

## Digital supplementary material to

SEPPÄ, P., HELANTERÄ, H., TRONTTI, K., PUNTTILA, P., CHERNENKO, A., MARTIN, S.J. & SUNDSTRÖM, L. 2011: The many ways to delimit species: hairs, genes and surface chemistry. – Myrmecological News 15: 31-41.

**Appendix 1:** Number of hairs on different body parts of *Formica fusca* and *F. lemni* according to different authors.

| Promesonotum & pronotum |                    |                     |  |                             |                              |               |                                    |                |                    |
|-------------------------|--------------------|---------------------|--|-----------------------------|------------------------------|---------------|------------------------------------|----------------|--------------------|
|                         | YARROW (1954)      | COLLINGWOOD (1958)  | DLUSSKY & PISARSKI (1971)                            | KUTTER (1977)               | COLLINGWOOD (1979)           | DOUWES (1995) | CZECHOWSKI & al. (2002)            | SEIFERT (1996) | SEIFERT (2007)     |
| <i>F. fusca</i>         | < 3                | at most 2 - 3       | ≤ 2  | usually 0, sometimes 1 - 4  | usually = 0, occasion. 1 - 2 | ≤ 2           | usually 0, rarely 1 - 5            | average < 1    | average 0 - 0.8    |
| <i>F. lemni</i>         | numerous           | numerous            | some ind. >10, in SE-Europ. populations, most ind. 0 | up to 20                    | "with erect hairs"           | ≥ 3           | > 6                                | average > 1    | average 1.2 - 13.5 |
| Femora                  |                    |                     |  |                             |                              |               |                                    |                |                    |
| <i>F. fusca</i>         | mid = 0            | mid = 0<br>hind = 0 |  | mid = 0<br>hind = 0         |                              | mid ≤ 1       | fore = 2 - 3<br>mid = rarely 1 - 2 |                |                    |
| <i>F. lemni</i>         | mid = "long hairs" | all = "hairy"       |  | mid = a few<br>hind = a few |                              | mid ≥ 2       | fore = 3 - 12<br>mid = 3 - 17      |                |                    |

## References

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**Appendix 2:** Morphological characters (mean; standard deviation, SD; minimum, Min; maximum, Max) measured for individual workers and averaged over nests. Sample size (N) given as the number of individuals and number of colonies, note that the same number of individuals was measured in all colonies, resulting in the same means in individuals and colonies. The characters were measured following EICHHORN (1972) and SEIFERT (1996a, b, 1997, 2003), and were the maximum head width across the eyes (KB), maximum head capsule length along the median line (KL), maximum straight line scapus length excluding the articular condyle (SL), and the number of standing hairs on pronotum (H<sub>PRON</sub>), mesonotum (H<sub>MESON</sub>), and the inner margin of femur of foreleg (H<sub>FORELEG</sub>), midleg (H<sub>MIDLEG</sub>) and hindleg (H<sub>HINDLEG</sub>). One measurement unit for KB, KL and SL corresponds to 0.019 mm. In addition, the presence of hairs on propodeum, gula and occipital and posterior margins of the head was checked, but no hairs were found except for one propodeal hair in one *Formica lemami* worker. Differences between the species were tested with one-way analysis of variance (normally-distributed size measures, F) or Kruskal-Wallis rank-sum test (non-normally distributed hair counts, KrW).

|             | Characters           | <i>Formica fusca</i> (N = 110, 22) |       |      |      | <i>Formica lemami</i> (N = 135, 27) |       |      |      | F / KrW | p       |
|-------------|----------------------|------------------------------------|-------|------|------|-------------------------------------|-------|------|------|---------|---------|
|             |                      | Mean                               | SD    | Min  | Max  | Mean                                | SD    | Min  | Max  |         |         |
| Individuals | KB                   | 67.268                             | 6.457 | 52   | 82   | 66.319                              | 6.364 | 51   | 79   | 1.33    | 0.26    |
|             | KL                   | 77.036                             | 6.276 | 62   | 93   | 75.444                              | 6.028 | 60   | 86   | 4.07    | 0.045   |
|             | SL                   | 77.186                             | 5.503 | 62   | 89   | 73.626                              | 5.770 | 60   | 85   | 24.1    | < 0.001 |
|             | H <sub>PRON</sub>    | 0.200                              | 0.521 | 0    | 2    | 8.519                               | 3.936 | 1    | 19   | 189.9   | < 0.001 |
|             | H <sub>MESON</sub>   | 0.0091                             | 0.095 | 0    | 1    | 1.830                               | 2.297 | 0    | 10   | 94.2    | < 0.001 |
|             | H <sub>FORELEG</sub> | 0.718                              | 0.978 | 0    | 4    | 3.326                               | 1.820 | 0    | 8    | 110.3   | < 0.001 |
|             | H <sub>MIDLEG</sub>  | 0.036                              | 0.232 | 0    | 2    | 0.674                               | 0.976 | 0    | 4    | 44.4    | < 0.001 |
|             | H <sub>HINDLEG</sub> | 0                                  | 0     | 0    | 0    | 0.133                               | 0.383 | 0    | 2    | 13.9    | < 0.001 |
| Nests       | KB                   | 67.268                             | 5.268 | 57.2 | 76.4 | 66.319                              | 4.055 | 56.4 | 71.4 | 0.51    | 0.48    |
|             | KL                   | 77.036                             | 5.078 | 67   | 85.2 | 75.444                              | 3.939 | 65.8 | 80.8 | 1.53    | 0.22    |
|             | SL                   | 77.186                             | 4.473 | 67.8 | 84.6 | 73.626                              | 3.900 | 65.2 | 79.3 | 8.86    | 0.005   |
|             | H <sub>PRON</sub>    | 0.200                              | 0.327 | 0    | 1    | 8.519                               | 2.579 | 2.6  | 12.2 | 36.4    | < 0.001 |
|             | H <sub>MESON</sub>   | 0.0091                             | 0.043 | 0    | 0.2  | 1.830                               | 1.425 | 0.2  | 5.4  | 38.3    | < 0.001 |
|             | H <sub>FORELEG</sub> | 0.718                              | 0.568 | 0    | 2    | 3.326                               | 1.053 | 1    | 5.2  | 33.6    | < 0.001 |
|             | H <sub>MIDLEG</sub>  | 0.036                              | 0.100 | 0    | 0.4  | 0.674                               | 0.448 | 0    | 1.8  | 32.5    | < 0.001 |
|             | H <sub>HINDLEG</sub> | 0                                  | 0     | 0    | 0    | 0.133                               | 0.157 | 0    | 0.4  | 13.8    | < 0.001 |

## References

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**Appendix 3:** Description of genetic variation in the allozyme and DNA microsatellite loci studied. Species identification was made by using surface chemistry. *n* is the sample size as the number of nests; *n*<sub>ALL</sub> is the number of alleles, *R*<sub>s</sub> is the allelic richness and *H*<sub>E</sub> is the expected heterozygosity according to the Hardy Weinberg equilibrium in each locus. *H*<sub>E</sub> was corrected for the sample size (*n*):  $H_E^* = (H_E \times 2n) / (2n - 1)$  (NEI 1987). In the test combining morphometrics and allozyme data, *Idh*, and *Pgi* (data from SEPPÄ & al. 2009) were studied in addition to the loci described below. Significant differences and differences approaching significance (Wilcoxon Signed Ranks Tests) were found in: 1) allelic richness between Hyytiälä and UK populations of *F. fusca*, *Z* = 1.84, *p* = 0.066; 2) allelic richness between *F. fusca* and *F. lemani* populations in the UK, *Z* = 1.89, *p* = 0.059 and 3) expected heterozygosity between *F. fusca* and *F. lemani* populations in the UK, *Z* = -2.14, *P* = 0.032. In all other comparisons, *P* > 0.093.

|                     | <i>F. fusca</i>         |                       |                       |                         |                       |                       | <i>F. lemani</i>        |                       |                       |                         |                       |                       |
|---------------------|-------------------------|-----------------------|-----------------------|-------------------------|-----------------------|-----------------------|-------------------------|-----------------------|-----------------------|-------------------------|-----------------------|-----------------------|
|                     | Hyytiälä                |                       |                       | UK                      |                       |                       | Hyytiälä                |                       |                       | UK                      |                       |                       |
|                     | <i>n</i> <sub>ALL</sub> | <i>R</i> <sub>s</sub> | <i>H</i> <sub>E</sub> | <i>n</i> <sub>ALL</sub> | <i>R</i> <sub>s</sub> | <i>H</i> <sub>E</sub> | <i>n</i> <sub>ALL</sub> | <i>R</i> <sub>s</sub> | <i>H</i> <sub>E</sub> | <i>n</i> <sub>ALL</sub> | <i>R</i> <sub>s</sub> | <i>H</i> <sub>E</sub> |
| DNA microsatellites | n = 22                  |                       |                       | n = 12                  |                       |                       | n = 26                  |                       |                       | n = 17                  |                       |                       |
| Fl20                | 7                       | 5.58                  | 0.42                  | 4                       | 3.96                  | 0.42                  | 10                      | 6.68                  | 0.68                  | 8                       | 7.02                  | 0.80                  |
| Fe17                | 5                       | 4.32                  | 0.57                  | 4                       | 4.00                  | 0.70                  | 10                      | 7.68                  | 0.79                  | 7                       | 6.71                  | 0.82                  |
| Fe19                | 5                       | 4.05                  | 0.30                  | 3                       | 2.88                  | 0.53                  | 5                       | 4.95                  | 0.78                  | 4                       | 3.84                  | 0.66                  |
| Fl12                | 3                       | 2.90                  | 0.56                  | 3                       | 2.98                  | 0.50                  | 3                       | 2.55                  | 0.48                  | 3                       | 2.99                  | 0.53                  |
| Fe51                | 7                       | 6.03                  | 0.77                  | 6                       | 5.82                  | 0.69                  | 3                       | 2.55                  | 0.40                  | 7                       | 6.69                  | 0.82                  |
| Fe13                | 10                      | 7.76                  | 0.82                  | 5                       | 5.00                  | 0.64                  | 14                      | 8.67                  | 0.77                  | 8                       | 7.25                  | 0.83                  |
| Fe21                | 8                       | 5.46                  | 0.64                  | 4                       | 3.52                  | 0.42                  | 4                       | 3.80                  | 0.57                  | 4                       | 3.44                  | 0.60                  |
| Fy13                | 2                       | 2.00                  | 0.37                  | 2                       | 2.00                  | 0.39                  | 5                       | 2.98                  | 0.49                  | 3                       | 2.75                  | 0.53                  |
| Fy4                 | 4                       | 2.27                  | 0.08                  | 2                       | 1.82                  | 0.03                  | 5                       | 3.79                  | 0.52                  | 3                       | 2.99                  | 0.45                  |
| Fy7                 | 3                       | 3.00                  | 0.56                  | 5                       | 4.25                  | 0.47                  | 4                       | 2.86                  | 0.21                  | 3                       | 2.88                  | 0.29                  |
| Allozymes           | n = 23                  |                       |                       |                         |                       |                       | n = 26                  |                       |                       |                         |                       |                       |
| <i>Pep</i>          | 1                       | 1.0                   | 0.00                  |                         |                       |                       | 3                       | 3.0                   | 0.49                  |                         |                       |                       |
| <i>Mdh</i>          | 2                       | 2.0                   | 0.05                  |                         |                       |                       | 3                       | 3.0                   | 0.50                  |                         |                       |                       |
| <i>Pgd</i>          | 2                       | 2.0                   | 0.51                  |                         |                       |                       | 2                       | 2.0                   | 0.06                  |                         |                       |                       |
| <i>Aco</i>          | 1                       | 1.0                   | 0.00                  |                         |                       |                       | 2                       | 2.0                   | 0.38                  |                         |                       |                       |

## References

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**Appendix 4:** The occurrence of private alleles in the 10 DNA microsatellite loci studied in *Formica fusca* and *F. lemani* populations in Hyytiälä and UK; the average number across loci ( $\bar{x} \pm SD$ ) and the average proportion of alleles that are private in each locus ( $\% \pm SD$ ) are shown. A significantly larger proportion of *F. lemani* alleles were private in Hyytiälä than in the UK (Wilcoxon Signed Ranks Test): *Z* = -2.37, *P* = 0.016; in all other comparisons, *P* > 0.13.

|                  | Populations combined |             | Hyytiälä         |             | UK               |             |
|------------------|----------------------|-------------|------------------|-------------|------------------|-------------|
|                  | $\bar{x} \pm SD$     | $\% \pm SD$ | $\bar{x} \pm SD$ | $\% \pm SD$ | $\bar{x} \pm SD$ | $\% \pm SD$ |
| <i>F. fusca</i>  | 1.6 ± 1.91           | 18 ± 18     | 0.93 ± 1.59      | 9 ± 14      | 0.29 ± 0.61      | 3 ± 7       |
| <i>F. lemani</i> | 2.8 ± 2.28           | 34 ± 19     | 1.2 ± 1.53       | 11 ± 13     | 0.43 ± 0.76      | 3 ± 6       |