

## NC-Clustering demonstrates heterospecificity of the cryptic ant species *Temnothorax luteus* (FOREL, 1874) and *T. racovitzai* (BONDROIT, 1918) (Hymenoptera: Formicidae)

With 6 figures

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Published on 2014-07-31

### Key words

Cryptic species, exploratory data analysis, thermal niche

### Summary

Evidence is presented that the W Mediterranean cryptic ant species *Temnothorax luteus* (FOREL, 1874) and *T. racovitzai* (BONDROIT, 1918) are clearly separable by exploratory and hypothesis-driven data analyses of complex morphological data sets. Investigation was concentrated to the sympatric range of both species. 18 morphological characters were evaluated in a total of 178 workers originating from 64 nest samples. Classifications by hierarchical NC-Ward clustering, non-hierarchical NC-K-Means clustering, NC-NMDS-K-Means clustering and linear discriminant analysis coincided by 100 % after reduction of the character set to the seven most discriminative characters. The type series of *T. massiliensis* (BONDROIT, 1918) and *T. racovitzai* (BONDROIT, 1918) were assigned to the same cluster with posterior probabilities of  $p = 1.000$  in the former and  $p = 0.9992$  in the latter. The type series of *T. tristis* (BONDROIT, 1918) and *T. luteus* were allocated to another, clearly separated cluster with  $p = 0.9999$  in each case. Accordingly, *T. tristis* is considered as junior synonym of *T. luteus* whereas *T. massiliensis* was established as junior synonym of *T. racovitzai*. The classification error of the LDA was 1.7 % in 178 individuals. Despite a broad sympatric range, there are no suggestions to any nest samples containing a mixture of both phenotypes. This rejects the hypothesis that *T. luteus* and *T. racovitzai* could represent an intraspecific dimorphism. Significant interspecific differences of the two species in geographic distribution and thermal niche were demonstrated. Mean air temperature from May to August of 24 sampling sites of *T. luteus* was by 3.77°C cooler than in 21 sites of *T. racovitzai* ( $p < 0.0001$ ).

### Zusammenfassung

Es wird gezeigt, dass die westmediterranen kryptischen Ameisenarten *Temnothorax luteus* (FOREL, 1874) und *T. racovitzai* (BONDROIT, 1918) mittels explorativer und hypothesengetriebener Analysen komplexer morphologischer Datensätze eindeutig unterscheidbar sind. Die Untersuchung konzentrierte sich auf das sympatrische Areal beider Arten. An 178 Arbeiterinnen aus 64 Nestproben wurden 18 morphologische Merkmale erfasst. Nach Reduktion auf die sieben meistseparierenden Merkmale ergab sich eine 100 %ige Übereinstimmung der Klassifizierungen durch

hierarchische NC-Ward-, nicht hierarchische NC-K-Means- und NC-NMDS-K-Means Clusteranalyse sowie durch eine lineare Diskriminanzanalyse. Die Typenserien von *T. massiliensis* (BONDROIT, 1918) und *T. racovitzai* (BONDROIT, 1918) wurden mit einer a posteriori Wahrscheinlichkeit von  $p = 1.0000$  bzw.  $p = 0.9992$  dem gleichen Cluster zugeordnet. Die Typenserien von *T. tristis* (BONDROIT, 1918) und *T. luteus* wurden beide mit  $p = 0.9999$  einem zweiten, deutlich vom ersteren separierten Cluster zugeordnet. Daraus ergibt sich, dass *T. tristis* ein jüngeres Synonym von *T. luteus* und *T. massiliensis* ein jüngeres Synonym von *T. racovitzai* ist. Der Klassifikationsfehler auf der Ebene der Individuen betrug 1.7 % an 178 Arbeiterinnen. Trotz einer breiten Überlappung der Verbreitungsgebiete gibt es keine Hinweise auf phänotypisch gemischte Nester, was die Hypothese widerlegt, dass *T. luteus* und *T. racovitzai* einen intraspezifischen Polymorphismus repräsentieren könnten. Es wurden signifikante interspezifische Unterschiede in der geographischen Verbreitung und der thermischen Nischenkomponente gezeigt. Die mittlere Lufttemperatur von Mai bis August von 24 Fundorten von *T. luteus* war im Mittel um 3.77°C kälter als die von 21 Fundorten von *T. racovitzai* ( $p < 0.0001$ ).

## Introduction

The identity of *Temnothorax luteus* (FOREL, 1874) described from Grand Salève/ France and Ticino /Switzerland has remained obscure until very recently when PLATEAUX & CAGNIANT (2012) discovered two type workers in the collection of Musée Zoologique Lausanne. These specimens carried no type labels but the argumentation of PLATEAUX & CAGNIANT, based on the original morphological description and the localities reported by Forel, was conclusive. However, because their paper was based on very few investigated specimens and poor investigation standards, they published several diffuse and problematic decisions. In a synonymic list on page 430 and following CASEVITZ-WEULERSSE & GALKOWSKI (2009), PLATEAUX & CAGNIANT published *Temnothorax massiliensis* (BONDROIT, 1918) as a synonym of *T. luteus* and they considered *T. racovitzai* (BONDROIT, 1918) as a subspecies of *T. luteus*. However, results of exploratory and hypothesis-driven data analyses of complex character sets, show that *T. massiliensis* is no synonym of *T. luteus* and that *T. racovitzai* cannot be a subspecies of *T. luteus*. Furthermore, we want to assess the status of all five European taxa belonging to the *T. luteus* species complex and to demonstrate that there are two reliably delimited species, *T. luteus* and *T. racovitzai*, which differ significantly in morphology, distribution and in the thermal component of their climatic niches.

## Material and Methods

### Material subject to numeric data recording

For both sibling species, numeric data recording was performed in a total of 64 samples containing 178 workers and originating from 38 localities.

#### *Temnothorax luteus*

Numeric data recording was performed in a total of 106 workers from 38 different samples and 24 localities. These are in detail with collecting date given in the format yyyy.mm.dd and coordinates in international decimal format:

**ANDORRA:** Andorra City -9 km NNW, 1991.05.18, samples No 500a, 502 [42.680°N, 1.470°E, 1750 m]. **FRANCE:** Cucuron -6 km NNE, Luberon Mts., 2009.03.15, samples No 574, 577, 584, 590, 593, 594 [43.816°N, 5.458°E, 1009 m]; Desaignes, 2008.04 (Darras) [44.983°N, 4.517°E, 500 m]; Grand Canyon du Verdon, 1992.05 [43.770°N, 6.210°E, 500 m]; Grand Saleve, pre 1874, type *T. luteus* [46.130°N, 6.190°E, 900 m]; La Tour de Carol, 2008.06.28, samples No d, h [42.466°N, 1.890°E, 1500 m]; Laruns, 2009.07.16 [42.917°N, 0.450°W, 800 m]; Le Vigan -15 km NNW, 1994.07.09 [44.023°N, 3.579°E, 900 m]; Mas de Tence, 2007.07 [45.100°N, 4.333°E, 1000 m]; Mt. Ventoux, 1917.08.12 [44.133°N, 5.282°E, 1000 m]; Nimes, 2008.07.19 [43.783°N, 4.283°E, 50 m]; Nyons, 1981.04.02, No 9714 [44.360°N, 5.150°E, 400 m]; Puy de Dome: Sayat, pre 1918, type *T. tristis* [45.830°N, 3.050°E, 500 m]; Puy de Dome: Sayat, 2006.06.29 [45.830°N, 3.050°E, 500 m]; Puy de Dome: Sayat, 2006.07.25 [45.830°N, 3.050°E, 500 m]; Rochepaule, 2008.07 [45.067°N, 4.450°E, 800 m]; S Col d'Izoard, Arvieux, 1994.07.29, No 396 [44.770, 6.730°E, 1750 m]; St. Andre les Alpes, 1995.05, samples No 727, 729 [43.974°N, 6.505°E, 1000 m]; Tournus -5 km W, 1994.07.08, samples No 1, 4, 5 and 007 [46.581°N, 4.834°E, 335 m]; Volvic, 2006.06.29 [45.871°N, 3.029°E, 573 m]. **SPAIN:** Chodos -1 km W, 1991.05.18, sample No 388 [40.250°N, 0.300°W, 1250 m]. **SWITZERLAND:** Brig, pre 1991, sample No 514 (A. Schulz) [46.320°N, 7.973°E, 900 m]; Locarno, below Mte. Bre, 1997.04.29, sample No 24.2 [46.181°N, 8.778°E, 880 m]; Locarno, below Mte. Bre, 1997.05.27, sample No 24.3 [46.181°N, 8.778°E, 880 m]; Locarno, below Mte. Bre, 2011.08.21, sample No 1, [46.181°N, 8.778°E, 880 m]; Locarno: Ai Sassi, 1997.06.24, sample No 25.2 [46.200°N, 8.800°E, 580 m]; Pfynwald, 1990.06 (A. Schulz) [46.294°N, 7.560°E, 590 m].

#### *Temnothorax racovitzai*

Numeric data recording was performed in a total of 72 workers from 26 different samples and 21 localities. These are in detail:

FRANCE: Aix-en-Provence, 1994.06 [43.517°N, 5.467°E, 200 m]; Aix-en-Provence -10 km N, 2009.03.13, sample No 535 [43.529°N, 5.586°E, 783 m]; Apt -10 km S, Luberon Mts., 2009.03.14, sample No 565 [43.787°N, 5.352°E, 384 m]; Banyuls, pre 1918, type *T. racovitza* [42.480°N, 3.130°E, 10 m]; Banyuls, 2009.06.13 [42.450°N, 3.083°E, 140 m]; Banyuls -4 km SE, 1995.05.31, sample No 799 [42.466°N, 3.155°E, 100 m]; Cerbere 2006.05.15 [42.433°N, 3.150°E, 156 m]; Cerbere 2007.09 [42.433°N, 3.150°E, 156 m]; Col de Banyuls, 2009.08 [42.450°N, 3.050°E, 350 m]; Col de Ceris, 2007.06.23 [42.468°N, 3.144°E, 140 m]; Cucuron -6 km NNE, Luberon Mts., 2009.03.15, samples No 596, 597 [43.816°N, 5.458°E, 1009 m]; Dieulefit, pre 1900 (Forel) [44.500°N, 5.113°E, 500 m]; Marseille, pre 1918 (Grouvelle), type *T. massiliensis* [43.290°N, 5.390°E, 40 m]; Mt. Ventoux, 2010.08.22 [44.133°N, 5.283°E, 1000 m]; Nyons, 1981.04.02, sample No 9712 [44.360°N, 5.150°E, 400 m]. SPAIN: Chodos -1 km W, 1991.05.18, sample No 380 [40.253°N, 0.299°W, 1250 m]; Colldejou, 2009.10.20, samples No -599, 600, 601 [41.108°N, 0.864°E, 874 m]; Montsant Mts., 2007.04.03, samples No 16, 18 [41.279°N, 0.875°E, 980 m]; Pauls vic., 2009.10.21, samples No 627, 628 [40.925°N, 0.368°E, 983 m]; Torroja del Priorat, 2007.04.03, sample No 15 [41.215°N, 0.810°E, 330 m]; Viella Baixa, 2009.10.23 [41.224°N, 0.754°E, 265 m].

#### The type material and its labeling

##### *Leptothorax tuberum* r. *luteus* FOREL, 1874

Two workers labeled “♀”, “Grd Salève, chez A. Varanen”, “Lectotype (bottom worker) *Temnothorax luteus* (Forel, 1874), des. Seifert 2011” [this labeling is consistent with the published lectotype designation of Plateaux & Cagniant (2012) who did not physically label types]; lectotype with CW = 588 µm; in Forel collection of MZ Lausanne.

##### *Leptothorax tristis* BONDROIT, 1918

One type worker labelled “Sayat Puy de Dome 650 m M.Avel, “*Leptothorax tristis* Type Bondr.”; IRSNB Bruxelles. Another worker in IRSNB Bruxelles, labelled “Mt. Ventoux V:S. 1250 m 12-VIII-1917\ A. Chobaut, coll. Minozzi” and “*Leptothorax tristis* Bondr.”; cannot be considered a type because the locality Mt. Ventoux was not mentioned by BONDROIT (1918).

##### *Leptothorax racovitza* BONDROIT, 1918

Five workers labeled “Banyuls”, “*Leptothorax racovitza* Type Bondr.”, “Lectotypus des: Schulz 1994” [A. Schulz did not specify the lectotype specimen, the top specimen with CW = 0.585 mm is published herewith as lectotype]; IRSNB Bruxelles.

##### *Leptothorax massiliensis* BONDROIT, 1918

Four syntype workers labeled “Marseille Grouvelle”, “*Leptothorax massiliensis* Type Bondr.”; IRSNB Bruxelles.

#### Investigation Methods

18 morphometric characters currently being used in taxonomy of Palaearctic *Temnothorax* (SEIFERT 2006, Csösz et al. 2013) were investigated. 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 120–384x. 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. Measurements of body parts always refer to real cuticular surface and not to the diffuse pubescence surface.

The process of species discrimination included four major routines:

- Reduction of errors in primary data recording as described in SEIFERT (2002).
- Removal of allometric variance (RAV) performed with the procedure described by SEIFERT (2008).
- Application of the exploratory data analyses Nest Centroid Clustering (NC-Clustering). This included a hierarchical method (NC-Ward), a non-hierarchical method (NC-K-Means) and a highly flexible ordination method (NC-NMDS-K-Means). These methods were described in more detail by SEIFERT et al. (2013) who also provided a script written in R and freely available under the GNU / GPL license from the following website: <http://sourceforge.net/projects/agnesclustering/>.
- Checking of the demonstrated species clusters by linear discriminant analysis (LDA) also provided by SPSS 16.0.

## The morphometric characters

- 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; the maximum is found in *Temnothorax* and *Leptothorax* usually across and including the eyes, exceptionally posterior of the eyes.
- EYE** - eye-size index: the arithmetic mean of the large (EL) and small diameter (EW) of the elliptic compound eye is divided by CS, i.e.  $EYE = (EL+EW)/(CL+CW)$ . All structurally visible ommatidia are considered.
- FRS** - distance of the frontal carinae immediately caudal of the posterior intersection points between frontal carinae and the lamellae dorsal of the torulus. If these dorsal lamellae do not laterally surpass the frontal carinae, the deepest point of scape corner pits may be taken as reference line. These pits take up the inner corner of scape base when the scape is fully switched caudad and produce a dark triangular shadow in the lateral frontal lobes immediately posterior of the dorsal lamellae of scape joint capsule.
- MGr** - depth of metanotal groove or depression, measured from the tangent connecting the dorsalmost points of promesonotum and propodeum; here given as per cent ratio of CS.
- MH** - in workers: with mesosoma in lateral view and measured orthogonal to “longitudinal mesosomal axis”; MH is the longest measurable *section* line of mesosoma at mesopleural level (not height above all). “Longitudinal mesosomal axis” in lateral view is defined as straight line from the centre of propodeal lobe to the border point between anterior pronotal shield and propleuron. In gynes it is the longest section line directed perpendicular to the straight dorsal profile line of mesosoma (formed by mesonotum and scutellum). The lower reference point is usually the lowest part of mesopleuron.
- MW** - maximum mesosoma width; in gynes measured anteriorly of the tegulae.
- ML** - in workers: 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 border-line between pronotal slope and pronotal shield as anterior reference point). In gynes: length from caudalmost point of propodeal lobe to the most distant point of steep anterior pronotal face.
- 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.
- PEL** - diagonal petiolar length in lateral view; measured from anterior corner of subpetiolar process to dorso-caudal corner of caudal cylinder.
- PEW** - maximum width of petiole.
- 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 the level of the posterior eye margin. Note that many heads are asymmetric and average the left and right postocular distance.
- PP** - maximum width of postpetiole.
- SL** - maximum straight line scape length excluding the articular condyle as 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 (Fig. 4 in SEIFERT 2006). This mode of measuring is less ambiguous than other methods but it results in some spine length in species with reduced spines.
- SPBA** - the smallest distance of the lateral margins of the spines at their base. This should be measured in dorsofrontal view, since the wider parts of the ventral propodeum do not interfere with the measurement in this position. If the lateral margins of spines diverge continuously from the tip to the base, a smallest distance at base is not defined. In this case, SPBA is measured at the level of the bottom of the interspinal meniscus.
- SPST** - distance between the centre of propodeal stigma and spine tip. The stigma centre refers to the midpoint defined by the outer cuticular ring but not to the centre of real stigma opening that may be positioned eccentrically.
- SPTI** - the distance of spine tips in dorsal view; if spine tips are rounded or thick, the centres of spine tips are taken as reference points.

## Removal of allometric variance (RAV)

In ant groups without extreme allometries removal of allometric variance (RAV) does not change the results of linear discriminant functions (SEIFERT 2008) but it may improve the success of exploratory data analyses. Exposing which deviations in shape characters or counts are no consequence of interspecific body size differences, RAV improves the explanatory power of comparative tables (Tab. 1). RAV was performed in workers as a mean of the parameters of 16 West Palaearctic *Temnothorax* species presented by 1443 evaluated specimens in the data files of the senior author. With CS given in mm, RAV was calculated for the assumption of all individuals having the same head size of CS = 0.62 mm:

$$CL/CW_{0.62} = CL/CW / (-0.0631 \cdot CS + 1.1366) \cdot 1.0975$$

$$SL/CS_{0.62} = SL/CS / (-0.1193 \cdot CS + 0.8298) \cdot 0.7559$$

$$\begin{aligned}
\text{PoOc/CL}_{0.62} &= \text{PoOc/CL} / (-0.0259 * \text{CS} + 0.4044) * 0.3883 \\
\text{EYE/CS}_{0.62} &= \text{EYE/CS} / (+0.0395 * \text{CS} + 0.1980) * 0.2225 \\
\text{FRS/CS}_{0.62} &= \text{FRS/CS} / (+0.0142 * \text{CS} + 0.3417) * 0.3505 \\
\text{MW/CS}_{0.62} &= \text{MW/CS} / (+0.1424 * \text{CS} + 0.5163) * 0.6046 \\
\text{MH/CS}_{0.62} &= \text{MH/CS} / (-0.0060 * \text{CS} + 0.5224) * 0.5186 \\
\text{ML/CS}_{0.62} &= \text{ML/CS} / (+0.1501 * \text{CS} + 1.1208) * 1.2139 \\
\text{SPBA/CS}_{0.62} &= \text{SPBA/CS} / (+0.1563 * \text{CS} + 0.1712) * 0.2681 \\
\text{SPTI/CS}_{0.62} &= \text{SPWI/CS} / (+0.1866 * \text{CS} + 0.2047) * 0.3204 \\
\text{PEW/CS}_{0.62} &= \text{PEW/CS} / (+0.1129 * \text{CS} + 0.1929) * 0.2629 \\
\text{PPW/CS}_{0.62} &= \text{PPW/CS} / (+0.0793 * \text{CS} + 0.3120) * 0.3611 \\
\text{SP/CS}_{0.62} &= \text{SP/CS} / (+0.1543 * \text{CS} + 0.1033) * 0.1989 \\
\text{SPST/CS}_{0.62} &= \text{SPST/CS} / (+0.1286 * \text{CS} + 0.1944) * 0.2741 \\
\text{PEH/CS}_{0.62} &= \text{PEH/CS} / (+0.0380 * \text{CS} + 0.3384) * 0.3620 \\
\text{PEL/CS}_{0.62} &= \text{PEL/CS} / (+0.2043 * \text{CS} + 0.3642) * 0.4909
\end{aligned}$$

### Calculation of the thermal niche component

As a rough measure of thermal niche, we estimated standard air temperature TAS in 2 meters above ground. Following the logic of SEIFERT & PANNIER (2007: p. 162), TAS was calculated for a sampling site as mean air temperature of the period 1 May to 31 August of the nearest three meteorological stations. As the samples originate from the long period 1874 to 2012 we selected a reference before the recent acceleration of global warming and used the mean of the years 1961–1990 provided by Klimaabteilung der Zentralanstalt für Meteorologie und Geodynamik (1996). We corrected the data for an altitudinal temperature decrease of 0.661°C per 100 m according to the equation of SEIFERT and PANNIER (2007):  $\text{TAS} = -0.694 \text{ LAT} + 0.078 \text{ LON} - 0.00661 \text{ ALT} + 52.20$  where TAS is the predicted standard air temperature in °C, LAT and LON are the geographical latitude and longitude in international decimal format, and ALT is the altitude above sea level in meters.

## Results and discussion

### Diagnosis of the *Temnothorax luteus* complex

Members of the *Temnothorax luteus* species complex (Figs 3–6) can be separated from other species by the character combination described below. Numeric data are given as overall means  $\pm$  standard deviation of individual workers without RAV-correction.

Whole ants light yellowish to yellowish brown, lacking any blackish or blackish brown pigmentation with exception of eyes; some body parts or the entire body may be infuscated. Dorsum of head capsule rather weakly longitudinally rugulose; small to very large areas of central vertex often completely smooth and shining. Relatively small-sized and long-headed in terms of the genus (CS  $608 \pm 45 \mu\text{m}$ , CL/CW  $1.133 \pm 0.020$ ). Scape longer than in most congeners (SL/CS  $0.819 \pm 0.018$ ). Dorsal profile of mesosoma convex, without a metanotal groove. Propodeal spines rather long and acute (SP/CS  $0.234 \pm 0.020$ , SPST/CS  $0.319 \pm 0.020$ ), significantly diverging in

dorsal view (SPBA/CS  $0.265 \pm 0.019$ , SPTI/CS  $0.346 \pm 0.026$ ) and in lateral view moderately erected, deviating by 40–45° from longitudinal mesosomal axis. Petiole in lateral view moderately pedunculate and of comparably low height (PEH/CS  $0.351 \pm 0.014$ ).

### Clustering, classification and synonymies

NC-Ward, NC-K-Means and NC-NMDS-K-Means clustering considering 18 characters (i.e., the absolute size measure CS and 17 RAV-corrected shape characters) resulted in the demonstration of two main clusters (Fig. 1). These clusters are interpreted to represent two species *Temnothorax luteus* (FÖREL, 1874) and *Temnothorax racovitzai* (BONDROIT, 1918). All three exploratory data analyses made a coincident assignment of the types series to either cluster: The type series of *T. massiliensis* (BONDROIT, 1918) shared a cluster with the type series of *T. racovitzai* and the type of *T. tristis* (BONDROIT, 1918) clustered with the *T. luteus* type series. As a consequence, we consider *T. massiliensis* as a junior synonym of *T. racovitzai* and *T. tristis* a junior synonym of *T. luteus*. NC-K-Means and NC-NMDS-K-Means provided identical classifications for each of the 64 samples but they differed from NC-Ward in the allocation of three samples to the *T. luteus* cluster. These samples, placed by NC-Ward in the *T. racovitzai* branch, are marked in Fig. 1 with arrows. Running these three samples as wild-cards in a linear discriminant analysis (LDA) confirmed their allocation to *T. luteus*. This 3 : 1 “majority decision” suggests that NC-Ward probably made three clustering errors when using the unselected set of all 18 characters. The LDA provided the following posterior probabilities for the type series: All four type specimens of *T. massiliensis* were assigned with  $p = 1.000$  to the *T. racovitzai* cluster, the three type specimens of *T. racovitzai* were allocated with  $p = 0.9997$ ,  $0.9998$  and  $0.9994$  to the same cluster, the single type specimen of *T. tristis* was allocated with  $p = 0.9998$  to the *T. luteus* cluster and the two type specimens of *T. luteus* with  $p = 0.9999$  and  $0.9999$  to the same cluster. The classification error of the LDA was 2.2 % in 178 worker individuals and 4.5 % in a leave-one-out cross-validation LDA.

Accepting the hypotheses provided by NC-K-Means, NC-NMDS-K-means and the LDA using the 18-character system and running a stepwise LDA, reduced the number of characters to seven: SL/CS<sub>0.62</sub>, FRS/CS<sub>0.62</sub>, EYE/CS<sub>0.62</sub>, PEW/CS<sub>0.62</sub>, SPST/CS<sub>0.62</sub>, PEL/CS<sub>0.62</sub>, ML/CS<sub>0.62</sub>. As a result, the classifications of all three exploratory data analyses and the LDA were 100 % congruent and the NC-Ward dendrogram became undisturbed (Fig. 2). With the number of individuals in the smallest class being 10.2 fold larger than the number of characters, an optimum condition for running an LDA was given. The type specimens of *T. massiliensis* were assigned with  $p = 0.9999$ ,  $1.000$ ,  $1.000$ , and  $1.000$  to the *T. racovitzai* cluster, the type specimens of *T. racovitzai* with

$p = 0.9994$ ,  $0.9996$  and  $0.9982$  to the same cluster, the type specimen of *T. tristis* was allocated with  $p = 0.9999$  to the *T. luteus* cluster and the two type specimens of *T. luteus* with  $p = 0.9999$  and  $0.9999$  to the same cluster. The classification error of the LDA was 1.7 % in 178 individuals and 3.4 % in a leave-one-out cross-validation LDA. These data give another clear indication for the presence of two well-separable species and the synonymies stated above. It might be asked if the different character syndromes of *T. luteus* and *T. racovitzai* could be the expression of a discrete intraspecific dimorphism rather than indicating different species. Discrete character syndromes feigning different species may be generated by the pleiotropic action of a single dominant – recessive gene encoding a transcription factor. Alternatively, but perhaps less likely, a non-recombinant cluster of linked genes may cause similar effects. Assuming conspecificity and panmictic behavior and considering that 83 % of all samples were collected in the sympatric zone (precisely 27 samples of *T. luteus* and 26 of *T. racovitzai*), one should expect a significant number of nest samples containing both morphs. Considering within-sample variance of the posterior probabilities of the 7-character LDA, we found no sample in the whole material indicating a morph mixture on the  $p > 0.90$  level. This is a strong argument against intraspecific dimorphism.

Having only very few specimens available and based on a subjective guess, PLATEAUX & CAGNIANT (2012) considered *T. racovitzai* as subspecies of *T. luteus* and published the new combination *T. luteus racovitzai* (BONDROIT). This idea is untenable because of the broadly overlapping geographical ranges of both taxa (see below). Subspecies, if a use of this category would make sense at all, are defined by replacing each other geographically and to show weak gene flow in contact zones.

We had no access to type specimens of *Leptothorax luteus* var. *subcingulata* EMERY, 1924. Type localities are Algeciras [36.13°N, 5.47°W] and Tibidabo [41.42°N, 2.12°E]. EMERY (1924) noted that only the specimens from Algeciras matched his description fully while those from Tibidabo had a more or less brown antennal club. PLATEAUX & CAGNIANT (2012) had a look on the types from Tibidabo and allocated these to their newly erected subspecies *T. luteus racovitzai* (BONDROIT). However, they did not see the types from Algeciras but assumed that there was a subspecies *T. luteus subcingulata* (EMERY) in southern Spain. We cannot assess this question and consider *Leptothorax luteus* var. *subcingulata* EMERY as a Nomen Dubium as long as there is no access to the types from Algeciras.

### Interspecific morphological differences and a simpler method for species delimitation

The two species are extremely similar, showing a large interspecific overlap for any investigated character even if nest sample means are considered (Tab. 1). A quick

species determination by subjective eye inspection seems impossible. *T. luteus* and *T. racovitzai* are truly cryptic species according to the definition of SEIFERT (2009). *T. racovitzai* differs from *T. luteus* by a relatively smaller mesosoma in all three dimensions, a smaller distance of frontal carinae and larger eyes. Pigmentation and sculpture characters are of no use: Both species show very light, almost entirely yellowish forms as well as darker forms with different body parts appearing infuscated while sculpture varies strongly even within nests and as a function of size. Figs 3–6 show two aspects of each species by example of type specimens.

The complex methods of species delimitation presented above appear unacceptable for practitioners of biodiversity research for reasons of time required for learning the methods and for processing of the samples. Hence, it should be asked how much simplification is possible while keeping the error rate at an acceptable level. The first simplification is using absolute measuring data instead of RAV-corrected shape variables and the second one a strong reduction of characters used. Running the 17 primary characters CL, CW, SL, PoOc, FRS, EYE, MW, MH, ML, SPBA, SPTI, SP, SPST, PEW, PPW, PEH and PEL in discriminant analyses with both forward and backward elimination of characters, we selected a discriminant function reduced to four characters:

$$D(4) = 74.496 * FRS - 206.142 * EYE + 57.695 * ML - 49.634 * CL + 2.636.$$

This method shows a classification error of 3.9 % on worker individual level and the scores of D(4) vary in the following way:

*T. racovitzai*:

$$D(4) = -2.044 \pm 0.88 [-4.363, 0.041] \quad n = 72$$

*T. luteus*:

$$D(4) = 1.388 \pm 1.07 [-1.051, 4.433] \quad n = 106$$

Nest sample means of D(4) showed no interspecific overlap:

*T. racovitzai*:

$$D(4) = -1.996 \pm 0.74 [-3.113, -0.435] \quad n = 26$$

*T. luteus*:

$$D(4) = 1.380 \pm 0.83 [-0.157, 3.779] \quad n = 38$$

This method requires some 10 minutes of investigation time per specimen and should be successful if character definitions are carefully considered and if a sufficient measuring accuracy is achieved.

### Distribution and thermal niche

The presented study intended to investigate the relations in the northern part of both species' geographic ranges, focusing on the situation in the sympatric area. Accordingly, our data are far from depicting the entire geographic ranges. They exclude Spain south of 40°N where *T. racovitzai* should predominate and entire Italy where *T. luteus*

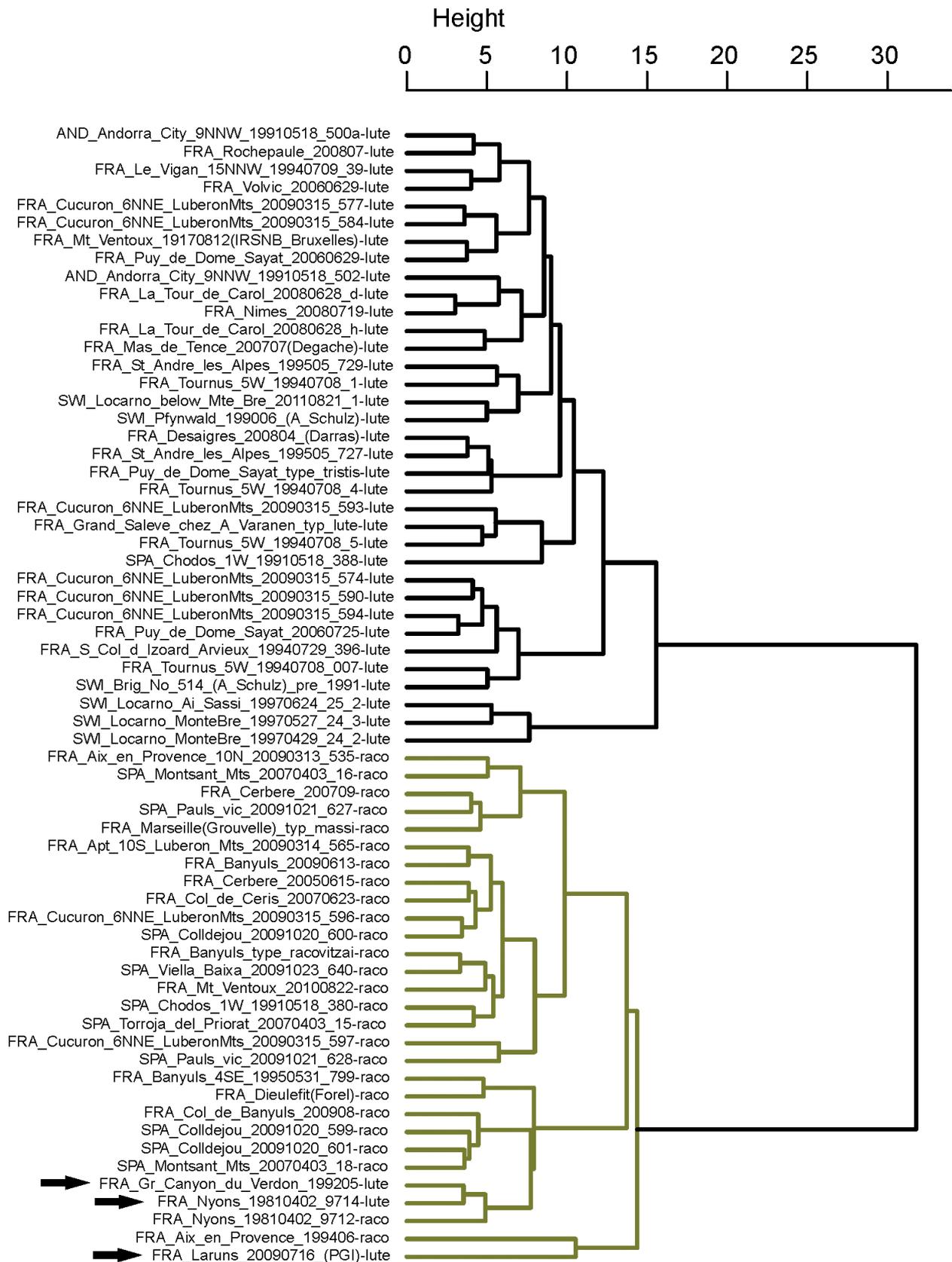


Fig. 1: NC-Ward clustering of 64 worker nest samples of *Temnothorax luteus* (FOREL, 1874) and *T. racovitzai* (BONDROIT, 1918) considering 18 unselected morphometric characters. Arrows point to three nest samples of *T. luteus* erroneously placed in the *T. racovitzai* cluster.

**Tab. 1:** Morphometric data of nest sample means of workers of *Temnothorax luteus* and *T. racovitzai*. Removal of allometric variance of shape variables was performed for the assumption of each individual having a head size of CS = 0.62 mm. Arrangement of data: arithmetic mean  $\pm$  standard deviation [minimum, maximum] and in falling order of significance in an univariate ANOVA.

|                              | <i>T. racovitzai</i> (n = 26)   | ANOVA F, p   | <i>T. luteus</i> (n = 38)       |
|------------------------------|---------------------------------|--------------|---------------------------------|
| ML/ CS <sub>0.62</sub>       | 1.171 $\pm$ 0.014 [1.151,1.197] | 109.93 0.000 | 1.214 $\pm$ 0.017 [1.156,1.244] |
| EYE/ CS <sub>0.62</sub>      | 0.226 $\pm$ 0.005 [0.215,0.238] | 74.40 0.000  | 0.216 $\pm$ 0.004 [0.206,0.226] |
| FRS/ CS <sub>0.62</sub>      | 0.319 $\pm$ 0.007 [0.306,0.330] | 67.44 0.000  | 0.337 $\pm$ 0.009 [0.322,0.355] |
| MH/ CS <sub>0.62</sub>       | 0.491 $\pm$ 0.011 [0.460,0.511] | 62.58 0.000  | 0.517 $\pm$ 0.014 [0.482,0.545] |
| MW/ CS <sub>0.62</sub>       | 0.597 $\pm$ 0.011 [0.573,0.623] | 52.28 0.000  | 0.616 $\pm$ 0.010 [0.592,0.637] |
| SPBA/ CS <sub>0.62</sub>     | 0.259 $\pm$ 0.011 [0.242,0.296] | 24.29 0.000  | 0.272 $\pm$ 0.009 [0.252,0.290] |
| PEW/ CS <sub>0.62</sub>      | 0.249 $\pm$ 0.005 [0.241,0.260] | 23.58 0.000  | 0.257 $\pm$ 0.006 [0.245,0.277] |
| PEH/ CS <sub>0.62</sub>      | 0.345 $\pm$ 0.010 [0.330,0.369] | 18.06 0.000  | 0.356 $\pm$ 0.010 [0.339,0.378] |
| CS [ $\mu$ m]                | 586 $\pm$ 28 [522,638]          | 15.07 0.000  | 623 $\pm$ 42 [504,692]          |
| SPST/ CS <sub>0.62</sub>     | 0.314 $\pm$ 0.012 [0.298,0.338] | 6.74 0.012   | 0.324 $\pm$ 0.016 [0.282,0.355] |
| SPTI/ CS <sub>0.62</sub>     | 0.341 $\pm$ 0.018 [0.308,0.379] | 6.32 0.015   | 0.352 $\pm$ 0.017 [0.327,0.388] |
| PPW/ CS <sub>0.62</sub>      | 0.339 $\pm$ 0.009 [0.316,0.355] | 6.19 0.016   | 0.346 $\pm$ 0.011 [0.317,0.368] |
| PoOc/ CL <sub>0.62</sub>     | 0.383 $\pm$ 0.005 [0.372,0.390] | 4.97 0.029   | 0.386 $\pm$ 0.006 [0.370,0.400] |
| CL/CW <sub>0.62</sub>        | 1.130 $\pm$ 0.012 [1.108,1.156] | 2.18 n.s.    | 1.135 $\pm$ 0.015 [1.103,1.172] |
| PEL/ CS <sub>0.62</sub>      | 0.521 $\pm$ 0.012 [0.498,0.545] | 0.57 n.s.    | 0.518 $\pm$ 0.013 [0.498,0.556] |
| SL/ CS <sub>0.62</sub>       | 0.815 $\pm$ 0.015 [0.792,0.842] | 0.38 n.s.    | 0.818 $\pm$ 0.021 [0.758,0.852] |
| MPGR/ CS <sub>0.62</sub> [%] | 0.08 $\pm$ 0.10 [0.00,0.27]     | 0.34 n.s.    | 0.10 $\pm$ 0.17 [0.00,0.67]     |
| SP/CS <sub>0.62</sub>        | 0.234 $\pm$ 0.018 [0.198,0.274] | 0.00 n.s.    | 0.235 $\pm$ 0.016 [0.186,0.266] |

**Tab. 2:** Distributional data of *Temnothorax luteus* and *T. racovitzai* collapsed to sites sampled. Arrangement of data: arithmetic mean  $\pm$  standard deviation [minimum, maximum]. Significance testing by an univariate ANOVA.

|                      | number of sites | LAT [°]                        | LON [°]                      | ALT [m]                 | TAS [°C]                       |
|----------------------|-----------------|--------------------------------|------------------------------|-------------------------|--------------------------------|
| <i>T. racovitzai</i> | 21              | 42.58 $\pm$ 1.23 [40.25,44.50] | 3.24 $\pm$ 2.00 [-0.30,5.59] | 479 $\pm$ 392 [10,1250] | 18.99 $\pm$ 2.30 [14.84,22.72] |
| ANOVA                |                 | F = 23.95 p < 0.0001           | F = 5.72 p < 0.021           | F = 9.30 p < 0.004      | F = 26.78 p < 0.0001           |
| <i>T. luteus</i>     | 24              | 44.65 $\pm$ 1.56 [40.25,46.58] | 4.92 $\pm$ 2.62 [-0.45,8.80] | 848 $\pm$ 417 [50,1750] | 15.22 $\pm$ 2.56 [9.43,21.26]  |

should abound. Yet, even this reduced data set revealed significant interspecific differences in geographic distribution and thermal niche (Tab. 2). *T. racovitzai* is the more southern and western species which occurs in the sympatric range at lower elevations. Its postglacial expansion most certainly started from an Iberian refuge while the Pleistocene refuge of *T. luteus* most probably was somewhere in the central or southern Apennine. The most striking interspecific difference exists in the thermal component of climatic niche: air temperature from May to August is by 3.77°C cooler in the sampling sites of *T. luteus* than in those of *T. racovitzai*.

Two experienced ant collectors, who were not able to distinguish the cryptic species and having studied the situation in S France, independently expressed the opinion that workers of the *T. luteus* complex grow to a larger size (and develop a darker color) in cooler environments. These impressions are true but they were generated by the

fact that the significantly larger species *T. luteus* (Tab. 1) occurs in significantly cooler environments (Tab. 2). Linear regression analyses within the species showed no significant negative dependence of the body-size indicator CS from environmental temperature TAS:

*T. luteus*:

$$CS = -0.0004 \text{ TAS} + 0.629 \quad (r = -0.0219, \text{ n.s., } n = 38).$$

*T. racovitzai*:

$$CS = -0.0024 \text{ TAS} + 0.631 \quad (r = -0.1897, \text{ n.s., } n = 26).$$

## Acknowledgements

We wish to thank Anne Freitag (MZ Lausanne) and Wouter Dekoninck (IRSNB Bruxelles) for allowing a loan of type specimens from the Forel and Bondroit collections.

## References

- CASEVITZ-WEULERSSE, J. & GALKOWSKI, C. 2009: Liste actualisée des Fourmis de France (Hymenoptera, Formicidae). – Bulletin de la Société entomologique de France **114** (4): 475-510.
- CSÖSZ, S.; SEIFERT, B.; MÜLLER, B.; TRINDL, A.; SCHULZ, A. & HEINZE, J. 2013: Cryptic diversity in the Mediterranean *Temnothorax lichtensteini* species complex (Hymenoptera: Formicidae). – Organisms, Diversity & Evolution **14**: 75-88. DOI: 10.1007/s13127-013-0153-3.
- EMERY, C. 1924: Formiche di Spagna, raccolte dal Prof. Filippo Silvestri. – Bolletino del Laboratorio di Zoologia generale e agraria della R. Scuola superiore d' Agricoltura in Portici **17**: 164-171.
- Klimaabteilung der Zentralanstalt für Meteorologie und Geodynamik 1996: Klimadaten der Welt auf CD-ROM. Version 1.0. Zentralanstalt für Meteorologie und Geodynamik, Vienna. 1 CD-ROM.
- PLATEAUX, L. & CAGNIANT, H. 2012: Quelques synonymies dans le genre *Temnothorax* MAYR, 1855 (Hymenoptera, Formicidae). – Bulletin de la Société entomologique de France **117** (4): 427-440.
- SEIFERT, B. 2002: How to distinguish most similar insect species - improving the stereomicroscopic and mathematical evaluation of external characters by example of ants. – The Journal of Applied Entomology **126**: 445-454.
- SEIFERT, B. 2006: *Temnothorax saxonicus* (SEIFERT, 1995) stat. nov., comb. nov. - a parapatric, closely-related species of *T. sordidulus* (MÜLLER, 1923) comb. nov., stat. nov. and description of two new closely-related species, *T. schoedli* sp. n. and *T. artvinense* sp. n. from Turkey (Hymenoptera: Formicidae). – Myrmecological News **8**: 1-12.
- SEIFERT, B. 2008: Removal of allometric variance improves species separation in multi-character discriminant functions when species are strongly allometric and exposes diagnostic characters. – Myrmecological News **11**: 91-105.
- SEIFERT, B. 2009: Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach. – Myrmecological News **12**: 149-166.
- SEIFERT, B. & PANIER, L. 2007: A method for standardized description of soil temperatures in terrestrial ecosystems. – Abhandlungen und Berichte des Naturkundemuseums Görlitz **78**: 151-182.
- SEIFERT, B.; RITZ, M. & CSÖSZ, S. 2013: Application of Exploratory Data Analyses opens a new perspective in morphology-based alpha-taxonomy of eusocial organisms. – Myrmecological News **19**: 1-15.

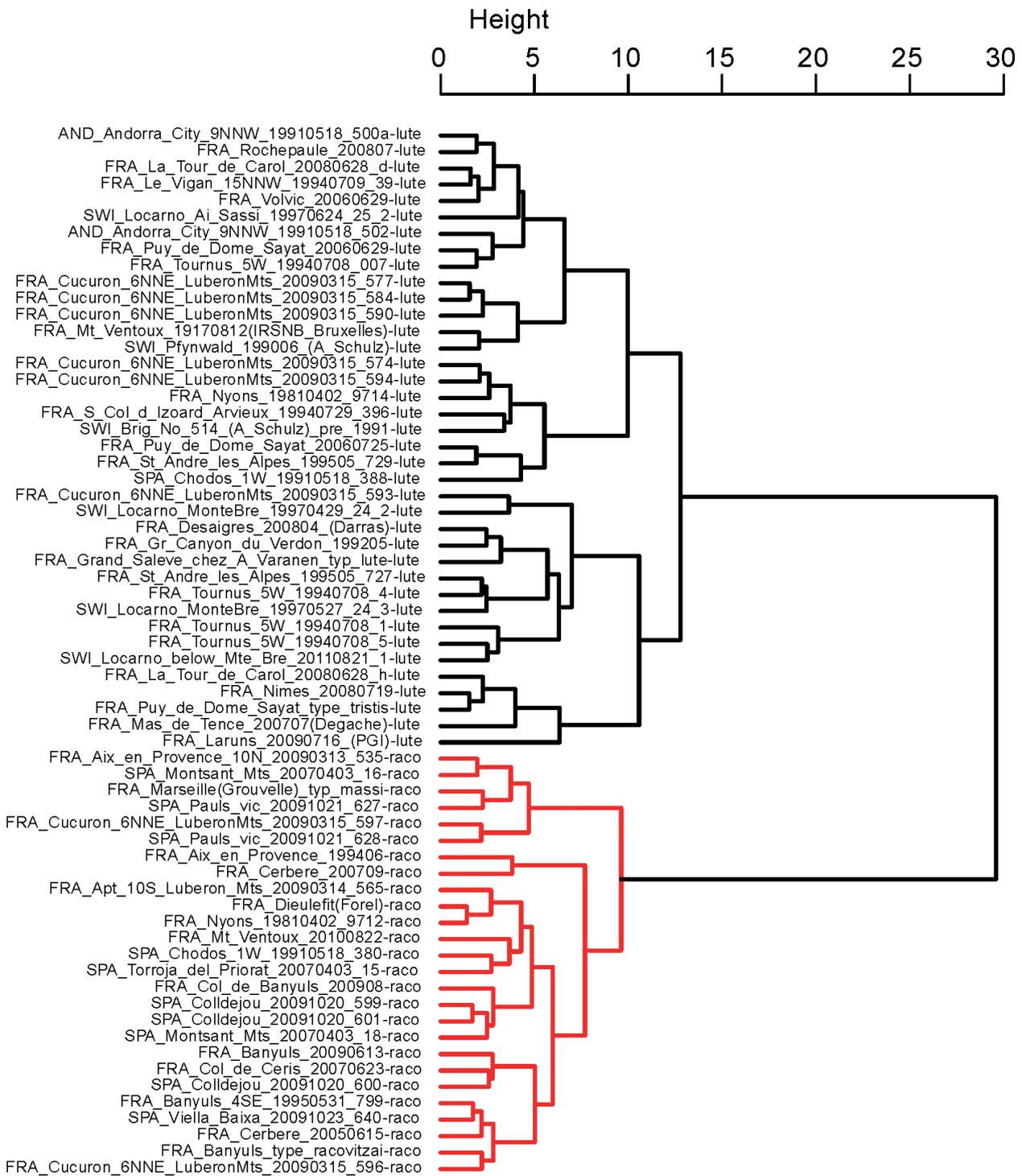


Fig. 2: NC-Ward clustering of 64 worker nest samples of *Temnothorax luteus* (FOREL, 1874) and *T. racovitza* (BONDROIT, 1918) considering 7 selected morphometric characters.



Fig. 3: Head of *Temnothorax luteus* (FOREL, 1874); shown is the type of the junior synonym *Temnothorax tristis* (BONDROIT, 1918). Fig. 5: Head of lectotype of *Temnothorax racovitzai* (BONDROIT, 1918).



Fig. 4: *Temnothorax luteus* (FOREL, 1874) in lateral view; shown is the type of the junior synonym *Temnothorax tristis* (BONDROIT, 1918).



Fig. 6: Lectotype of *Temnothorax racovitzai* (BONDROIT, 1918) in lateral view.