 KU749812

Montpellier 1 *Proformica nasuta* Montpellier Montpellier (34) France 8Mar.2012 43.62914 3.89072 38 Rumsais Blatrix 2 KU749711

Montpellier 2 *Proformica nasuta* Montpellier Montpellier (34) France 8Mar.2012 43.62925 3.89072 40 Rumsais Blatrix 1 KU749712

Orange 1 *Proformica longipilosa* sp. nov. Orange Orange (84) France 14Jul.2011 44.13486 4.80817 93 Rumsais Blatrix 8 2 KU749625, KU749626 KU749713 KU749778 KU749813

Orange 2 *Proformica longipilosa* sp. nov. Orange Orange (84) France 14Jul.2011 44.1345 4.8084 96 Rumsais Blatrix 1

Orange 3 *Proformica longipilosa* sp. nov. Orange Orange (84) France 14Jul.2011 44.13394 4.80864 99 Rumsais Blatrix 4 KU749627 KU749714 KU749779 KU749814

Sainte-Baume 1 *Proformica nasuta* Sainte-Baume Riboux (83) France 3Jun.2011 43.32738 5.77002 950 Rumsais Blatrix 3 KU749628, KU749629, KU749630 KU749721, KU749722 KU749780 KU749815

Sainte-Baume 11 *Proformica nasuta* Sainte-Baume Cuges-les-pins (13) France Jul.2012 43.31528 5.71057 1012 Claude Lebas 4 1 KU749715

Sainte-Baume 12 *Proformica nasuta* Sainte-Baume Cuges-les-pins (13) France Jul.2012 43.31495 5.70795 1009 Claude Lebas 4 KU749716

Sainte-Baume 13 *Proformica nasuta* Sainte-Baume Cuges-les-pins (13) France Jul.2012 43.31528 5.71057 1012 Claude Lebas 3 KU749717

Sainte-Baume 14 *Proformica nasuta* Sainte-Baume Plan-d’Aups-Sainte-Baume (83) France Jul.2012 43.31582 5.70975 1017 Claude Lebas KU749718

Sainte-Baume 15 *Proformica nasuta* Sainte-Baume Cuges-les-pins (13) France Jul.2012 43.31468 5.70517 996 Claude Lebas KU749719

Sainte-Baume 16 *Proformica nasuta* Sainte-Baume Cuges-les-pins (13) France Jul.2012 43.31468 5.70517 996 Claude Lebas KU749720

Sainte-Baume 2 *Proformica nasuta* Sainte-Baume Riboux (83) France 3Jun.2011 43.32738 5.77002 950 Rumsais Blatrix KU749632, KU749633 KU749725

Sainte-Baume 21 *Proformica nasuta* Sainte-Baume Plan-d’Aups-Sainte-Baume (83) France Jul.2012 43.31937 5.68035 864 Claude Lebas KU749631 KU749723 KU749781 KU749816

Sainte-Baume 22 *Proformica nasuta* Sainte-Baume Plan-d’Aups-Sainte-Baume (83) France Jul.2012 43.31937 5.68035 864 Claude Lebas 3 KU749724

Sainte-Baume 3 *Proformica nasuta* Sainte-Baume Riboux (83) France 3Jun.2011 43.32738 5.77002 950 Christophe Galkowski 4 KU749726

Sainte-Baume 4 *Proformica nasuta* Sainte-Baume Riboux (83) France 3Jun.2011 43.32738 5.77002 950 Christophe Galkowski 5 KU749727

Sainte-Baume 5 *Proformica nasuta* Sainte-Baume Riboux (83) France Aug.2011 43.32738 5.77002 950 Claude Lebas 4 KU749728

Sainte-Baume 6 *Proformica nasuta* Sainte-Baume Riboux (83) France Aug.2011 43.32738 5.77002 950 Claude Lebas 4 KU749729, KU749730

Sainte-Baume 7 *Proformica nasuta* Sainte-Baume Riboux (83) France 10Sep.2011 43.32738 5.77002 950 Rumsais Blatrix 4 KU749731

Sainte-Baume 8 *Proformica nasuta* Sainte-Baume Riboux (83) France 10Sep.2011 43.32738 5.77002 950 Rumsais Blatrix 4 KU749732

Sainte-Baume 9 *Proformica nasuta* Sainte-Baume Riboux (83) France 10Sep.2011 43.32738 5.77002 950 Rumsais Blatrix 4 KU749733

Pompignan 1 *Proformica nasuta* Pompignan Pompignan (30) France May2010 43.89747 3.82489 199 Rumsais Blatrix 1 KU749636 KU749735 KU749782 KU749817

Pompignan 10 *Proformica nasuta* Pompignan Pompignan (30) France 10Jul.2011 43.899 3.82611 196 Rumsais Blatrix, Philippe Wegnez 2 KU749634, KU749635 KU749734

Pompignan 2 *Proformica nasuta* Pompignan Pompignan (30) France May2010 43.89747 3.82489 199 Rumsais Blatrix 1 KU749637 KU749736, KU749737 KU749783 KU749818

Pompignan 3 *Proformica nasuta* Pompignan Pompignan (30) France May2010 43.89747 3.82489 199 Rumsais Blatrix 1

Sauteyrargues 1 *Proformica nasuta* Sauteyrargues Sauteyrargues (34) France 28May2011 43.8275 3.91917 118 Rumsais Blatrix 2 KU749641 KU749739 KU749788 KU749823

Sauteyrargues 2 *Proformica nasuta* Sauteyrargues Sauteyrargues (34) France 28May2011 43.8275 3.91917 118 Rumsais Blatrix 3 KU749740

Sisteron 1 *Proformica longipilosa* sp. nov. Sisteron Sisteron (04) France 17Jun.2012 44.19895 5.94151 554 Rumsais Blatrix 3 KU749642 KU749741 KU749789 KU749824

Sisteron 2 *Proformica longipilosa* sp. nov. Sisteron Sisteron (04) France 17Jun.2012 44.19872 5.94217 558 Rumsais Blatrix 3 KU749742

Sisteron 3 *Proformica longipilosa* sp. nov. Sisteron Sisteron (04) France 17Jun.2012 44.19898 5.9419 558 Rumsais Blatrix 3 KU749743

Sisteron 4 *Proformica longipilosa* sp. nov. Sisteron Sisteron (04) France 17Jun.2012 44.1991 5.94187 558 Rumsais Blatrix 3 KU749643 KU749744 KU749790 KU749825

Sumène 1 *Proformica nasuta* Montagne de la Fage Sumène (30) France 9Apr.2012 43.99044 3.7714 929 Rumsais Blatrix 3 KU749644 KU749745

Sumène 2 *Proformica nasuta* Montagne de la Fage Sumène (30) France 9Apr.2012 43.99044 3.7714 929 Rumsais Blatrix 3 KU749746

Tarascon 1 *Proformica nasuta* Tarascon Tarascon (13) France 9Jul.2011 43.84189 4.73778 15 Rumsais Blatrix, Claude Lebas 4 1 KU749645 KU749747

Tarascon 2 *Proformica nasuta* Tarascon Tarascon (13) France 9Jul.2011 43.84186 4.73744 16 Rumsais Blatrix, Claude Lebas 4 KU749748

Tarascon 3 *Proformica nasuta* Tarascon Tarascon (13) France 9Jul.2011 43.84247 4.7395 16 Rumsais Blatrix, Claude Lebas 4 KU749646 KU749749

Mont Ventoux 1 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 2009 44.15158 5.32319 1466 Christophe Galkowski 3 KU749652 KU749756 KU749791 KU749826

Mont Ventoux 10 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 14Jul.2011 44.15261 5.32081 1442 Rumsais Blatrix 4 KU749647, KU749648 KU749750

Mont Ventoux 11 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 14Jul.2011 44.15158 5.32319 1466 Rumsais Blatrix 4 1 KU749649 KU749751

Mont Ventoux 12 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 14Jul.2011 44.15231 5.31939 1425 Rumsais Blatrix 4 KU749650 KU749752

Mont Ventoux 13 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 14Jul.2011 44.15183 5.32328 1466 Rumsais Blatrix 4 KU749651 KU749753

Mont Ventoux 14 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 14Jul.2011 44.1521 5.3232 1473 Philippe Wegnez KU749754

Mont Ventoux 15 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France Aug.2011 44.1521 5.3232 1473 Claude Lebas 4 KU749755

Mont Ventoux 16 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France Aug.2011 44.1521 5.3232 1473 Claude Lebas 4

Mont Ventoux 2 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 2009 44.15158 5.32319 1466 Christophe Galkowski 8 1 KU749653 KU749757 KU749792 KU749827

Mont Ventoux 3 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 2009 44.15158 5.32319 1466 Christophe Galkowski 3

Mont Ventoux 4 *Proformica longipilosa* sp. nov. Mont Ventoux, Chalet Reynard Bedoin (84) France 2009 44.15158 5.32319 1466 Christophe Galkowski 3

Vinsobre 1 *Proformica longipilosa* sp. nov. Vinsobre Vinsobre (84) France Aug.2012 44.33333 5.05972 297 Philippe Wegnez KU749654 KU749758 KU749793 KU749828

Appendix 2. Measurements of morphological characters for 321 workers of *Proformica* from southern France.

colony CW nCH nCU nTx nSc nG PDG GHL

Aurons 1 1190 1 2 2 2 5 17 70

Aurons 1 850 0 2 1 0 5 18 50

Aurons 1 545 0 1 1 1 1 11 35

Aurons 1 580 0 0 0 0 0 14 0

Beaucaire 1 1240 0 1 0 0 2 14 42

Beaucaire 1 1150 0 2 0 0 1 14 60

Beaucaire 1 990 0 1 0 0 2 14 50

Beaucaire 1 715 0 0 0 0 2 15 50

Beaucaire 2 815 0 0 0 0 1 17 60

Beaucaire 2 600 0 0 0 0 0 20 0

Beaucaire 2 650 0 0 0 0 2 15 50

Beaucaire 3 885 0 0 0 0 2 17 35

Beaucaire 3 595 0 0 0 0 0 11 0

Beaucaire 3 615 0 0 1 0 2 15 50

Beaucaire 4 935 0 1 0 0 3 14 50

Beaucaire 4 920 0 0 0 0 2 13 50

Beaucaire 4 680 0 0 0 0 0 17 0

Beaucaire 4 615 0 0 0 1 1 15 50

Bonnieux 1 600 0 0 0 0 0 17 0

Bonnieux 1 680 0 0 0 0 2 18 35

Bonnieux 1 700 0 0 1 0 0 21 0

Bonnieux 1 680 0 0 0 0 2 17 35

Bonnieux 2 730 0 0 2 0 0 24 0

Bonnieux 2 615 0 0 0 0 2 28 42

Bonnieux 2 615 0 0 0 0 0 17 0

Bonnieux 2 715 0 0 0 0 2 21 42

Plateau de Calern 10 1020 7 8 20 3 18 42 155

Plateau de Calern 10 900 5 6 23 4 10 42 136

Plateau de Calern 10 731 7 2 10 3 5 42 102

Plateau de Calern 10 663 5 3 6 4 7 42 145

Plateau de Calern 18 1530 10 13 75 22 38 56 221

Plateau de Calern 18 1275 8 8 37 11 28 56 187

Plateau de Calern 18 1225 6 5 33 7 22 68 204

Plateau de Calern 18 612 1 2 7 4 9 42 85

Plateau de Calern 7 1070 6 8 27 4 28 56 170

Plateau de Calern 7 663 6 5 13 2 10 42 155

Plateau de Calern 2 1100 7 15 34 8 20 42 200

Plateau de Calern 2 715 4 5 11 2 15 38 100

Plateau de Calern 2 680 2 4 11 3 10 38 100

Plateau de Calern 3 1200 11 10 27 8 39 38 220

Plateau de Calern 3 835 9 7 21 5 27 42 170

Plateau de Calern 3 715 2 8 12 6 16 42 170

Plateau de Calern 4 1225 10 5 48 7 43 38 200

Plateau de Calern 4 765 9 4 18 5 17 42 170

Plateau de Calern 4 765 8 7 9 3 13 38 100

Plateau de Calern 6 1395 13 17 65 8 36 42 200

Plateau de Calern 6 700 8 5 21 4 15 38 100

Plateau de Calern 6 735 4 5 18 5 11 42 170

Plateau de Caussols 1 1350 8 6 45 9 26 56 190

Plateau de Caussols 1 1170 9 8 40 5 22 48 170

Plateau de Caussols 1 715 6 4 18 2 13 42 102
Plateau de Caussols 19 1275 4 6 36 9 32 56 221
Plateau de Caussols 19 918 3 8 24 6 25 68 155
Plateau de Caussols 19 714 1 4 16 5 7 45 130
Plateau de Caussols 2 1325 12 9 57 10 38 48 170
Plateau de Caussols 2 1445 8 6 42 5 37 48 200
Plateau de Caussols 2 1120 6 9 30 5 29 48 170
Plateau de Caussols 3 1170 3 4 30 6 32 56 155
Plateau de Caussols 3 830 6 4 21 5 22 42 120
Plateau de Caussols 3 545 3 2 11 2 6 42 68
Plateau de Caussols 4 1260 6 7 44 7 32 56 190
Plateau de Caussols 4 730 4 5 14 4 12 42 102
Plateau de Caussols 5 1460 9 17 54 14 37 56 220
Plateau de Caussols 5 950 10 6 31 8 26 42 155
Plateau de Caussols 5 650 3 3 4 2 11 42 100
Plateau de Caussols 6 1325 15 13 60 12 35 56 190
Plateau de Caussols 6 1020 12 7 26 4 15 38 140
Plateau de Caussols 6 615 2 3 10 2 10 38 120
Plateau de Caussols 7 1360 10 11 46 7 31 48 170
Plateau de Caussols 7 780 5 3 12 4 18 42 100
Plateau de Caussols 7 680 3 3 9 3 8 42 100
Collias 1 1160 0 2 2 0 7 14 76
Collias 1 580 0 0 1 0 2 12 50
Collias 1 660 0 0 0 0 1 12 68
Collias 1 731 0 0 0 0 1 11 75
Collias 1 650 0 0 0 0 0 11 0
Plaine de la Crau 1 1000 0 2 7 0 2 18 50
Plaine de la Crau 1 870 0 0 0 0 2 14 35
Plaine de la Crau 1 815 0 0 2 0 0 14 0
Plaine de la Crau 1 665 0 0 0 0 0 14 0
Plaine de la Crau 1 615 0 0 0 0 2 12 45
Plaine de la Crau 2 885 0 3 3 0 1 15 60
Plaine de la Crau 2 850 0 2 5 2 0 17 0
Plaine de la Crau 2 600 0 0 1 0 0 14 0
Plaine de la Crau 2 575 0 0 0 0 0 14 0
Plaine de la Crau 2 650 0 0 0 0 1 14 50
Plaine de la Crau 3 900 0 3 3 3 3 17 50
Plaine de la Crau 3 575 0 0 0 1 1 9 35
Plaine de la Crau 3 615 0 0 0 0 0 11 0
Plaine de la Crau 3 715 0 1 0 0 1 11 35
Plaine de la Crau 3 600 0 1 0 1 2 12 50
Plaine de la Crau 4 1090 0 0 4 2 3 17 60
Plaine de la Crau 4 800 0 0 0 0 1 14 35
Plaine de la Crau 4 850 0 0 1 0 3 14 60
Gréolières 7 1240 13 7 33 9 23 48 170
Gréolières 7 850 8 4 11 5 15 42 155
Gréolières 7 665 2 3 10 3 10 42 100
Gréolières 8 1325 8 9 59 8 35 48 240
Gréolières 8 815 6 7 17 5 9 42 155
Gréolières 8 645 3 4 12 3 7 38 140
Grospierres 1 850 0 2 3 2 1 10 50
Grospierres 1 715 0 1 0 0 1 10 35
Grospierres 1 560 0 0 0 0 0 10 0
Grospierres 10 815 2 1 3 1 4 10 50
Grospierres 10 715 0 0 0 0 0 12 0
Grospierres 10 600 0 0 0 0 1 14 35
Grospierres 2 870 0 0 1 0 2 10 35
Grospierres 2 615 0 0 1 0 0 12 0
Grospierres 2 615 0 0 0 0 0 9 0
Grospierres 3 850 0 0 0 0 2 9 35
Grospierres 3 680 0 1 0 1 1 12 35
Grospierres 3 545 0 0 0 0 0 12 0
Grospierres 4 920 0 1 5 1 3 14 60
Grospierres 4 715 0 0 0 0 0 12 0
Grospierres 4 650 0 0 0 0 1 10 35
Grospierres 5 730 0 0 4 0 3 10 50
Grospierres 5 650 0 0 0 0 1 12 35
Grospierres 5 580 0 0 1 0 1 9 35
Grospierres 6 920 1 0 5 0 4 10 50
Grospierres 6 650 0 0 0 0 0 9 0
Grospierres 6 615 0 0 0 0 1 12 35
Grospierres 7 935 1 0 6 4 3 10 35
Grospierres 7 750 0 0 3 0 2 9 35
Grospierres 7 665 0 0 1 0 1 12 35
Grospierres 8 850 1 0 6 0 3 12 50
Grospierres 8 810 0 0 0 1 0 12 0
Grospierres 8 715 0 0 2 0 2 12 35
Grospierres 9 870 2 0 4 1 4 12 50
Grospierres 9 575 0 0 0 0 0 10 0
Grospierres 9 550 0 0 0 0 0 10 0
Jonquières 1 1120 0 0 0 0 0 12 0
Jonquières 1 663 0 0 0 0 1 12 68
Jonquières 2 1050 0 0 0 0 2 14 50
Jonquières 2 714 0 0 0 0 1 12 68
Jonquières 3 952 0 0 1 0 1 14 60
Jonquières 3 1020 0 0 0 0 3 14 50
Jonquières 3 663 0 0 1 0 1 12 50
Grand Luberon 1 1395 6 5 28 3 10 14 85
Grand Luberon 1 1070 2 2 16 4 8 21 68
Grand Luberon 1 750 2 2 8 4 6 14 68
Grand Luberon 2 1000 4 5 14 5 8 14 68
Grand Luberon 2 970 4 6 16 1 6 21 68
Grand Luberon 2 665 4 1 8 1 3 21 50
Grand Luberon 2 580 2 0 5 0 2 24 50
Grand Luberon 3 1290 7 4 21 2 7 17 85
Grand Luberon 3 800 3 4 10 3 7 34 68
Grand Luberon 3 680 4 3 4 3 1 34 35
Grand Luberon 4 1020 3 4 13 2 3 14 68
Grand Luberon 4 665 4 1 3 4 5 24 85
Grand Luberon 4 630 4 2 5 3 5 34 60
Grand Luberon 5 1200 8 6 16 4 14 14 68
Grand Luberon 5 935 4 0 3 1 7 34 68
Grand Luberon 5 760 4 3 6 2 7 34 60
Grand Luberon 6 1260 6 3 19 5 6 17 68
Grand Luberon 6 1020 2 3 12 5 9 17 68
Grand Luberon 6 665 3 2 7 1 3 14 50

Grand Luberon 7 1190 7 5 18 2 9 19 85
Grand Luberon 7 865 7 3 14 3 5 19 50
Grand Luberon 7 700 2 0 0 0 0 34 0
Grand Luberon 8 1190 5 5 18 3 9 14 75
Grand Luberon 8 850 4 4 15 3 7 21 93
Grand Luberon 8 650 4 0 6 0 4 28 50
Grand Luberon 9 1120 6 6 28 6 8 17 68
Grand Luberon 9 830 2 2 9 3 4 13 68
Grand Luberon 9 580 1 0 8 1 4 21 68
Montagne de Lure1 1225 6 4 12 7 10 14 68
Montagne de Lure 1 765 2 1 9 4 8 14 50
Montagne de Lure 1 700 0 1 5 0 7 14 42
Montagne de Lure 2 970 3 3 13 4 5 14 76
Montagne de Lure 2 815 1 1 7 2 9 14 50
Montagne de Lure 2 700 1 2 7 3 5 14 50
Montagne de Lure 3 1325 2 4 19 5 7 14 60
Montagne de Lure 3 815 2 3 11 1 6 14 50
Montagne de Lure 3 670 2 1 4 2 6 14 35
Montagne de Lure 4 1325 3 5 23 1 6 14 50
Montagne de Lure 4 850 5 5 9 2 12 14 65
Montagne de Lure 4 700 2 3 5 2 6 14 42
Montagne de Lure 5 1260 9 6 25 6 7 14 85
Montagne de Lure 5 1020 3 2 15 3 9 12 50
Montagne de Lure 5 700 2 1 7 2 4 15 42
Montpellier 1 715 0 0 0 0 1 14 35
Montpellier 1 615 0 0 0 0 0 12 0
Montpellier 2 630 0 0 0 0 1 17 35
Orange 1 715 0 0 11 2 7 48 120
Orange 1 884 1 0 5 1 8 42 140
Orange 1 680 1 2 10 0 4 42 120
Orange 1 715 2 1 9 1 5 42 135
Orange 1 1350 0 1 28 2 16 37 120
Orange 1 1140 0 2 24 4 19 42 120
Orange 1 865 0 1 12 3 8 56 135
Orange 1 900 1 2 13 3 7 42 120
Orange 2 580 0 0 3 1 1 42 85
Orange 3 935 2 2 15 2 9 42 130
Orange 3 815 1 0 4 2 4 42 110
Orange 3 700 1 2 10 1 6 37 95
Orange 3 715 2 2 5 2 5 42 110
Saint-Baume 1 1140 6 6 21 4 7 15 60
Saint-Baume 1 1105 2 5 12 4 5 18 50
Saint-Baume 1 815 2 6 5 3 2 13 75
Saint-Baume 11 1200 1 4 23 4 12 34 70
Saint-Baume 11 1100 3 1 20 1 5 21 50
Saint-Baume 11 630 1 3 6 1 5 34 50
Saint-Baume 11 650 1 1 3 1 4 17 50
Saint-Baume 12 780 2 3 5 0 6 37 68
Saint-Baume 12 680 2 1 8 2 4 17 42
Saint-Baume 12 730 1 3 2 1 1 14 35
Saint-Baume 12 650 2 0 7 3 2 17 50
Saint-Baume 13 985 4 2 7 2 7 14 35
Saint-Baume 13 730 0 3 2 1 4 17 25
Saint-Baume 13 680 0 2 4 1 4 21 35
Saint-Baume 22 1080 2 4 16 5 8 15 60
Saint-Baume 22 865 1 3 11 1 7 15 50
Saint-Baume 22 630 0 0 5 1 3 17 35
Saint-Baume 3 1190 2 3 19 3 3 20 50
Saint-Baume 3 833 0 5 8 3 2 17 50
Saint-Baume 3 850 0 4 8 3 2 18 50
Saint-Baume 3 600 0 0 6 1 1 24 35
Saint-Baume 4 1140 6 5 12 3 6 15 70
Saint-Baume 4 1120 1 5 13 1 4 20 68
Saint-Baume 4 1160 0 5 11 3 7 15 50
Saint-Baume 4 645 0 0 2 1 2 12 50
Saint-Baume 4 612 0 0 4 1 0 12 0
Saint-Baume 5 1275 2 4 19 2 7 14 60
Saint-Baume 5 1190 1 1 15 5 6 17 50
Saint-Baume 5 884 1 3 5 4 1 11 35
Saint-Baume 5 665 0 3 5 0 2 14 50
Saint-Baume 6 985 1 5 8 4 9 17 60
Saint-Baume 6 900 2 3 9 4 5 18 50
Saint-Baume 6 630 2 2 6 2 5 14 50
Saint-Baume 6 650 0 0 6 1 4 17 42
Saint-Baume 7 1050 3 3 9 4 10 13 50
Saint-Baume 7 870 2 2 7 3 6 17 50
Saint-Baume 7 680 0 1 6 2 3 11 35
Saint-Baume 7 630 0 1 4 2 2 17 42
Saint-Baume 8 1155 4 4 17 4 10 14 50
Saint-Baume 8 935 2 4 7 2 6 15 42
Saint-Baume 8 800 3 3 5 2 5 17 68
Saint-Baume 8 665 0 1 5 3 3 14 35
Saint-Baume 9 730 2 4 9 2 7 17 50
Saint-Baume 9 815 1 4 8 3 8 14 60
Saint-Baume 9 730 0 3 6 3 5 17 76
Saint-Baume 9 650 0 3 6 3 5 14 50
Pompignan 1 1200 0 0 0 0 1 12 76
Pompignan 2 1170 0 2 2 0 3 11 68
Pompignan 3 1090 0 0 2 0 1 11.7 60
Sauteyrargues 1 750 0 0 0 0 0 12 0
Sauteyrargues 1 730 0 0 0 0 0 12 0
Sauteyrargues 2 600 0 0 0 0 1 13 35
Sauteyrargues 2 560 0 0 0 0 0 11 0
Sauteyrargues 2 730 0 0 0 0 2 12 50
Sisteron 1 800 10 5 18 3 11 34 130
Sisteron 1 645 8 3 13 4 10 34 120
Sisteron 1 700 10 5 15 4 11 34 102
Sisteron 2 1240 10 8 41 5 24 38 155
Sisteron 2 950 6 6 21 6 17 34 145
Sisteron 2 750 8 5 11 2 6 34 68
Sisteron 3 1035 10 9 35 6 18 34 160
Sisteron 3 800 10 3 13 4 10 42 102
Sisteron 3 680 6 2 12 4 16 34 110
Sisteron 4 815 10 7 20 4 16 34 155
Sisteron 4 750 6 4 11 2 8 34 95
Sisteron 4 665 8 3 14 1 9 42 85

Sumène 1 630 0 0 0 0 0 11 0
Sumène 1 665 0 0 0 0 0 11 0
Sumène 1 560 0 0 0 0 0 11 0
Sumène 2 1100 0 2 0 0 0 12 0
Sumène 2 615 0 1 0 0 0 11 0
Sumène 2 615 0 0 0 0 0 14 0
Tarascon 1 1120 1 4 3 0 5 17 60
Tarascon 1 920 0 2 1 0 2 17 50
Tarascon 1 920 0 2 1 0 2 14 45
Tarascon 1 715 0 0 2 0 2 12 50
Tarascon 2 990 0 0 0 0 3 14 90
Tarascon 2 870 0 0 0 0 2 15 50
Tarascon 2 650 0 0 0 0 0 12 0
Tarascon 2 730 0 0 0 0 1 14 50
Tarascon 3 1070 0 0 2 0 0 15 0
Tarascon 3 715 0 0 0 0 1 14 50
Tarascon 3 615 0 0 0 0 0 12 0
Tarascon 3 615 0 0 0 0 2 13 50
Mont Ventoux 1 1156 5 4 31 6 18 42 187
Mont Ventoux 1 646 3 2 11 7 13 42 170
Mont Ventoux 1 646 4 2 13 3 10 37 85
Mont Ventoux 10 1395 10 2 34 8 24 37 170
Mont Ventoux 10 950 4 7 20 8 17 42 190
Mont Ventoux 10 815 5 1 4 2 11 48 120
Mont Ventoux 10 665 4 4 9 1 7 37 85
Mont Ventoux 11 1310 9 6 47 8 26 42 170
Mont Ventoux 11 850 6 5 18 5 11 48 170
Mont Ventoux 11 700 6 4 13 4 10 37 135
Mont Ventoux 11 665 3 2 13 5 7 37 170
Mont Ventoux 12 1445 12 9 55 8 27 42 170
Mont Ventoux 12 1100 8 5 34 5 19 42 170
Mont Ventoux 12 750 6 5 16 4 13 34 140
Mont Ventoux 12 600 5 4 11 3 7 34 150
Mont Ventoux 13 1240 13 4 41 7 24 34 190
Mont Ventoux 13 1190 14 6 33 5 12 37 170
Mont Ventoux 13 730 5 4 16 5 7 42 140
Mont Ventoux 13 665 5 3 14 4 7 42 140
Mont Ventoux 15 1300 13 5 38 9 22 28 205
Mont Ventoux 15 1150 10 2 37 10 16 34 190
Mont Ventoux 15 650 4 2 9 2 10 42 105
Mont Ventoux 15 680 1 0 11 3 9 37 85
Mont Ventoux 16 1020 8 4 32 7 25 42 190
Mont Ventoux 16 850 8 5 15 6 11 48 170
Mont Ventoux 16 640 8 3 11 3 9 48 155
Mont Ventoux 16 650 6 2 12 4 9 48 170
Mont Ventoux 2 750 2 4 16 4 10 42 153
Mont Ventoux 2 697 2 3 11 5 10 42 153
Mont Ventoux 2 586 1 3 14 5 7 34 85
Mont Ventoux 2 1240 6 8 43 9 17 42 170
Mont Ventoux 2 1326 9 6 46 12 17 42 195
Mont Ventoux 2 1088 7 5 39 8 19 42 170
Mont Ventoux 2 697 2 4 17 3 10 34 93
Mont Ventoux 2 730 3 4 19 4 12 42 136
Mont Ventoux 3 1411 4 5 41 7 23 48 204
Mont Ventoux 3 1122 7 0 30 7 16 48 187
Mont Ventoux 3 850 4 2 17 6 8 42 102
Mont Ventoux 4 1326 8 9 44 9 21 56 187
Mont Ventoux 4 1258 8 7 34 8 28 24 187
Mont Ventoux 4 833 4 3 16 4 11 42 170

Appendix 3. Measurements of morphological characters for 10 queens of *Proformica* from southern France.

colony CW SL nCH nCU nPn nMes nPP nSc nG PDG GHL MW ML ScW
Sainte-Baume 1410 1130 0 2 14 14 8 5 10 8 85 1070 2000 660
Tarascon 1 1500 1260 0 6 6 5 8 2 1 14 85 1090 2175 620
Beaucaire 1 1530 1240 0 2 1 1 2 0 0 9 0 1020 2040 620
Beaucaire 1 1630 1275 0 4 0 0 2 0 2 11 70 1190 2245 665
Pompignan 10 1665 1235 1 2 1 10 6 2 3 11 70 1190 2245 800
Pompignan 10 1690 1240 0 2 1 11 5 2 2 11 85 1240 2310 865
Orange 1 1700 1275 3 2 11 26 10 2 25 15 240 1275 2340 765
Orange 1 1530 1225 1 3 15 30 6 3 24 17 210 1190 2245 700
Mont Ventoux 11 1770 1395 4 5 13 38 16 8 26 12 220 1410 2510 815
Mont Ventoux 2 1750 1325 13 7 20 53 15 13 36 14 240 1320 2450 850

Appendix 4. Relative proportions of cuticular hydrocarbons of *Proformica* workers. Hydrocarbons were identified using gas chromatography – mass spectrometry.

Peak	Species	*P. longipilosa* (Mont Ventoux + Sisteron)		*P. nasuta* (Montagne de Lure + Grand Luberon)		*P. nasuta*		*P. longiseta*	*C. cursor*	
no.	compounds	mean	SE	mean	SE	mean	SE	-	mean	SE
1	C23	0.82	0.37	0.23	0.14	0.00	0.00	2.29	1.34	0.21
2	11C23	0.29	0.12	0.06	0.05	0.00	0.00	0.00	0.00	0.00
3	7C23	0.35	0.12	0.00	0.04	0.00	0.00	0.00	0.00	0.00
4	5C23	0.69	0.12	0.71	0.17	0.00	0.00	0.00	0.00	0.00
5	3C23	0.28	0.10	0.00	0.03	0.00	0.00	0.00	0.03	0.00
6	5,11+5,13C23	0.43	0.11	0.41	0.15	0.00	0.00	0.00	0.00	0.00
7	C24:1	0.00	0.00	0.00	0.00	0.00	0.00	4.41	0.00	0.00
8	C24	0.30	0.16	0.60	0.11	0.04	0.03	3.14	0.24	0.00
9	3,7+3,10C23	0.08	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00
10	8+9+10+11+12C24	0.35	0.15	0.04	0.06	0.00	0.00	0.00	0.57	0.00
11	6C24	0.42	0.14	0.00	0.05	0.00	0.00	0.82	0.00	0.00
12	4C24	0.17	0.05	0.00	0.02	0.00	0.00	1.10	0.00	0.00
13	8,12C24	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.00
14	C25:1	0.21	0.10	0.00	0.03	0.02	0.02	0.00	0.00	0.00
15	6,8+6,10C24	0.16	0.06	0.02	0.07	0.03	0.03	0.00	0.00	0.00
16	C25	7.42	0.74	0.49	0.63	0.48	0.19	4.41	3.01	0.00
17	9+11+13C25	9.58	0.98	0.20	0.82	0.08	0.05	0.80	0.16	0.00
18	7C25	0.27	0.27	0.00	0.06	0.00	0.00	0.00	0.00	0.00
19	5C25	10.16	1.03	0.16	0.88	0.00	0.00	1.51	0.00	0.00
20	9,13+9,15C25	0.17	0.06	0.32	0.08	0.09	0.06	0.53	0.00	0.00
21	3C25	1.73	0.37	0.16	0.17	0.02	0.02	0.30	0.26	0.00
22	7,15+7,17C25	0.15	0.15	0.01	0.04	0.05	0.04	0.00	0.00	0.00
23	5,9+5,13+5,15C25	13.37	1.78	0.20	1.19	0.00	0.00	0.00	0.00	0.00
24	C26:1	0.00	0.00	0.00	0.01	0.02	0.02	1.01	0.00	0.00
25	C26	0.36	0.10	0.64	0.11	0.02	0.01	2.92	0.37	0.00
26	3,7 C25 + 3,8+3,10C25	0.88	0.59	0.09	0.15	0.00	0.00	0.00	0.00	0.00

Peak	Species	*P. longipilosa* (Mont Ventoux + Sisteron)		*P. nasuta* (Montagne de Lure + Grand Luberon)		*P. nasuta*		*P. longiseta*	*C. cursor*	
no.	**compounds**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**-**	**mean**	**SE**
27	8+9+10+11C26	2.91	0.54	0.22	0.27	0.05	0.04	0.24	0.00	0.00
27b	11,13,15+13,15,17TriMeC25	0.00	0.00	0.06	0.10	0.00	0.00	0.00	0.00	0.00
28	8,12 DiMe C26	0.00	0.00	0.00	0.01	0.00	0.00	0.63	0.00	0.00
29	6C26	0.79	0.14	0.04	0.08	0.05	0.03	0.55	0.00	0.00
30	5C26	0.04	0.04	0.00	0.02	0.02	0.02	0.00	0.00	0.00
31	4 Me C26	0.68	0.11	0.00	0.06	0.00	0.00	2.36	0.00	0.00
32	11,13,15+13,15,17C25	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00
33	C27:1	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00
34	6,8+6,10C26	0.75	0.25	0.13	0.11	0.14	0.08	0.00	0.00	0.00
35	4,8+4,10C26	1.79	0.47	1.30	0.27	0.03	0.03	0.00	0.00	0.00
36	C27	2.58	0.43	1.97	0.31	0.45	0.14	7.14	2.98	0.00
36b	4,8,12 TriMeC26	0.00	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00
37	9+11+13 Me C27	2.20	0.85	0.85	0.33	0.12	0.04	8.69	1.12	0.00
38	7C27	0.12	0.10	0.35	0.07	0.07	0.06	1.05	0.00	0.00
39	5 Me C27	4.83	0.79	3.01	0.49	0.03	0.02	1.70	0.72	0.00
40	9,13+11,13C27	0.00	0.00	0.00	0.13	0.07	0.03	0.36	0.00	0.00
41	3 Me C27	0.78	0.18	0.23	0.61	0.17	0.09	2.33	1.39	0.00
42	5,9+5,15+5,17C27	25.22	3.15	4.30	2.15	0.02	0.01	0.72	0.62	0.00
43	3,9C27	0.00	0.00	0.16	0.03	0.00	0.00	0.00	0.00	0.14
44	C28	0.03	0.03	0.69	0.13	0.05	0.02	0.92	0.97	0.08
45	5,9,13TriMeC27	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00
46	3,7+3,9C27	0.81	0.23	0.22	0.11	0.00	0.00	0.00	0.46	0.00
47	8+9+10+12+14 Me C28	0.00	0.00	0.11	0.25	0.00	0.00	2.07	0.97	0.00
48	13,15,17TriMeC27	0.00	0.00	0.05	0.10	0.00	0.00	0.00	0.00	0.00
49	8,12+8,14+10,12 +10,14DiMe C28	0.00	0.00	1.05	0.27	0.00	0.00	0.00	0.00	0.00
50	7 Me C28	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.00	0.00
51	6 Me C28	0.28	0.06	3.71	0.43	0.03	0.03	0.00	0.73	0.00
52	4 Me C28	0.29	0.07	1.99	0.27	0.00	0.00	0.00	0.33	0.00

Peak	Species	*P. longipilosa* (Mont Ventoux + Sisteron)		*P. nasuta* (Montagne de Lure + Grand Luberon)		*P. nasuta*		*P. longiseta*	*C. cursor*	
no.	compounds	mean	SE	mean	SE	mean	SE	-	mean	SE
53	9,13C28	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
54	10,12+10,14 DiMeC28	0.00	0.00	0.22	0.27	0.03	0.02	1.84	0.00	0.00
55	7,11,19 TriMe C27	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00
56	C29:1	0.00	0.00	0.00	0.07	0.02	0.02	0.00	0.00	0.00
57	6,10+6,12+6,14 DiMe C28	0.45	0.15	2.40	0.30	0.00	0.00	0.00	1.07	0.00
58	C29:1	0.00	0.00	0.00	0.15	0.25	0.10	0.00	0.00	0.00
59	4,8+4,10+4,12 DiMe C28	0.50	0.11	9.49	1.16	0.84	0.27	0.00	0.00	0.00
60	C29	0.31	0.10	5.16	0.60	1.41	0.34	2.57	6.60	0.00
61	4,8,11+4,8,14+4,10,14C28	0.02	0.02	6.05	1.29	0.05	0.05	0.00	0.00	0.00
62	9+11+13+15 Me C29	0.11	0.04	0.99	1.74	0.77	0.24	4.60	0.00	0.00
63	7 Me C29	0.48	0.45	0.26	0.25	0.85	0.23	6.37	10.72	0.39
64	5 Me C29	0.50	0.15	4.20	0.48	0.39	0.15	0.00	3.70	0.89
65	11,15+13,15 DiMe C29	0.00	0.00	0.02	0.79	0.17	0.06	3.49	0.00	0.00
66	9.13C29	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00
67	3 Me C29	0.01	0.01	0.39	0.67	1.07	0.46	0.00	0.00	0.00
68	7,11+7,15+7,17 DiMe C29	0.05	0.05	0.00	0.01	0.00	0.00	16.41	19.53	0.00
69	5,9+5,15+5,17 Di Me C29	1.41	0.30	6.57	0.75	0.04	0.02	1.98	2.86	0.58
70	C30	0.13	0.06	0.00	0.29	1.44	0.33	0.00	0.00	0.00
71	3,9+3,11C29	0.00	0.00	0.14	0.08	0.00	0.00	0.00	4.54	0.00
72	9,13,15C29	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00
73	7,11,13+7,11,15C29	0.00	0.00	0.00	0.42	0.02	0.02	0.00	0.00	0.00
74	5,11,13+3,7,11C29	0.01	0.01	0.00	0.13	0.00	0.00	0.00	0.00	0.00
75	8+9+10+11+12 Me C30	0.00	0.00	7.04	0.84	0.87	0.38	1.27	1.30	0.16
76	11,13,15TriMeC29	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00
77	3,11,13C29	0.01	0.01	0.02	0.18	0.01	0.01	0.00	0.00	0.00
78	8,12+8,14 DiMe C30	0.14	0.06	6.61	0.84	0.09	0.06	0.10	3.23	0.00
79	3+4 Me C30	0.00	0.00	0.63	0.25	0.29	0.10	0.00	0.00	0.00
80	9,11,17+9,13,15C29	0.10	0.05	1.14	0.23	0.00	0.00	1.74	0.00	0.00

Peak	Species	*P. longipilosa* (Mont Ventoux + Sisteron)		*P. nasuta* (Montagne de Lure + Grand Luberon)		*P. nasuta*		*P. longiseta*	*C. cursor*	
no.	**compounds**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**-**	**mean**	**SE**
81	6,10+6,12+6,14C30	0.00	0.00	0.82	0.21	0.09	0.04	0.00	0.00	0.00
82	8,12,16C30	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00
83	C31:1	0.01	0.01	0.00	0.05	0.03	0.02	0.00	0.92	0.00
84	4,8+4,10+4,12+4,14+4,16 DiMe C30	0.23	0.14	11.32	1.36	0.35	0.15	0.00	0.48	0.05
85	C31	0.23	0.07	0.12	0.32	1.58	0.39	0.54	1.52	0.03
86	8,12,13C30	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
87	6,14,16+6,10,12+6,12,16C30	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00
88	4,10,12+4,12,16+4,14,16 TriMe C30	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00
89	9+11+13+15+16 Me C31	0.00	0.00	1.14	1.31	5.68	1.18	0.75	2.24	0.00
90	7C31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.84	0.00
91	13,15+13,17Me C31	0.00	0.00	0.00	0.65	0.04	0.04	0.00	0.00	0.00
92	11,13+11,15+11,17C31	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00
93	9,11+9,13+9,15 DiMe C31	0.00	0.00	0.00	0.59	0.23	0.13	3.71	0.00	0.00
94	5C31	1.74	0.90	0.01	0.70	4.98	0.56	0.00	0.00	0.00
95	7,11+7,15C31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.26	0.00
96	5,9+5,13+5,15+5,17 DiMe C31	0.50	0.23	7.53	1.52	9.64	1.63	0.00	0.00	0.00
96b	11.15.19+13.15.19C31	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00
97	9,11,13+9,13,15+9,13,17C31	0.00	0.00	0.00	0.85	2.29	1.26	0.00	0.00	0.00
98	7,11,17+7,13,17C31	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
99	C32	0.00	0.00	0.44	0.58	3.11	0.67	0.00	1.24	0.00
100	3,11C31	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00
101	5,10,14+5,10,12+7,11,15TriMeC31	0.17	0.07	0.30	0.54	1.95	0.74	0.00	0.00	0.00
102	8 +10 +11+12+13+14Me C32	0.01	0.01	0.65	0.43	2.81	0.45	0.00	1.14	0.00
103	11,13,15C31	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00
104	8,14 + 8,16 +10,12+10,14DiMe C32	0.09	0.09	0.62	0.36	1.39	0.45	0.07	0.00	0.00
105	9,13,15C31	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
106	6,10+6,14DiMe C32	0.00	0.00	0.32	0.13	0.81	0.12	0.00	1.02	0.00
107	4,12+4,14+4,16C32	0.00	0.00	0.08	0.05	0.00	0.00	0.00	0.00	0.00

Peak	Species	*P. longipilosa* (Mont Ventoux + Sisteron)		*P. nasuta* (Montagne de Lure + Grand Luberon)		*P. nasuta*		*P. longiseta*	*C. cursor*	
no.	compounds	mean	SE	mean	SE	mean	SE	-	mean	SE
108	C33:1	0.00	0.00	0.00	0.10	0.21	0.14	0.00	0.50	0.00
109	C33	0.00	0.00	0.24	0.11	0.67	0.11	0.00	0.03	0.00
110	4,12,xC32	0.00	0.00	0.00	0.13	0.50	0.18	0.00	0.00	0.00
111	11+13+15+17 Me C33	0.02	0.02	0.04	0.47	3.03	0.45	0.00	0.55	0.02
112	13,15 C33	0.03	0.03	0.05	1.07	5.35	1.26	0.15	0.00	0.00
113	11,13+11,15C33	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00
114	5C33	0.00	0.00	0.00	0.42	1.00	0.58	0.00	0.00	0.00
115	7,11+7,15C33	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.99	0.00
116	5,11+5,13+5,15C33	0.02	0.02	0.03	1.12	8.47	0.68	0.00	0.14	0.08
117	7,11,17C33	0.00	0.00	0.00	0.07	0.27	0.09	0.00	0.00	0.00
118	C34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
119	5,11,15+5,13,15C33	0.00	0.00	0.01	1.29	9.74	0.76	0.00	0.00	0.00
120	10+12C34	0.00	0.00	0.00	1.86	9.89	2.10	0.00	0.14	0.04
121	10,14+10,16+12,14C34	0.00	0.00	0.01	0.23	1.65	0.18	0.00	0.00	0.00
122	8,12,16+8,12,18+8,14,16+8,14,16C34	0.00	0.00	0.00	0.17	0.94	0.19	0.00	0.00	0.00
123	6,12+6,14+6,16C34	0.00	0.00	0.01	0.09	0.41	0.11	0.00	0.00	0.00
124	C35	0.00	0.00	0.00	0.30	1.48	0.34	0.00	0.00	0.00
125	11+13 C35	0.02	0.02	0.00	0.64	2.55	0.78	0.00	0.00	0.00
126	11,15+13,15+13,17C35	0.00	0.00	0.00	0.30	1.35	0.37	0.00	0.00	0.00
127	5,13+5,14+5,15+5,16C35	0.00	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00
128	C36	0.01	0.01	0.00	0.52	2.59	0.59	0.00	0.00	0.00
129	5,13,15C35	0.00	0.00	0.00	0.26	1.56	0.24	0.00	0.00	0.00
130	10+12+13+14+15+16+17+18C36	0.00	0.00	0.00	0.15	0.41	0.17	0.00	0.00	0.00
131	12,16C36	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
132	C37	0.00	0.00	0.00	0.16	0.85	0.18	0.00	0.00	0.00
133	MeC37:1	0.00	0.00	0.00	0.10	0.34	0.12	0.00	0.00	0.00
134	11+13+17 Me C37	0.00	0.00	0.00	0.07	0.10	0.09	0.00	0.00	0.00
135	DiMeC37	0.00	0.00	0.00	0.20	0.79	0.27	0.00	0.00	0.00
-	n =	8		16		22		1	5	

Appendix 5. Examples of chromatograms from cuticular extracts of *Proformica* workers analysed with gas chromatography.