

Digital supplementary material to

SEIFERT, B. 2016: Analyzing large-scale and intranidal phenotype distributions in eu-social Hymenoptera – a taxonomic tool to distinguish intraspecific dimorphism from heterospecificity. – *Myrmecological News* 23: 41-59.

Case 1: *Camponotus lateralis*

The following characters and conditions were considered:

All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y, and Z axes. A Leica high-performance stereomicroscope M165C equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) was used at magnifications of 120 - 384×. A Schott KL 1500 LCD cold-light source equipped with two flexible, focally mounted light-cables, providing 30° inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimum resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating the voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. The mean relative measuring error over all magnifications and systems was 0.4%. To avoid rounding errors, all measurements were recorded in µm even for characters for which this precision is impossible. Setae counts are restricted to setae with distinctly larger diameters compared to long standing pubescence hairs. The counts included pits of seta bases in which remains of broken-off setae could be detected at highest resolution.

- CL maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of posterior vertex reduce CL.
- CS cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- CW maximum cephalic width – either across, anterior or posterior of eyes, whichever yields the maximum measurement.
- SL maximum straight line scape length excluding the articular condyle.
- SCI Index of scape diameter extension near scape base. SCI is the maximum scape diameter near scape base divided by minimum scape diameter distal of the point with the maximum diameter. The adjustment of scape remains the same in both measurements. SCI gets the value 1.0 if no extension is visible.
- MW maximum width of mesosoma.
- MGR depth of metanotal groove relative to the tangent going through the dorsalmost points of promesonotum and propodeum.
- PRL *in worker*: maximum length of upper propodeal surface from the posterior margin of dorsal plane to the centre of the metanotal suture (that may be wide in major workers). *In gynes*: Distance from hind margin of subtegular shield to hind profile of propodeum measured in lateral view at the horizontal level of the centre of "subtegular shield" and parallel to the straight profile line of dorsal mesosoma.
- PRW maximum width of dorsal plane of propodeum.
- PEW maximum width of petiole.
- nSC unilateral number of standing setae on dorsal plane of scape as mean value of both scapes.
- nMn number of standing setae on mesonotum.
- nPR number of standing setae on propodeum dorsal of the spiracle.
- RipD mean distance of fine transversal ripples on dorsal surface of 1st gaster tergite; at least 50 ripple distances are averaged.

Removal of allometric variance was performed in workers by overall functions computed as average of species specific functions of seven *Camponotus* species with sufficient data available: *C. dalmaticus* (NYLANDER, 1849), *C. atricolor* (NYLANDER, 1849), *C. piceus* (LEACH, 1825), *C. sp. "longiscapus"* (undescribed), *C. candiotes* EMERY, 1895, *C. lateralis* (OLIVIER, 1792), *C. rebecca* FOREL, 1913. This overall correction was preferred to enable a direct comparison of data in tables and because pair-specific corrections did not provide significant improvement. The overall allometric corrections for workers transform data for the assumption of each individual having a head size CS of 1.25 mm. Due to an expressed minor vs. major dimorphism in this group of ants, removal of allometric variance was performed in a number of characters by a diphasic function.

For workers with $CS \leq 1.25$ mm:

$$CL/CW_{cor} = CL/CW / (-0.0998 * CS + 1.2064) * 1.0817$$

$$SL/CS_{cor} = SL/CS / (-0.4314 * CS + 1.4566) * 0.9394$$

$$\begin{aligned} MW/CS_{cor} &= MW/CS / (-0.1046 * CS + 0.8775) * 0.7468 \\ SCI_{cor} &= SCI / (-0.2035 * CS + 1.4001) * 1.1458 \\ PRW/CS_{cor} &= PRW/CS / (-0.0100 * CS + 0.2991) * 0.2867 \\ PRL/CS_{cor} &= PRL/CS / (-0.1141 * CS + 0.5405) * 0.3979 \\ PEW/CS_{cor} &= PEW/CS / (-0.0207 * CS + 0.4097) * 0.3838 \\ MGR/CS_{cor} &= MGR/CS / (-0.0258 * CS + 0.0966) * 0.0644 \end{aligned}$$

For workers with $CS > 1.25$ mm:

$$\begin{aligned} CL/CW_{cor} &= CL/CW / (-0.2506 * CS + 1.3895) * 1.0817 \\ SL/CS_{cor} &= SL/CS / (-0.3983 * CS + 1.4374) * 0.9394 \\ MW/CS_{cor} &= MW/CS / (-0.1672 * CS + 0.9558) * 0.7468 \\ SCI_{cor} &= SCI / (-0.2091 * CS + 1.4073) * 1.1458 \\ PRW/CS_{cor} &= PRW/CS / (-0.0302 * CS + 0.3244) * 0.2867 \\ PRL/CS_{cor} &= PRL/CS / (-0.1324 * CS + 0.5634) * 0.3979 \\ PEW/CS_{cor} &= PEW/CS / (-0.1021 * CS + 0.5114) * 0.3838 \\ MGR/CS_{cor} &= MGR/CS / (-0.0039 * CS + 0.0693) * 0.0644 \end{aligned}$$

In the following characters a monophasic removal of allometric variance was a good approximation over the full size range:

$$\begin{aligned} RipD_{cor} &= RipD / (+1.68 * CS + 6.935) * 9.04 \\ nSC_{cor} &= nSC / (+8.84 * CS - 2.402) * 8.65 \\ nMn_{cor} &= nMn / (+8.30 * CS - 4.218) * 6.16 \\ nPr_{cor} &= nPr / (+10.71 * CS - 4.41) * 8.98 \\ nPn_{cor} &= nPn / (+6.96 * CS - 4.82) * 3.88 \end{aligned}$$

These characters were finally used in exploratory and hypothesis-driven data analyses.

Case 2: *Lasius umbratus*

The following conditions and characters were used:

All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y, and Z axes. Two high-performance stereomicroscopes, a Leica Wild M10 equipped with a 1.6× planapochromatic objective (resolution 650 lines/mm) and Leica M165C equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) were used at magnifications of 120 - 384×. A Schott KL 1500 LCD cold-light source equipped with two flexible, focally mounted light-cables, providing 30° inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimum resolution of tiny structures and micro-sculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating the voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. The mean relative measuring error over all magnifications and systems was 0.4%. To avoid rounding errors, all measurements were recorded in μm even for characters for which this precision is impossible.

Any metric measurement refers to real cuticular surface and not to the diffuse pubescence surface (most important in CW, IF2, Smax, Smin, HTmax). All setae counts include setae projecting $> 10 \mu\text{m}$ from cuticular profile with no change of viewing position. In cases of ambiguous projecting distance or unclear hair type (is it a fine seta or only a larger pubescence hair?) countings of 0.5 are applied.

- CL maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of posterior vertex reduce CL.
- CS cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- CW maximum cephalic width either across, anterior or posterior of eyes, whichever yields the maximum measurement.
- GHL length of longest seta on dorsal plane of first gaster tergite excluding setae near the posterior margin. The anterior area of dorsal plane where it slopes down to anterior face is included, but do never include areas sloping $> 50^\circ$ (here setae are significantly longer!).
- HTmax maximum width of hind tibia at midpoint. The reference points to determine the midpoint are the distal end of tibia and the point of strongest constriction of the tibia's flexor side at the tibio-femoral joint (arrow in Fig. 5). In some species with dense and long pubescence (as exemplaric in *L. mixtus*) visualization of cuticular surface is difficult and measuring errors are expected. However, control measurements after removal of pubescence have shown subjective and real HTmax not to differ considerably. Users should test their subjective errors at the beginning of a measuring series.
- nHT number of setae projecting more than $10 \mu\text{m}$ above the extensor profile of hind tibia. Apical setae at distal end are excluded.

- PEAS bilateral height asymmetry of petiolar crest. Height difference of the corners of petiolar crest. If only one side shows a corner, height measurement on the other side is performed at a sagittal level which distance from the median is equal to that of the sagittal level of the corner from the median (Fig. 4).
- PEH petiolar height measured from centre of spiracle to dorsalmost point of petiolar crest. In case of asymmetric crests, the average of both sides is taken (Fig. 4).
- PEW maximum width of petiole.
- PECR width of petiolar crest. In case of an average *Chthonolasius* scale shape (Fig.1), PECR is measured from corner centre to corner centre. If the crest is straight, PECR is measured between the lateralmost points of the straight section (Fig. 2). If only one side shows a corner in asymmetric scales, PECR is twice the distance of the corner centre from the median (Fig. 3). PECR is 0 in case of symmetric crests without any corners (rounded or tipped crests).
- SL maximum straight line scape length excluding the articular condyle.
- Smax maximum scape diameter at midpoint.
- Smin minimum scape diameter at midpoint.
- sqPDF square root of transverse pubescence distance on vertex frontal of mid ocellus. To avoid damaged and oily surface areas, countings are done as average of three or four shorter sections of 90 μm . A section crossing the frontal line is always included. Hairs crossing / just touching the 90 μm reference lines are counted as 1 / 0.5. A rather flat incidence of light (30°) is advantageous. To visualize the full length of pubescence hairs, short to-and-fro rotations of the light-cables around the optical axis are permanently performed during counting. Use objectives with numeric apertures > 0.2.
- sqPDG square root of transverse pubescence distance on dorsum of first gaster tergite. Measuring is basically done as in PDF. In species with high pubescence distance and undamaged, clean surfaces, larger sections of up to 400 μm should be combined but do not exceed this length to minimize errors caused by surface convexity. In gynae, the square root of PDG (sqPDG) is applied to normalize positively skewed distributions.

Removal of allometric variance was performed in workers by overall functions computed as average of species specific functions of 12 *Chthonolasius* entities with sufficient data available: *Lasius balcanicus* SEIFERT, 1988, *L. bicornis* (FÖRSTER, 1850), *L. citrinus* EMERY, 1922, *L. distinguendus* (EMERY, 1916), *L. jensi* SEIFERT, 1982, *L. meridionalis* (BONDROIT, 1920), *L. mixtus* (NYLANDER, 1846), *L. nitidigaster* SEIFERT, 1996, *L. sabularum* (BONDROIT, 1918), *L. umbratus* (NYLANDER, 1846), *L. jensi* \times *umbratus*, *L. meridionalis* \times *umbratus*. This overall correction was preferred to enable a direct comparison of data in tables and because pair-specific corrections did not provide significant improvement. The overall allometric corrections for workers transform data for the assumption of each individual having a head size CS of 1.05 mm:

$$\begin{aligned} \text{CL}/\text{CW}_{\text{cor}} &= \text{CL}/\text{CW} / (-0.13684 * \text{CS} + 1.1938) * 1.0501 \\ \text{SL}/\text{CS}_{\text{cor}} &= \text{SL}/\text{CS} / (-0.03646 * \text{CS} + 0.9235) * 0.8852 \\ \text{GHL}/\text{CS}_{\text{cor}} &= \text{GHL}/\text{CS} / (-0.06093 * \text{CS} + 0.1578) * 0.09380 \\ \text{Smax}/\text{CS}_{\text{cor}} &= \text{Smax} / \text{CS} / (+0.00236 * \text{CS} + 0.09299) * 0.09546 \\ \text{Smax}/\text{Smin}_{\text{cor}} &= \text{Smax}/\text{Smin} / (+0.4138 * \text{CS} + 1.0482) * 1.4827 \\ \text{HTmax}/\text{CS}_{\text{cor}} &= \text{HTmax} / \text{CS} / (-0.00344 * \text{CS} + 0.1418) * 0.13814 \\ \text{sqPDF}_{\text{cor}} &= \text{sqPDF} / (-0.079 * \text{CS} + 2.88) * 2.79 \\ \text{sqPDG}_{\text{cor}} &= \text{sqPDG} / (-1.172 * \text{CS} + 4.254) * 3.024 \\ \text{nHT}_{\text{cor}} &= \text{nHT} / (+25.42 * \text{CS} - 13.71) * 12.99 \\ \text{PECR}/\text{CS}_{\text{cor}} &= \text{PECR} / \text{CS} / (+0.0632 * \text{CS} + 0.0170) * 0.0833 \\ \text{PEAS}/\text{CS}_{\text{cor}} &= \text{PEAS} / \text{CS} / (+0.436 * \text{CS} - 0.029) * 0.428 \\ \text{PEW}/\text{CS}_{\text{cor}} &= \text{PEW} / \text{CS} / (+0.0072 * \text{CS} + 0.2741) * 0.2817 \\ \text{PEH}/\text{CS}_{\text{cor}} &= \text{PEH} / \text{CS} / (+0.0946 * \text{CS} + 0.1749) * 0.2514 \end{aligned}$$

These characters were finally used in exploratory and hypothesis-driven data analyses.

Case 3: *Formica lugubris*

The following conditions and characters were considered:

All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y, and Z axes. Two high-performance stereomicroscopes, a Leica Wild M10 equipped with a 1.6 \times planapochromatic objective (resolution 650 lines/mm) and Leica M165C equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) were used at magnifications of 120 - 384 \times . A Schott KL 1500 LCD cold-light source equipped with two flexible, focally mounted light-cables, providing 30° inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimum resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating the voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. The mean relative measuring error over all magnifications and systems was 0.4%. To avoid rounding errors, all measurements were recorded in μm even for characters for which this precision is impossible.

Setae, also called pilosity or simply "hairs", are differentiated from pubescence hairs in having at least twice the basal diameter of neighboring pubescence hairs. All seta counts (acronyms beginning with "n") are restricted to standing setae projecting > 10 µm from cuticular surface. Setae counts and scape measurements are, if not otherwise stated, averages of both body halves with exception of strongly and symmetrically haired specimens. The description of the evaluated numeric characters is given in the following.

- CL maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of occiput and/or clypeus reduce CL.
- CS cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- CW maximum cephalic width.
- MetHL length of the longest seta on a metapleuro-propodeal area below a straight reference line that is directed parallel to the straight section of lower metapleural margin and touches the lower margin of propodeal spiracle. The same-level area of caudal propodeal slope is included, weir hairs protecting the orifice of metapleural gland and proprioceptive setae immediately at the caudal margin near petiole junction are excluded. Care is taken to visualize the lateral suture between meso- and metapleuron.
- mPnHL mean pronotal hair length. Applied measuring schedule: select one of the longest hairs on dorsal pronotum and calculate the arithmetic mean length of this hair and of its seven nearest neighbors. Proprioceptive setae on anterior pronotal shield are excluded.
- nMet bilateral mean of the number of standing setae on a metapleuro-propodeal area below a straight reference line that is directed parallel to the straight section of lower metapleural margin and that touches the lower margin of propodeal spiracle. Include also the infraspicular area of caudal propodeal slope and take care to visualize the antero-lateral suture between meso- and metapleuron. Setae positioned directly on the suture are counted as 0.5. Definitely excluded from count are weir hairs protecting the orifice of the metapleural gland, proprioceptive setae immediately at the caudal margin near petiole junction and hairs standing on the ventrolateral edge of metapleuron (hatched area in Fig. A282 in SEIFERT 2007: p. 175).
- nOcc bilateral mean of the number of standing hairs protruding more than 10 µm from head silhouette as seen in full face view. Counting begins at the level of anterior eye margin and ends at median occiput. The full depth of focus is used for counting and the parallax error is considered.
- nSC bilateral mean of the number of setae on the dorsal plane of scape.
- OccHL apparent protrusion length of the longest standing seta over the postocular head contour in full face view. This value does not indicate the real setae length because setae bases are often concealed and setae axes are inclined compared to visual plane. This measuring mode was selected to save time.
- PEW maximum width of petiole scale measured in dorsal, frontodorsal or caudodorsal view.
- SL maximum scape length excluding articular condyle and its neck.
- SMAX maximum scape width at midpoint; data of both scapes are averaged.

Removal of allometric variance: In most species groups of *Formica*, morphological characters are strongly influenced by allometric growth. In order to make characters directly comparable in synoptic tables (side-by-side comparisons), a removal of allometric variance (RAV) was performed with the procedure described by SEIFERT (2008). As standard for all members of the *Formica rufa* group, RAV was calculated assuming that all individuals have an identical cephalic size of 1.75 mm. RAV functions used collective parameters calculated as the arithmetic mean of the species-specific functions of twelve Palearctic *Formica rufa* group species. Inspection of scatter plots indicated the use of monophasic linear RAV functions. The RAV functions were

$$\begin{aligned} CL/CW_{1.75} &= CL / CW / (-0.1085 * CS + 1.2922) * 1.1023 \\ SL/CS_{1.75} &= SL / CS / (-0.0762 * CS + 1.0556) * 0.9222 \\ SL/SMAX_{1.75} &= SL / SMAX / (-0.1561 * CS + 9.995) * 9.722 \\ PEW/CS_{1.75} &= PEW / CS / (0.0227 * CS + 0.4395) * 0.4792 \\ nOCC_{1.75} &= nOCC / (7.20 * CS + 2.28) * 14.87 \\ nSC_{1.75} &= nSC / (0.59 * CS + 1.88) * 2.90 \\ OCCHL_{1.75} &= OCCHL / (41.23 * CS + 17.0) * 89.1 \\ mPNHL_{1.75} &= mPNHL / (27.07 * CS + 34.12) * 81.5 \\ nMET_{1.75} &= nMET / (5.19 * CS - 2.16) * 6.93 \\ METHL_{1.75} &= METHL / (44.01 * CS + 65.69) * 142.7 \end{aligned}$$

These characters were finally used in exploratory and hypothesis-driven data analyses.

Case 4: intraspecific dimorphism in *Cardiocondyla elegans*

In bilaterally recorded characters, arithmetic means of both body sides were calculated. All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y, and Z axes. A Leica M165C high-performance stereomicroscope equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) was used at magnifications of 120 - 384×. The mean relative measuring error over all magnifications was 0.3%. A Schott KL 1500 cold-light source equipped with two flexible, focally mounted light-cables, providing 30°-inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of sil-

houette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimal resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks ranging over 52% of the visual field was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. Measurements of body parts always refer to real cuticular surface and not to the diffuse pubescence surface.

Definition of recorded characters:

- CL maximum cephalic length in median line; the head must be carefully tilted to the position yielding the true maximum; excavations of hind vertex and/or clypeus reduce CL.
- CW maximum cephalic width; the maximum in *Cardiocondyla* is found usually across and including the eyes, exceptionally posterior of the eyes.
- CS cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- dFOV mean inner diameter of foveolae or mesh-like surface structures on vertex at about half way between the median line of head and the inner eye margin. These structures are either real foveolae or meshes of a reticulum and usually have the base of a decumbent pubescence hair in their centre. In species whose foveolae or mesh-like structures are reduced (e.g., in the *C. stambuloffii* group) the mean diameter of the small punctures or tubercles at hair bases is measured as dFOV. At least six measurements are averaged. Use magnifications $\geq 250\times$ and light diffusers to suppress irritating reflections.
- EYE eye-size: the arithmetic mean of the large (EL) and small diameter (EW).
- MpGr Depth of metanotal groove or depression, measured from the tangent connecting the dorsalmost points of promesonotum and propodeum.
- PEH maximum petiole height. The straight section of ventral petiolar profile at node level is the reference line perpendicular to which the maximum height of petiole node is measured at node level.
- PEW maximum width of petiole.
- PLG mean length of pubescence hairs on dorsum of first gaster tergite as arithmetic mean of 6 measurements at least. Use magnifications > 250 and light diffusers to suppress irritating reflections.
- PPH maximum postpetiole height; the lateral suture of dorsal and ventral sclerites is the reference line perpendicular to which the maximum height of postpetiole is measured.
- PPW maximum width of postpetiole.
- PoOc postocular distance. Use a cross-scaled ocular micrometer and adjust the head to the measuring position of CL. Caudal measuring point: median occipital margin; frontal measuring point: median head at level of posterior eye margin. Note that many heads are asymmetric; therefore average the left and right postocular distance.
- SL maximum straight line length of scape excluding the articular condyle given as the arithmetic mean of both scapes.
- SP maximum length of propodeal spines; measured in dorsofrontal view along the long axis of the spine, from spine tip to a line, orthogonal to the long axis, that touches the bottom of the interspinal meniscus. Left and right SP are averaged. This mode of measuring is less ambiguous than other methods but yields higher spine length values in species with reduced spines.
- sqPDG square root of pubescence distance on dorsum of first gaster tergite. The number of pubescence hairs n crossing a transverse measuring line of length L is counted; hairs just touching the line are counted as 0.5. The pubescence distance PDG is then given by L/n . In order to normalise the positively skewed distributions, the square root of PDG is calculated. Exact counts are promoted by clean surfaces and flat, reflection-reduced illumination directed slightly skew to the axis of the pubescence hairs. Counting is performed at a magnification of $320\times$. Tergite pubescence is easily torn-off in *Cardiocondyla*. An effort should be made to evaluate undamaged surface spots. In specimens with mostly removed pubescence, PDG can be calculated from the mean distance of hair base pits (BD) and PLG using the formula $PDG = BD^2 / PLG$.

Case 5 : *Formica foreli* and *F. pressilabris*

The following conditions and characters were considered:

All measurements were made on mounted and dried specimens using a pin-holding stage, permitting full rotations around X, Y, and Z axes. Two high-performance stereomicroscopes, a Leica Wild M10 equipped with a $1.6\times$ planapochromatic objective (resolution 650 lines/mm) and Leica M165C equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) were used at magnifications of 120 - $384\times$. A Schott KL 1500 LCD cold-light source equipped with two flexible, focally mounted light-cables, providing 30° inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimum resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating the voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field. The mean relative measuring error over all magnifications and systems was 0.4%. To avoid rounding errors, all measurements were recorded in μm even for characters for which this precision is impossible.

Setae, also called pilosity or simply "hairs", are differentiated from pubescence hairs in having at least twice the basal diameter of neighboring pubescence hairs. All seta counts (acronyms beginning with "n") are restricted to standing setae projecting > 10 µm from cuticular surface. Setae counts and scape measurements are, if not otherwise stated, averages of both body halves with exception of strongly and symmetrically haired specimens. The description of the evaluated numeric characters is given in the following.

- CL maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of occiput and/or clypeus reduce CL.
- CS cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- CW maximum cephalic width.
- SL maximum scape length excluding articular condyle and its neck.
- sqrtPDF square root of pubescence distance in the ocellar triangle. The number of pubescence hairs n crossing a transverse measuring line of length L is counted, hairs just touching the line are counted as 0.5. Pubescence distance PDF is then L/n . Four measuring lines equal in length to the distance between the inner margins of lateral ocellae are positioned at different transversal levels: the first line is between hind ocellae, the second line a short distance frontal to the hind ocellae, the third line between the hind and the frontal ocellus, and the fourth a short distance caudal of the frontal ocellus. The four counts are averaged. Exact counting is only possible with clean surfaces, high-resolution stereomicroscopy at magnifications $\geq 280\times$ and reflexion-reduced illumination visualising the full length of hairs. Surface spots with torn-off pubescence were excluded from measuring. In order to normalise the positively skewed distributions, the square root of PDF is calculated.
- sqrtPDG square root of pubescence distance on the dorsomedian part of first gaster tergite. Principles of counting as in sqrtPDF. In case of surface damage or deformation, the second tergite may be used. To reduce accidental errors, 6 countings along 6 differently positioned, transverse measuring lines of 400 µm length are averaged. In order to normalise the positively skewed distributions, the square root of PDG is calculated.
- TERG the ordinal number of the frontalmost gaster tergite with at least one standing seta. In species with reduced pilosity, the posterior tergite margins must be scrutinised carefully in search of a hair fitting the definition of seta. Intermediate scores may be applied when the classification of a particular hair is undecided. For instance "3.5" means that there is a hair of doubtful classification on third tergite but clear seta on the fourth.

As there is no significant allometry detectable in this group of ants, the following characters were run in exploratory and hypothesis-driven data analyses without removal of allometric variance: CS, CL/CW, SL/CS, sqrtPDF, sqrtPDG, TERG.

Case 8 : two cryptic species of the *Pheidole pallidula* complex

A pin-holding stage, permitting full rotations around X, Y, and Z axes and a Leica M165C high-performance stereomicroscope equipped with a 2.0 planapochromatic objective (resolution 1050 lines/mm) were used for spatial adjustment of specimens at magnifications of 120 - 384 \times . The mean relative measuring error over all magnifications was 0.3%. A Schott KL 1500 cold-light source equipped with two flexible, focally mounted light-cables, providing 30°-inclined light from variable directions, allowed sufficient illumination over the full magnification range and a clear visualization of silhouette lines. A Schott KL 2500 LCD cold-light source in combination with a Leica coaxial polarized-light illuminator provided optimal resolution of tiny structures and microsculpture at highest magnifications. Simultaneous or alternative use of the cold-light sources depending upon the required illumination regime was quickly provided by regulating voltage up and down. A Leica cross-scaled ocular micrometer with 120 graduation marks ranging over 52% of the visual field was used. To avoid the parallax error, its measuring line was constantly kept vertical within the visual field.

The morphometric characters: 16 morphometric characters were investigated in major ("soldier") workers. In bilaterally developed characters, arithmetic means of both body sides were calculated. All measurements were made on mounted and fully dried specimens. Measurements of body parts always refer to real cuticular surface and not to the diffuse pubescence surface.

APrMn angle between integrated dorsal profile of propodeum and the straight posterior profile of mesonotum.

- CL maximum cephalic length in median line; the head must be carefully tilted to the position with the true maximum. Excavations of hind vertex and/or clypeus reduce CL. Surface irregularities due to sculpture, carinae in particular, are considered by averaging between peaks and valleys of sculpture.
- CS cephalic size; the arithmetic mean of CL and CW, used as a less variable indicator of body size.
- CW maximum cephalic width.
- dAN minimum distance between margins of antennal sockets measured in almost frontal view.
- EL maximum large diameter of the elliptic eye. All structurally defined ommatidia, pigmented or not, are included.
- ExOcc with maximum cephalic length in measuring plane, depth of excavation of posterior border of head. Surface irregularities due to sculpture are considered by averaging between peaks and valleys of sculpture.
- Fe3L Length of hindfemur on extensor side; measured from the distalmost point of femur to borderpoint between femur and trochantellus, equivalent measurements possible with view on plane or edge of femur and intermediate viewing positions.
- GuHL length of longest seta on underside of head (gula) posterior of the level of anterior eye margin.

Mesosomal longitudinal axis is defined in lateral view from centre of metapleural lobe to lowest point of anterior pronotal shield.

ML mesosoma length from caudalmost point of propodeal lobe to transition point between anterior pronotal slope and anterior propodeal shield (preferentially measured in lateral view; if the transition point is not well defined, use dorsal view and take the centre of the dark-shaded borderline between pronotal slope and pronotal shield as anterior reference point).

MW maximum pronotal width in dorsal view.

PEH petiole height; maximum height of section line measured perpendicular to the linearization of ventral profile line.

PEW maximum width of petiole.

PnHaa pronotal height above all, measured perpendicular to longitudinal mesosomal axis from lowest point of pronotal sclerite to highest point of promesonotum.

PoOc postocular distance; using the cross-scaled ocular micrometer, the head is adjusted to the measuring position of CL; caudal measuring point: median occipital margin as average between peaks and valleys of microsculpture; frontal measuring point: median head at the level of the posterior eye margin; average left and right postocular distance.

PPW maximum width of postpetiole.

SL maximum straight line scape length excluding the articular condyle as arithmetic mean of both scapes.

PrOc preocular distance in lateral view; measured as the shortest distance between the anterior eye margin and the sharp frontal margin of the gena.

Removal of allometric variance: In major workers of *Pheidole*, morphological characters are strongly influenced by allometric growth. In order to reveal in comparative tables which shape variables do really differ between the species independent from body size, a removal of allometric variance (RAV) was performed with the procedure described by SEIFERT (2008). As standard for all members of the *Pheidole* group, RAV was calculated assuming that all individuals have an identical cephalic size of 1.25 mm. RAV functions used collective parameters calculated as the arithmetic mean of the species-specific functions of the three species of which more than 80 individuals were measured. Inspection of scatter plots indicated the use of monophasic linear RAV functions. The RAV functions of 15 shape and one seta characters were

$$CL/CW_{1.25} = CL/CW / (-0.0475 * CS + 0.9985) * 0.9391$$

$$SL/CS_{1.25} = SL/CS / (-0.3007 * CS + 0.9509) * 0.5750$$

$$Fe3L/CS_{1.25} = Fe3L/CS / (-0.2549 * CS + 1.0601) * 0.7415$$

$$PoOc/CS_{1.25} = PoOc/CS / (+0.1244 * CS + 0.3821) * 0.5376$$

$$PrOc/CS_{1.25} = PrOc/CS / (-0.0075 * CS + 0.2334) * 0.2241$$

$$ExOc/CS_{1.25} = ExOc/CS / (+0.0585 * CS + 0.0160) * 0.0892$$

$$dAN/CS_{1.25} = dAN/CS / (-0.0462 * CS + 0.3509) * 0.2931$$

$$EL/CS_{1.25} = EL/CS / (-0.0562 * CS + 0.2113) * 0.1411$$

$$MW/CS_{1.25} = MW/CS / (-0.0318 * CS + 0.4965) * 0.4568$$

$$ML/CS_{1.25} = ML/CS / (-0.2013 * CS + 1.0980) * 0.8463$$

$$PnHaa/CS_{1.25} = PnHaa/CS / (-0.0496 * CS + 0.4023) * 0.3403$$

$$APrMn/CS_{1.25} = APrMn/CS / (-6.77 * CS + 128.5) * 120.0$$

$$PEW/CS_{1.25} = PEW/CS / (-0.0145 * CS + 0.1562) * 0.1381$$

$$PPW/CS_{1.25} = PPW/CS / (+0.0064 * CS + 0.2599) * 0.2679$$

$$PEH/CS_{1.25} = PEH/CS / (-0.0279 * CS + 0.2233) * 0.1884$$

$$GuHL/CS_{1.25} = GuHL/CS / (-0.0899 * CS + 0.3022) * 0.1898$$

These RAV-corrected variables were used in the exploratory and hypothesis-driven data analyses.