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Four species within the supercolonial ants of the *Tapinoma nigerrimum* complex revealed by integrative taxonomy (Hymenoptera: Formicidae)*

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Abstract

The West and Central Mediterranean ants known for 50 years under the name *Tapinoma nigerrimum* (NYLANDER, 1856) have attracted attention because of their efficient chemical weapons, impressive supercolonies and potential to limit the spreading of the Argentine Ant *Linepithema humile* (MAYR, 1868). The paper shows that the *T. nigerrimum* complex consists of at least four clearly separable species which differ in morphology of all castes, colony demography, geographic distribution, invasive potential and mtDNA data. Species delimitation by means of Nest Centroid Clustering, considering 20 quantitative phenetic characters in 159 nest samples, resolved four coincident clusters in both female and male castes which are classified as *T. nigerrimum*, *T. magnum* MAYR, 1861, *T. ibericum* SANTSCHI, 1925, and *T. darioi* sp.n. The exploratory data analyses NC-Ward clustering and NC-k-means clustering showed a mean disagreement from the final species hypothesis between 0 and 2.7% in workers on the nest sample level, whereas the classification error of a linear discriminant analysis was 4.2% in 533 worker individuals. The four phenetic clusters were basically confirmed by analysis of the COI segment of mtDNA with the smallest mean K2p genetic distance of 1.8% observed in *T. darioi* sp.n. against *T. magnum*, and the largest one of 4.0% in *T. nigerrimum* against *T. ibericum*. These data suggest a species divergence between late Pliocene and early Pleistocene (3.3 - 1.5 Ma). The mtDNA haplotypes of nine phenotypically ideal *T. darioi* sp.n. supercolonies, found at three sites in southern France, and Italy were placed within the *T. magnum* cluster. Among four alternative scenarios discussed for these mismatches, hybridization events in the younger evolutionary history with subsequent unidirectional genomic purging of nuDNA was proposed to be the most likely explanation. *Tapinoma nigerrimum* is monodomous to moderately polydomous with aggression between neighbouring colonies, whereas *T. magnum*, *T. ibericum*, and *T. darioi* sp.n. are supercolonial with a potential to become invasive pest ants through introduction by human commerce. For Europe north of 48° N, *T. magnum* could establish populations in nine cities in Germany, Belgium, and the Netherlands, whereas *T. ibericum* is known so far from one site in South England only, and *T. darioi* sp.n. from one city in the Netherlands. The differential zoogeography and biology of the four species and ways of species delimitation are outlined and discussed. *Tapinoma darioi* sp.n. is described as new.

Key words: Nest Centroid Clustering, cryptic species, invasive species, interspecific hybridization, new species, unidirectional genomic purging.

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Introduction

The ants collected throughout the last 50 years under the name *Tapinoma nigerrimum* (NYLANDER, 1856) have repeatedly attracted the interest of myrmecologists. Their fighting and interference behaviour repeatedly stood in the focus of interest. The defensive secretion produced by the anal gland is highly poisonous to probably all ant species, is a mixture of ketones and a dialdehyde and polymerizes if sprayed on the cuticular surface of an enemy which prevents the evaporation of the toxic ketones (HABERMEHL 1976). These dominant, frequently supercolonial ants are distributed in the West and Central Mediterranean parts of Europe and Africa but seem to be absent for unknown reasons from the Balkans and Asia Minor. They are particularly abundant in open unstable or degraded areas with significant to very strong anthropogenic influence and a weakly developed tree layer, are typical for coastal areas and are frequent in city centers. As a rather recent development, these ants became strongly invasive in urban areas

* This study would have been part of the PhD thesis of Dario D'Eustacchio who died on 14 October 2014 in a terrible car accident. This paper is dedicated to his memory.

north of 48° N to which they were introduced with plant material. They developed supercolonies in the introduction area and are on the way to become a significant pest species rivaling *Lasius neglectus* VAN LOON, BOOMSMA & ANDRASFALVY, 1990. The identification method introduced in this paper showed that all three cryptic species with a supercolonial demography basically have an invasive potential but nine of the eleven checked introductions in Germany, Belgium, England, and the Netherlands have to be referred to a single species. At least two of the invasive species may forage at ground temperatures of 3 - 7 °C and at least one species shows a high frost resistance. Ants of the *T. nigerrimum* complex are known from Corsica and southern France to limit the spread of the invasive Argentine ant, *Linepithema humile* (MAYR, 1868). In space and food competition assays of BLIGHT & al. (2010) ants of this species complex appeared to be more efficient than *Linepithema* in both interference and exploitative competition, clearly superior in direct fighting, dominated food in 100% of the replicates after one hour, and invaded *Linepithema* nests while the reverse was never observed.

This multitude of interesting biological properties, which are also relevant for humans from an economic point of view, generates a high interest for an in-depth taxonomic study of these ants. This paper aims to show that the ants collected under the name *Tapinoma nigerrimum* consist of at least four clearly separable species which differ in morphology of all castes, colony demography, geographic distribution, invasive potential and mtDNA data. These discoveries became possible after the recent development of high-resolution methods of Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) in which Nest Centroid Clustering (SEIFERT & al. 2014) plays a central role.

The extreme difficulty of phenotypical species delimitation made it sensorially and mentally impossible to form any prejudice on possibly existing cryptic species because even experienced and skilled morphology-based taxonomists apparently can not discriminate them by simple eye-inspection. The idea for the existence of more than a single species was developed during running routines of hierarchical exploratory data analyses of multiple morphological characters in the large material of more than 250 *Tapinoma nigerrimum* complex samples collected by Dario D'Eustacchio in the West Mediterranean. In order to elucidate this point, an integrative study has been carried out considering NUMOBAT data, zoogeography, and mtDNA as a genetic marker.

Material

A detailed account of the samples is given below in the sequence site, date in the format yyyy.mm.dd, sample number [latitude in decimal format, longitude in decimal format, altitude in meters]. Missing data in the date string are given by "xx".

***Tapinoma nigerrimum* (NYLANDER, 1856):** A total of 19 nest samples with 69 workers, 5 gynes, and 23 males originating from France (15 samples) and Spain (4 samples). France: Armissan - 1.5 km SSE, 2015.05.08, No 3, No 4, No 5 [43.177, 3.104, 121]; Bouches du Rhone: Charleval, 1987.07.11 [43.715, 5.245, 162]; Gigean - 3.3 km ESE, 2012.04.29, No T1S4, No T2S4 [43.489, 3.748, 210]; Prades-le-Lez - 1.5 km SE, 2012.04.xx, No K, No K-GP, No K-PN [43.684, 3.876, 88]; St. Mathieu de Treviers-N, 2014.09.15, No 25F [43.773, 3.869, 106]; St. Mathieu de Treviers-S, 2014.09.15, No 25G [43.773, 3.869, 106]; Uzes-North, 2014.09.14, No 25H [44.029, 4.416, 119]; Uzes-South, 2014.09.14, No 25D [44.028, 4.416, 119]; Villen. les-Maguelonne, 2012.04.29, No T1S2, No T2S2 [43.515, 3.834, 1]. Spain: Almaden, Sierra Magina, 1994.xx.xx, No AM1 [37.772, - 3.562, 1097]; Cruce de la Fuensanta,1996, No CF15 [37.652, - 3.829, 1206]; Torres - 1.8 km SSE, 2014.10.31, No SM1 [37.772, - 3.497, 1031]; Torres - 2.2 km SSE, 2014.10.31, No SM2 [37.768, -3.496, 1016].

***Tapinoma magnum* MAYR, 1861:** A total of 79 nest samples plus the type male and gyne with 252 workers, 25 gynes and 31 males originating from the following countries: Algeria (7 samples), Belgium (1), France (21), Germany (8), Italy (32), Morocco (7), the Netherlands (1), Spain (1), and Tunisia (1). Algeria: Ain Taya, 201211, No 7 [36.790, 3.290, 27]; Alger, El Harrach, ESNA, 2012.xx.xx, No 2 [36.700, 3.140, 8]; Alger, El Harrach, ESNA, 2014.11.25 [36.700, 3.140, 8]; Alger, Zeralda, 2012.09.10, No 1, No 2 [36.710, 2.850, 43]; Blida, Citrus orchard, 2012.12.14, No4 [36.480, 2.830, 230]; Qued Isser, 1988.05.21 [36.84, 3.67, 5] . Belgium: Oostende, 2015.03.25 [51.209, 2.909, 3]. France: Bordeaux, 2008.03.08, No 10 [44.783, -0.517, 4]; Corse: Ajaccio (coll. Santschi), pre 1925.xx.xx [41.910, 8.740, 16]; Corse: Monacia de Auliene, 2012.xx.xx, No 2 [41.513, 9.011, 104]; Corse: Olmeto, 2009.07.19, No 16 [41.700, 8.837, 2]; Corse: Solenzara, 2009.07.23, No 14 [41.850, 9.400, 2]; Corse: St. Florent, 2012.07.19, No 14 [42.674, 9.000, 1]; Frejus-Plage, city, 2014.10.09, No TFra5 [43.418, 6.745, 1]; Gruissan, 2014.09.17, No 25I [43.097, 3.112, 2]; Le Grau du Roi, 2014.09.19, No 38L, No 38O [43.546, 4.124, 3]; Le Muy - 2 km W, 2014.10.09, No TFra4 [43.466, 6.544, 41]; Lyon, Villeurbanne, 2011.xx.xx, No LM224 [45.749, 4.877, 181]; Lyon, city, 2011.xx.xx, No LM220 [45.726, 4.828, 167]; Marseillan, 2014.09.18, No 7F [43.311, 3.548, 3]; Meyzieu - 2.6 km ESE, 2014.07.18, No DUMP [45.760, 5.035, 208]; Meyzieu - 2.9 km ESE, 2014.07.18, No field [45.758, 5.039, 209]; Nizza, city, 2014.10.08, No TFra2 [43.695, 7.267, 7]; Port Camargue, 2014.09.19, No 38N [43.514, 4.124, 2]; Saintes-Marie, city, 2013.08.13, No TFra1 [43.453, 4.634, 0]; Saintes Maries de la Mer, 2014.09.19, No 38R [43.450, 4.413, 2]; Sete, 2014.09.18, No 38E [43.367, 3.617, 1]. Germany: Edesheim, 2009.06.xx [49.263, 8.135, 153]; Ginsheim-Gustavsburg, 2012.11.01 [49.967, 8.354, 88]; Hanhofen, 2011.08.31 [49.316, 8.338, 106]; Ingelheim, 2009.06/09.xx [49.967, 8.050, 122]; Ingelheim, 2009.10.xx, No K2 [49.967, 8.050, 122]; Neustadt, 2009.10.28, No 1, No 3, No 6 [49.350, 8.150, 136]; Speyerdorf 2016.04.21 [49.338, 8.195, 123]; Weinheim, 2016.xx.xx [49.548, 8.632, 100]. Italy: Brindisi - 3 km NW, 2013.08.10, No TPu2 [40.652, 17.909, 15]; Capo Comino - 2 km E, 2014.04.14; No TSa2 [40.535, 9.817, 2]; Casoli di Atri, 2013.08.19, No TAb8 [42.619, 13.984, 152]; Castelporziano, 2013.05.14, No TLa11 [41.672, 12.399, 15]; Castelporziano, 2014.05.16, No TLa47 [41.752, 12.430, 56]; Cosenza: Cratital, 1936.05.21 [39.30, 16.20, 210]; Eraclea Minoa - 1 km S, 2013.04.01, No TSi17 [37.390, 13.293, 17]; Genzano di Lucania - 1 km S, 2013.04.06, No TBa1 [40.837, 16.036, 560]; Grava - 1 km SE, 2013.03.28, No TSi8 [37.883, 15.157, 400]; Lerici-city, 2014.10.06, No TLi1 [44.082, 9.906, 6]; Letojanni - 2 km NE, 2013.03.27, No TSi1 [37.891, 15.321,

41]; Lipari - 1 km N, 2014.06.12, No TEo1 [38.472, 14.954, 3]; Marina di Ascea city, 2013.10.22, No TCa5 [40.158, 15.157, 7]; Marina di Lesina, 2013.09.02, No TPu6 [41.916, 15.334, 2]; Marina di Tortora - 1 km N, 2013.08.13, No TBa3 [39.925, 15.756, 16]; Marzanemi - 3 km S, 2013.04.02, No TSi26 [38.710, 15.120, 46]; Mercatale, city, 2014.04.18 No TMa6 [43.783, 12.484, 245]; Motta Camastra - 1 km S, 2013.03.28, No TSi4 [37.881, 15.173, 25]; Ogliastro Marina, city, 2013.10.22, No TCa3 [40.230, 14.936, 14]; Pisa - 6 km E, 2013.07.30, No TTo7 [43.682, 10.448, 7]; Pisa, type *Tapinoma magnum* (leg. Savi), pre 1861.xx.xx [43.75, 10.40, 7]; Potenza, Basilicata, 1936.05.12 [40.667, 15.917, 953]; Potenza, 2014.07.15, [40.654, 15.809, 600]; Punta Grande - 1 km W, 2013.04.01, No TSi20 [37.289, 13.479, 32]; Roma city, 2013.04.30, No TLa4 [41.905, 12.600, 32]; Roma, city, 2013.05.09, No TLa1b [41.907, 12.516, 45]; Roma: Meda park, 2013.06.04, No TLa17 [41.913, 12.546, 90]; Roscigno - 1 km N, 2013.10.21, No TCa1 [40.409, 15.342, 592]; San Basilio - 1 km E, 2013.03.29, No TSi11 [38.012, 14.791, 818]; San Paolo - 5 km E, 2013.04.03, No TSi30 [36.822, 15.084, 34]; Scerne - 1 km SE, 2013.05.25, No TAb3 [42.641, 14.046, 1]; Varezze-city, 20141008, No TLi7 [44.357, 8.571, 10]; Viareggio - 6 km S, 2013.07.29, No TTo6 [43.814, 10.260, 1]. Morocco: Essaouira city, 2014.03.05, No TMor4 [31.512, -9.768, 7]; Essaouira city, 2014.03.05, No TMor5 [31.511, -9.771, 4]; Hamrawa - 4 km NE, 2014.03.09, No TMor19 [34.007, -5.557, 398]; Hamrawa - 4 km NE, 2014.03.09, No TMor20 [34.007, -5.556, 398]; Meknes - 2 km S, 2014.03.09, No TMor18 [33.910, -5.556, 467]; Torres-de-Alcala, 2014.03.08, No TMor14 [35.157, -4.327, 4]; Torres-de-Alcala, 2014.03.08, No TMor15 [35.158, -4.326, 4]. Netherlands: Ulft, 2016.06.17, [51.887, 6.383, 18]. Spain: Donana National Park, 2014.03.05, No 1 [36.981, -6.484, 7]. Tunesia: Cheri-Cheri, 1930.08.16 [35.640, 9.810, 235].

***Tapinoma* cf. *magnum*:** One nest sample of six workers with conflicts between NUMOBAT, mtDNA and geographic distribution patterns from Italy: Roma, Cerveletta park, 2013.06.13, No TLa24 [41.917, 12.585, 40].

***Tapinoma ibericum* SANTSCHI, 1925:** A total of 23 nest samples plus the type male with 83 workers, 4 gynes, and 18 males originating from England (1 sample), Portugal (2 samples), and Spain (20 samples). England: Isle of Wight: Ventnor Botanic Garden, 2016.09.07 [50.5891, -1.2293, 26]. Portugal: Burgau - 0.8 km E, 2016.02.10, No 2 [37.073, -8.766, 47]; Montinhos da Luz - 1.5 km SSW, 2016.02.10, No 5 [37.075, -8.756, 46]. Spain: Almagro - 2.3 km SSE, 2014.04.24, No PCA7 [38.871, -3.697, 655]; Almagro - 5.2 km W, 2014.04.24, No PCA6 [38.896, -3.768, 676]; Cabo de Gata - 1 km WNW, 1995.xx.xx, No CG20, No CG24 [36.756, -2.121, 27]; El Portichuelo, 2013.10.20, No CV - 12, No CV - 13 [37.727, -3.803, 745]; Jaen, University Campus, 2015.05.xx [37.787, -3.775, 430]; La Lambra, Rus, 2004.xx.xx, No AH9 [38.072, -3.488, 435]; Los Villares - 0.8 km NE, 2013.09.xx, No CV - 6 [37.692, -3.812, 625]; Los Villares - 1.1 km NE, 2013.10.20, No CV - 11 [37.698, -3.811, 585]; Madrid, Bolivard (coll. Santschi), pre 1925.xx.xx [40.400, -3.700, 650]; Moral de Calatrava 2.4 km SE, 2014.04.24, No PCA8 [38.818, -3.556, 630]; Navaconcejo - 2.9 km SW, 2015.08.13 [40.161, -5.856, 434]; Pozuelo de Calatrava, type *Tapinoma ibericum* (coll. Santschi), pre 1925.xx.xx [38.91, -3.84, 630]; Pozuelo de Calatrava - 1.6 km SSW, 2014.04.24, No PCA5 [38.898, -3.844, 636]; Pozuelo de Calatrava - 0.6 km N, 2014.04.24, No PCA2 [38.917, -3.838, 622]; Pozuelo de Calatrava - 1 km ESE, 2014.04.24, No PCA1 [38.909, -3.826, 627]; Pozuelo de Calatrava - 1 km S, 2014.04.24, No PCA4 [38.903, -3.839, 630].

***Tapinoma darioi* sp.n.:** A total of 40 nest samples with 141 workers, 24 gynes, and 29 males originatig from the following countries: France (19 samples), Italy (13), the Netherlands (1), and Spain (7). France: Argeles, 2014.09.17, No 7M [42.550, 3.049, 2]; Canet Plage, 2014.09.17, No 7O [42.680, 3.034, 1]; Chalon Sur Saone, 2013.07.27 [46.775, 4.857, 175]; Corse: Bonifacio - 5.5 km E, 2012.xx.xx, No 1 [41.371, 9.222, 4]; La Grande Motte, 2012.04.30, No GM [43.560, 4.084, 8]; La Grande Motte, 2015.05.07 [43.561, 4.084, 8]; La Grande Motte, 20140918, No 38B, No 38D [43.557, 4.030, 4]; Le Grau d'Agde-East, 2014.09.18, No 7R [43.282, 3.454, 2]; Le Grau d'Agde-West, 2014.09.18, No 7G [43.282, 3.456, 2]; Port Leucate-East, 2014.09.17, No 7P [42.862, 3.047, 3]; Port Leucate-Mid, 2014.09.17, No 7J [42.862, 3.046, 2]; Port Leucate-West, 2014.09.17, No 7E [42.860, 3.046, 1]; Port Leucate, 2014.09.17, No 7H [42.863, 3.046, 2]; Port la Nouvelle, 2014.09.17, No 7N [43.007, 3.057, 2]; St. Pierre la Mer-E, 2014.09.16, No 7A [43.179, 3.193, 3]; St. Pierre la Mer-W, 2014.09.16, No 25M [43.177, 3.192, 7]; Valras Plage, 2015.05.08 [43.241, 3.283, 3]; Valras Plage, 2014.09.18, No 7I [43.242, 3.285, 1]. Italy: Castelporziano, 2013.06.14, No TLa27 [41.698, 12.352, 13]; Castelporziano, 2013.06.18, No TLa29 [41.698, 12.354, 7]; Castelporziano, 2013.06.19, No TLa31 [41.694, 12.357, 0]; Castelporziano, 2013.06.28, No TLa36 [41.684, 12.376, 9]; Castelporziano, 2014.04.23, No TLa45 [41.699, 12.350, 1]; Drignana - 1 km NE, 2014.10.06, No TLi3 [44.149, 9.685, 489]; Marina di Alberese - 1 km NW, 2013.07.27, No TTo2 [42.653, 11.024, 5]; Marina di Alberese - 2 km SE, 2013.07.28, No TTo3 [42.642, 11.054, 8]; Marina di Alberese - 3 km SE, 2014.10.09, No TTo10 [42.641, 11.060, 2]; Marina di Alberese - 5 km SE, 2013.07.28, No TTo4 [42.630, 11.082, 1]; Marina di San Nicola, 2013.07.13, No TLa38 [41.920, 12.136, 1]; Orbetello - 3 km SE, 2013.07.26, No TTo1 [42.417, 11.235, 1]; Principina - 1 km S, 2013.07.29, No TTo5 [42.683, 11.001, 8]. Netherlands: Wageningen, 2016.10.06 [51.9784, 5.6701, 12]. Spain: Bellaterra, University Campus, 2015.11.13 [41.501, 2.110, 128]; Castellon (coll. Dusmet), pre 1925.xx.xx [39.980, -0.040, 38]; Mallorca: San Torella, pre 1975.xx.xx (leg. Collingwood) [39.80, 2.70, 250]; Menorca: Cala Porter, 1976.04.19 [39.873, 4.136, 37]; Platja de Garbet, 2003.09.30, No 16160 [42.392, 3.152, 7]; Talamanca - 1.1 km N, 2015.09.28 [41.746, 1.972, 417]; Val de Ramio, 2000.07.11 [41.714, 2.622, 232].

***Tapinoma erraticum* (LATREILLE, 1798):** Two samples with six workers were used as outgroups in the phylogenetic tree. Italy: Percile - 2 km E, 2013.06.23, No TLa34 [41.090, 12.938, 799]; Pineto - 3 km SE, 2013.05.26, No TAb4 [42.586, 14.088, 4].

***Tapinoma madeirense* FOREL, 1895:** Two samples with six workers were used as outgroups in the phylogenetic tree. Italy: Fonni - 9 km SE, 2014.04. 15, No TSa10 [40.082, 9.349, 1184]. Spain: Sierra de Huetor, 2004.04.19, No FT3 [37.258, - 3.490, 1280].

***Tapinoma subboreale* SEIFERT, 2012:** Two samples with six workers were used as outgroups in the phylogenetic tree. Italy: Cesacastina - 2 km W, 2013.06.16, No

TAB6 [42.588, 13.424, 1460]; Civita - 7 km E, 2013.08. 24, No TLa37 [41.774, 12.490, 1146].

***Tapinoma simrothi* KRAUSSE, 1911:** Three samples with nine workers were used as outgroups in the phylogenetic tree. Morocco: Aghorizme - 3 km S, 2014. 03.04, No TMor2, TMor3 [30.058, -9.656, 10]. Spain: Jaraicejo - 3 km E, 2013.04.05 [39.672, -5.781, 566].

Type material

***Tapinoma nigerrimum* (NYLANDER, 1856):** Neotype, major worker, top specimen on a pin with four workers, labelled "FRA: 43.6843°N, 3.8763°E, Prades-le-Lez - 1.5 SE, 88 m, monodomous colony, Kaufmann 2012.04.30 – K" and "Neotype (top specimen) *Tapinoma nigerrimum* (NYLANDER, 1856), des. B. Seifert 2016". The neotype nest sample totals 18 males, 2 gynes, and 24 workers. All material is stored in SMN Görlitz. Comment: As types are not present in the Nylander collection in Helsinki, a neotype was fixed. The descriptive statements in the original description allow to conclude on a *Tapinoma nigerrimum* complex species but not which of the four cryptic species Nylander could have seen. Yet, the type locality "Locus aridis prope Monspelium" (Dry locality near Montpellier) gives an indication. Accordingly, we fixed a neotype in the species accounting for any of the nine *Tapinoma nigerrimum* complex colonies found within a radius of less than 20 km around Montpellier under exclusion of the immediate shore line.

***Tapinoma magnum* MAYR, 1861:** Lectotype male labelled "Savi Pisa.", "v. nigerrim. Magnum det. Mayr" [both labels in Mayr's handwriting], "Lectotype *Tapinoma magnum* (Mayr, 1861) des. B. Seifert 2012" and "AntWeb CASENT 0915547". One paralectotype gyne on another pin labelled "Savi Pisa.", "v. nigerrim. Magnum det. Mayr" [both labels in Mayr's handwriting] and "Paralectotype *Tapinoma magnum* (MAYR, 1861) des. B. Seifert 2012". Both specimens stored in NHM Wien.

***Tapinoma ibericum* SANTSCHI, 1925:** Type male labelled "Type", "Tapinoma nigerrimum Nyl. v ibericum Sants. type" [Santschi's handwriting], "POZUELO La Fuente" and "ANTWEB CASENT 0911578", NHM Basel. The type was remounted and the genital prepared in an in situ position.

***Tapinoma darioi* sp.n.:** Holotype, a major worker on the same pin with three paratype workers, labelled "ITA: 41.69858°N, 12.34985°E, Roma, Castelporziano, 1 m, Grotta di Piastra, dune, D'Eustacchio 20140423-TLa45", "Holotype (top specimen) and paratypes *Tapinoma darioi* SEIFERT & al."; two paratype gynes and two paratype males on other pins with the same collecting data. Another two pins with 8 paratype workers from a nest situated 240 m east, labelled "ITA: 41.69823°N, 12.35240°E, Roma, Castelporziano, 13 m, Tor Paterno parcel, dune, under shrub, D'Eustacchio 20130614-TLa27". All material stored in SMN Görlitz.

Methods

NUMOBAT: Equipment and measurement procedures

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 - 360 ×. The mean relative measuring error over all magnifications was 0.2%. 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. In order to achieve a sharp visualization of contour lines of cuticle, transmitted-light conditions were generated by directing the light stream of a light-cable on a white reflector plane at the base of the pin-holding stage. 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.

NUMOBAT: The morphometric characters

15 NUMOBAT characters were investigated in workers (CL, CW, dAN, EL, ExCly, ExClyW, ExOcc, Fu2, IFu2, MpGr, ML, MW, nExCly, PoOc, and SL), 13 in males (ALPH, CL, CW, dAN, EL, ExBasi, ExCly, ExClyW, Fu2, IFu2, ML, WSPL, and SL), and 14 in gynes (CL, CW, dAN, EL, ExCly, ExClyW, ExOcc, Fu2, IFu2, ML, MW, nExCly, PoOc, and SL). 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. The reproducibility of NUMOBAT data recording in general is strongly dependent from carefully considering the character definitions. In very small structures (such as FU2) the resolution of the microscope and illumination of the object are also important.

ALPH The angle formed by the sides of the caudal lobes of the subgenital plates as a function of the bilateral mean of WSPL and a constant distance measure (see below, Fig. 1). ALPH = arcus tan (WSPL / 200 µm).

CL Maximum cephalic length measured between points A and B; A is the posteromedian margin point of head capsule; B is an imagined median point situated at the same transversal level as the most anterior points of clypeus left and right of clypeal excision. Bilateral asymmetries are averaged.

CS Cephalic size; the arithmetic mean of CL and CW.

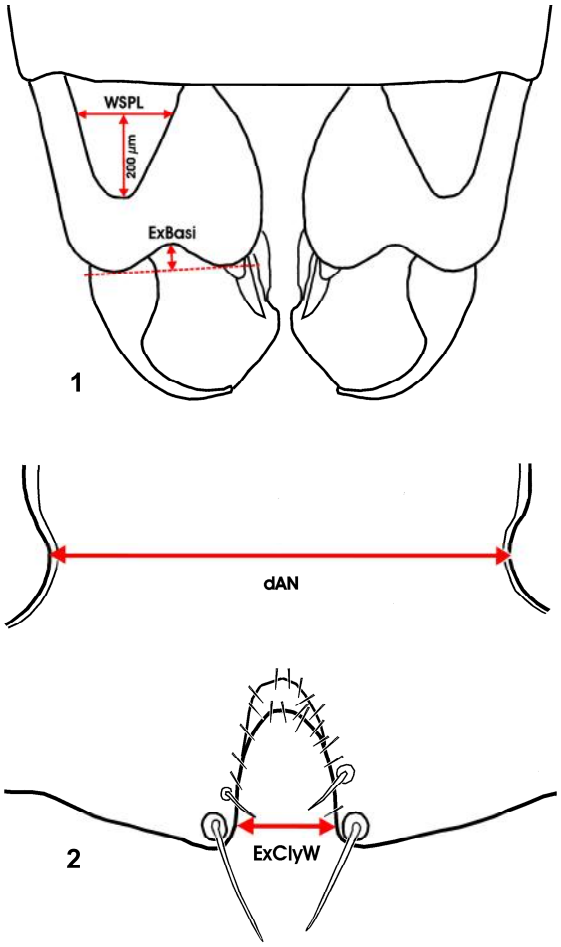
CW Maximum cephalic width.

dAN Minimum distance of the inner (centripetal) margins of antennal socket rings which is best measurable in dorsofrontal view (Fig. 2).

EL Eye length; maximum diameter of the compound eye over all structurally defined ommatidiae; bilateral mean.

ExBasi Depth of the excavation of distal basimere margin in ventral view. Adjust median basimere margin in visual plane (Fig. 1).

ExCly Maximum depth of anteromedian clypeal excision as it appears in frontodorsal view and with median



Figs. 1 - 2: (1) Male genital of a *Tapinoma nigerrimum* complex species in ventral view. (2) Measuring of clypeal excision width ExClyW and of minimum distance of the inner (centripetal) margins of antennal socket rings dAN.

line of head positioned perpendicular in the visual field; bilateral asymmetries are averaged (Fig. 2).

ExClyW Width of clypeal excision at the level of the base centres of the two most apical and largest setae (Fig. 2).

ExOcc Depth of excavation of posterior vertex. Procedure: Focus both posterior corners of vertex until they form a sharp contour, adjust them to equal horizontal level within the visual field and superimpose the corners with the horizontal line of the cross-scale. Change the focal level upwards until the median part of posterior vertex forms a sharp contour. Read the depth.

Fu2 Median length of second funiculus segment in dorsal view. (Dorsal view is given when the swiveling plane of 1st funiculus segment is positioned in the visual plane). Take care to really measure median length (the segment's sides have unequal length!) and to recognize the real distal margin of the segment. The latter has a very thin cuticle, frequently producing a narrow, shining ribbon that seems to be, by optical impression, demarcated from the rest of the segment. The median line of the segment is visualized by centre of the patch reflecting the coaxial light.

Fu2W Median width of second funiculus segment in dorsal view. Use of transmitted-light is important to visualize the real cuticular surface.

IFu2 Index Fu2 / Fu2W.

ML Mesosoma length from the caudalmost point of lateral metapleuron to rear margin of anterior pronotal fringe (in workers) or to the anteriormost point of anterior mesosomal face (in males and gynes). In workers, if anterior measuring point is concealed, keep the orientation of measuring line, choose a higher magnification, measure from the caudalmost point of lateral metapleuron to the level of promesonotal margin and multiply by 1.427 in the *Tapinoma nigerrimum* species complex (1.397 in the *T. erraticum* species complex, 1.422 in the *T. madeirense* species complex).

MPGr Depth of metanotal groove / depression in lateral view; the upper reference line extends between the highest points of mesonotum and propodeum perpendicular to which depth measuring is performed.

MW Maximum pronotal width.

nExCly Bilateral sum of pubescence hairs and smaller setae protruding at a few micron across margin of clypeal excision. The two large anteriormost setae are not counted (Fig. 2). If there is, at the bottom of the excision, a more dorsal, suggested margin (thinner line in Fig. 2) in addition to the more ventral main margin (thicker line), hairs protruding across the dorsal margin are included. This dorsad extension of excision is typically seen in *T. nigerrimum*, *T. magnum*, and *T. ibericum*. The correct count in the example of Figure 2 is 21.

PoOc Postocular distance: distance from the transversal level of posterior eye margin to hind margin of head measured in median line; bilateral asymmetries are averaged.

SL Scape length excluding articulatory condyle.

WSPl Width of the lobes of subgenital plate 200 µm below their tips (Fig. 1). Average the width of both lobes and try to visualize the real cuticular margin (which is often badly visible due to dense pubescence). Adjustment: median line of subgenital plate horizontal in visual field, tips of the lobes at equal perpendicular level and ventral surface of subgenital plate in visual plane.

Terminology of male genitalia

We follow the terminology used by YOSHIMURA & FISHER (2011) with the exception of retaining the name "subgenital plate" for the terminal abdominal sternum. The paramere usually consists of a basal part named here basimere (= squamula in the terminology of KUTTER 1977) and a distal part named here harpago (= stipes in the terminology of KUTTER 1977). The parameres are not separated in all ant groups in a basal and distal part. The digitus is the hook of the volsella (usually the most distal part) while the cuspis is another distal part of volsella (usually subdistal). The aedeagus is the most median genital segment and is named by other authors sagitta or penis valve.

NUMOBAT: removal of allometric variance

Many shape characters in *Tapinoma* are significantly influenced by allometric growth (SEIFERT 2012). This effect is

particularly strong in workers of the *T. nigerrimum* complex where cephalic width of the largest majors amounts 225% of the value in the smallest minors. 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). Since the evaluation of scatter plots showed differing parameters of allometric functions in minor and major workers, RAV was performed by a biphasic linear function with a breaking point at CS = 950 µm. RAV was calculated assuming all individuals to have an cephalic size of CS = 900 µm. RAV functions were calculated as the arithmetic mean of the species-specific functions of 15 Westpalaearctic *Tapinoma* species with currently 1300 evaluated workers. The RAV functions of 13 shape and one seta characters are given in the following. The variables ExCly / CS_{900}, ExClyW / CS_{900}, and $MpGr_{900}$ / CS are given in per cent.

For specimens with CS ≤ 950 µm:

CL / CW_{900} = CL/CW / (- 0.4590 * CS + 1.4691) * 1.0560
SL / CS_{900} = SL/CS / (- 0.2101 * CS + 1.1557) * 0.9666
$ExOcc / CS_{900}$ = ExOcc/CS / (3.796 * CS - 1.5861) * 1.830
$ExCly / CS_{900}$ = 100 * ExCly/CS / (2.7074 * CS + 6.869) * 9.305
$ExClyW / CS_{900}$ = 100 * ExClyW/CS / (-1.337 * CS + 7.645) * 6.442
$ExCly / ExClyW_{900}$ = ExCly/ExClyW / (0.7157 * CS + 1.1608) * 1.8049
$nExCly_{900}$ = nExCly / (15.07 * CS - 1.828) * 11.74
EL / CS_{900} = EL/CS / (-0.0647 * CS + 0.3112) * 0.2530
MW / CS_{900} = MW/CS / (0.0652 * CS + 0.5816) * 0.6403
ML / CS_{900} = ML/CS / (-0.0387 * CS + 1.3250) * 1.2902
dAN / CS_{900} = dAN/CS / (-0.0042 * CS + 0.2950) * 0.2988
$MpGr / CS_{900}$ = 100 * MpGr/CS / (3.581 * CS + 0.292) * 3.515
$Fu2 / CS_{900}$ = Fu2/CS / (-0.00513 * CS + 0.14518) * 0.14056
$IFu2_{900}$ = IFu2 / (0.5748 * CS + 1.2905) * 1.8078
$PoOc / CL_{900}$ = PoOc/CL / (-0.0576 * CS + 0.4372) * 0.3854

and for specimens with CS > 950 µm:

CL / CW_{900} = CL/CW / (-0.2955 * CS + 1.3138) * 1.0560
SL / CS_{900} = SL/CS / (-0.2515 * CS + 1.1950) * 0.9666
$ExOcc / CS_{900}$ = ExOcc/CS / (4.318 * CS - 2.030) * 1.830
$ExCly / CS_{900}$ = 100 * ExCly/CS / (-1.574 * CS + 10.936) * 9.305
$ExClyW / CS_{900}$ = 100 * ExClyW/CS / (-0.374 * CS + 6.730) * 6.442
$ExCly / ExClyW_{900}$ = ExCly/ExClyW / (-0.1536 * CS + 1.9866) * 1.8049
$nExCly_{900}$ = nExCly / (17.61 * CS - 3.455) * 11.74
EL / CS_{900} = EL/CS / (-0.0777 * CS + 0.3236) * 0.2530
MW / CS_{900} = MW/CS / (0.0346 * CS + 0.6106) * 0.6403
ML / CS_{900} = ML/CS / (-0.0965 * CS + 1.3800) * 1.2902
dAN / CS_{900} = dAN/CS / (-0.0031 * CS + 0.2961) * 0.2988
$MpGr / CS_{900}$ = 100 *MpGr/CS / (4.770 * CS - 0.838) * 3.515
$Fu2 / CS_{900}$ = Fu2/CS / (-0.02001 * CS + 0.15932) * 0.14056
$IFu2_{900}$ = IFu2 / (0.2974 * CS + 1.5541) * 1.8078
$PoOc / CL_{900}$ = PoOc/CL / (-0.0254 * CS + 0.4066) * 0.3854

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

NUMOBAT: Explorative and supervised data analyses, classification and statistical testing

The delimitation of the cryptic species was done by an interaction of Nest-Centroid Clustering (NC clustering) and a controlling linear discriminant analysis (LDA). NC Clustering was run both as hierarchical NC-Ward clustering, non-hierarchical NC-K-means clustering and NC-NMDS-K-means clustering. These methods were described in more detail by SEIFERT & al. (2014) who also provided a script written in R and freely available under the GNU / GPL license from the following website: https://sourceforge.net/projects/agnesclustering/. The script is also supplied as a text file in Appendix S1, as digital supplementary material to this article, at the journal's web pages.

NC-Ward clustering was run first to indicate the putative number of K main clusters. In the second step, NC-K-means was performed with the setting of K classes suggested by NC-Ward. Classifications being coincident between the hierarchical and non-hierarchical clustering formed the hypothesis for the controlling LDA that was subsequently run. Samples with classifications disagreeing between NC-clustering methods were run in this LDA as wild-cards. The final classification ("final species hypothesis") was established by the LDA in the iterative procedure described by SEIFERT & al. (2014). There remained no undecided cases also if their posterior probabilities were close to 0.5. The classification of particular type specimens was checked by a "Leave-One-Out Cross-Validation" analysis LDA (LOOCV-LDA, LACHENBRUCH & MICKEY 1968, LESAFFRE & al. 1989). LDA, LOOCV-LDA and ANOVA tests were performed with the software package SPSS 15.0. Nest distributions along shore and inland were compared by Fisher's two-tailed exact test run with the software package R (R DEVELOPMENT CORE TEAM 2012).

The decision if a cluster can be recognized as a valid species was found according to the criterion of the Pragmatic Species Concept (SEIFERT 2014) which requires that the mean error of the applied exploratory data analyses determined by the controlling LDA must be < 4%. If more than two clusters are in a data set and if the clusters are extremely similar as in the presented case, it is advised (see next section) to run the clustering in a stepwise procedure. The first step runs exploratory data analyses of all samples involved and determines the most clearly separable cluster. The samples of this cluster are then excluded from the 2nd EDA-LDA run in which the next most clearly separable cluster is identified and excluded from the 3rd run. In theory, the analysis has to be terminated when no cluster with an error rate < 4% remains. However, the procedure can be stopped by the supervising researcher if the sample size of a separated cluster becomes too small inducing an increased risk for premature taxonomic decisions and over-splitting.

The senior author made the experience during some 800 runs of NC-clustering in 10 ant genera that stepwise cluster exclusion clearly boosts the performance in separation of most similar species. This finding is supported by NILSEN & al. (2013). They have shown problems in identifying clusters by the usually applied horizontal cuts in dendrograms of datasets containing many entities. They emphasized that such global analyses of all samples / entities in a single step induce the risk that true or reasonable subclusters within major clusters are not correctly shown when some of the clusters are more dispersed than others, and when there are cases that do not fit in any clusters (outliers). NILSEN & al. (2013) presented as solution of the problem a stepwise, fully automated procedure which optimizes the functions on each level beginning with the demonstration of major clusters and ending with the smallest subclusters. The termination of their analysis was decided according to a mathematically defined threshold.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from isolated whole individuals using a standard proteinase K – phenol / chloro-

form method with ethanol precipitation and for most German, French, Spanish, and North African samples using Chelex® as described in CASQUET & al. (2012). The DNA-barcode fragment of the mitochondrial Cytochrome C Oxidase subunit I (COI) was amplified by PCR using the primers LEP-F1 (5'-ATTCAACCAATCATAAAGATAT-3') and LEP-R1 (5'-TAAACTTCTGGATGTCCAAAAA-3') (HEBERT & al. 2004). Amplification reactions were carried out in a total volume of 25 or 30 µl, with 0.2 µM dNTPs, 0.16 µM of each primer, 1.20 U Taq Polymerase (Eurobio or Bioline), 1× PCR Buffer, and 50 ng DNA. Cycling parameters were as follows: initial denaturation (94 °C, 5 min), 40 cycles (94 °C, 30 s; 48 °C, 30 s; 72 °C, 30 s) and final extension (72 °C, 5 - 10 min). In some cases 0.1 µg / µl BSA (New England Biolabs, Ipswich, USA) was added to the reaction mixture.

PCR products from French and German specimens were sequenced and run on a 3730xl DNA Analyzer (Applied Biosystems) by BIOFIDAL (Vaulx-en-Velin, France). After purification with EXO-SAP, according to manufacturer's instructions, PCR products from Italian and Spanish specimens were sequenced by Macrogen Inc. (The Netherlands). All sequences data were deposited in the European Nucleotide Archive, with accession numbers reported in Appendix S2.

All ants from France and Germany: PCR Reactions were carried out in 30 µl solutions with 0.2 µM / µl dNTPs, 0.1 µg / µl BSA (New England Biolabs, Ipswich, USA), 0.16 µM / µl of each primer, 1.20 U Taq Polymerase (Eurobio; GAETAQ00), 1× PCR Buffer (Eurobio), and 2 µl of DNA. Cycling was conducted on a PTC-200 (MJ Research) thermal cycler with following parameters: (I) initial denaturation for 5 min at 94 °C; (II) 40 cycles with denaturation for 30 s at 94 °C, annealing for 30 s at 48 °C, and extension for 30 s at 72°C; (III) final extension for 5 min at 72 °C. All PCR products were purified, sequenced and ran on a 3730xl DNA Analyzer (Applied Biosystems) by a service provider for French and German ants (BIOFIDAL, Vaulx-en-Velin).

All ants from Italy and Spain: PCR were carried out in 25 µl reaction volumes containing 1 × MilliQ (15.55 µl), Buffer (3 µl), MgCl2 (2.5 µl), dNTP (1 µl), LEP-F1 (0.4 µl), LEP-R1 (0.4 µl), BioTaq (0.15 µl) and DNA (2 µl). The PCR program was set as follows: initial denaturation (94 °C, 5 min), 40 cycles (94 °C, 30 s; 48 °C, 30 s; 72 °C, 30 s), and final extension (72 °C, 10 min). For DNA purification we used a PCR mixture of PCR products (15 µl) and Exosap (6 µl) for 37 °C (15 min) and 80 °C (15 min). Sequencing was carried out in Holland by Macrogen Sequencing.

All sequences have been deposited in Genbank with accessions reported in Appendix S2.

Sequence analysis and inference of phylogeny

Both forward and reverse sequences were analyzed in Geneious R7.0.6 (Biomatters Inc.) or Seaview 4.0 (GOUY & al. 2010) to complete contigs that were easily aligned by hand. The invertebrate mitochondrial code table was used to deduce encoded amino acid sequences and check for stop codons. Alignments were generated using Geneious R7.0.6 and ClustalW program with 122 sequences (from one specimen from colonies which were subject to NUMOBAT procedures). DNA polymorphism was analyzed using DnaSP 5.10 (LIBRADO & ROZAS 2009) while genetic distances were estimated in MEGA 6 (TAMURA & al. 2013) using Kimura 2-parameters model (K2p), which despite recent criticisms (e.g., SRIVATHSAN & MEIER 2011) is widely used in barcoding studies. Mean between-groups and within-group K2p distances were calculated in MEGA for the major clades defined in the phylogenetic analysis. Phylogeny was inferred out using two approaches, Maximum Likelihood (ML) and Bayesian Inference (BI). Ten sequences (one of these from Genbank, accession number: GU373568.1) of species closely related to the *Tapinoma nigerrimum* complex were used as outgroups. The best nucleotide substitution model was selected in jModelTest 2.1.5 (POSADA 2008), using the Bayesian Information Criterion (BIC). ML analysis was carried out with PhyML 3.0 (GUINDON & al. 2010). Support values for the nodes were estimated with 100 bootstrap replicates. BI was carried out with MrBayes v. 3.1.2 (RONQUIST & al. 2012) running a four-chain Metropolis-coupled Markov chain Monte Carlo for 10^6 generations. Trees were sampled every 1000 generations; convergence of each run was evaluated using Tracer 1.4.1 (RAMBAUT & DRUMMOND 2007), and analyses were terminated when ESS values of all parameters were above 200. A consensus tree was then calculated after omitting the first 25% trees as burn-in. FigTree Tree Figure Drawing Tool Version 1.4.2 was used to draw the mtDNA trees.

Results and discussion

Diagnosis of the *Tapinoma nigerrimum* complex

The species of the *Tapinoma nigerrimum* complex are easily separated from other Palaearctic species by cluster analyses of multiple characters. The following characters show the largest differences and may provide a simpler way for identification.

- Workers: Large-sized and strongly size-polymorphic: The largest major workers of mature colonies have twice the cephalic width (CW) and ninefold the body mass of the smallest minor workers. CW may reach 1385 µm. Species of the *Tapinoma simrothi* complex are also rather size-polymorphic but CW does not exceed 1050 µm – this allows a separation of both species complexes in 20% of the individuals. Length to width ratio of second funiculus segment larger than in other species including the *T. simrothi* complex, $IFu2_{900}$ 1.74 - 2.06. The bilateral sum of pubescence hairs and smaller setae protruding at a few micron across margin of clypeal excision is larger than in other species, $nExCly_{900}$ frequently 7 - 21.
- Gynes: Much larger than in other species. CW in 58 specimens 1369 ± 59 [1251, 1530]. Species of the *Tapinoma simrothi* complex are next similar in size and show a small size overlap: CW in 29 specimens 1155 ± 67 [998, 1290]. With all measurements in mm, a safe separation of both species complexes is given by the discriminant D(5) = 30.01 CW - 19.27 CL + 27.42 dAN - 29.71 ExCly - 215.5 Fu2W - 2.05. Gynes of the *T. nigerrimum* complex have D(6) < -1 and those of the *T. simrothi* complex D(6) > 1.
- Males: the genitalia show in ventral aspect a very broad basimere and a broad blade-like harpago (Fig. 11; SEIFERT 1984a: figs. 8, 9) which easily separates it from all other species complexes (SEIFERT 1984a: figs. 3-7). The members of the *Tapinoma simrothi* complex show in ven-

tral aspect a gripper-like harpago and a narrower basimere (SEIFERT 1984a: figs. 6, 7).

NUMOBAT clustering: workers form four separate clades

We present here three steps of exploratory data analyses in which four clades of the *Tapinoma nigerrimum* complex are resolved. The reasons for the taxonomic naming of these entities are explained in the next section but we anticipate the decisions to have an easier presentation. In a first step of analysis, considering the whole material of 159 nest samples and all 15 NUMOBAT characters unselectively, NC-Ward clustering suggested the existence of three main clusters. Setting K = 3, this result was basically confirmed by NC-K-means and NC-NMDS-K-means but there were, over all samples, 4.4% disagreement with NC-Ward clustering. However, the three exploratory data analyses showed 100% agreement regarding one cluster composed of 19 samples which was determined to represent *T. nigerrimum* (see below). The *T. nigerrimum* cluster was fully confirmed by running a three class LDA with no sample having posterior probabilities of $p < 0.999$. The classification success in 69 individual workers of this cluster was 98.6%. *T. nigerrimum*, as the most easily separable species of the complex was excluded from further analysis.

In the second step of analysis, again with the full set of characters, the NC-Ward dendrogram of the remaining 140 nest samples presented two major clusters (Fig. 3). NC-NMDS-K-means clustering and NC-K-means clustering with a setting of K = 2 disagreed in 6.4% of the samples from the classification by NC-Ward. These samples were run as wild-cards in an iterative controlling LDA. This re-classification, showing a cluster 1 (later identified as *Tapinoma magnum*) and a cluster 2 (later identified as *T. darioi* sp.n. + *T. ibericum*), agreed by 95.0% with NC-Ward and by both 99.3% with NC-NMDS-K-means and NC-K-means clustering. The heterospecificity of the two clusters can be accepted because the mean error of the three exploratory data analyses 2.1% [(5.0 + 0.7 + 0.7) / 3] is below the 4% threshold recommended by the Pragmatic Species Concept (SEIFERT 2014, SEIFERT & CSŐSZ 2015).

As Figure 3 suggested that cluster 2 could be composed of two sub-clusters 2a and 2b, we analyzed this cluster separately. NC-Ward clustering without character reduction confirmed the two subclusters (Fig. 4). NC-NMDS-K-means clustering and NC-K-means clustering with K = 2 disagreed in 1.4% and 8.2% of the 61 samples from the NC-Ward classification. The five disagreeing samples were run as wild-cards in an iterative controlling LDA which confirmed the classification by NC-Ward, NC-NMDS-K-means and NC-K-means clustering by 98.6, 100% and 93.4% respectively. Cluster 2a is identified as *Tapinoma darioi* sp.n. and cluster 2b as *T. ibericum* (see below). The mean clustering error of the three exploratory methods of 2.7% is below the 4.0% threshold and heterospecificity can be accepted. A significant evolutionary divergence of *T. darioi* sp.n. and *T. ibericum* is also supported by their placement in different mtDNA clades with a mean K2p genetic distance of 3.6% (see results of DNA analysis) and by the clear clustering of males (see next section).

NUMOBAT clustering and the 4% threshold of the Pragmatic Species Concept would support a fifth cluster: The 39 samples of *Tapinoma darioi* sp.n. can be subdivided into two clusters (see Fig. 4) with error rates of 5.1% in NC-Ward and 0% in NC-K-means giving a mean error of 2.6%. However, we refrained here from naming a fifth taxon because we consider the sample size too small for deciding such a critical case and because the mismatch with mtDNA clustering was 39%. We are aware that such a big mtDNA disagreement may not necessarily indicate a wrong phenotypic species separation. This was shown by the example of two species of Australian Wood Swallows, *Artamus personatus and A. superciliosus*, where phenotypes and behaviour differ so strikingly that no ornithologist raised doubts on heterospecificity. In this species pair, the mtDNA tree showed the maximum possible degree of paraphyly but the species, most remarkably, did not share a single haplotype (JOSEPH & al. 2006). In order to avoid the risk of over-splitting, we postpone a decision after further material will have been studied and when most informative nuDNA markers will be available. These subentities are provisionally named in the NUMOBAT files as the clusters "dari1" and "dari2" (Appendix S3).

A significant problem arises with sample TLa24 from Italy: Roma: Cerveletta Park which is placed by NC-Ward in the *Tapinoma darioi* sp.n. cluster, by NC-K-means in *T. ibericum* and by a wild-card 2-class LDA in *T. ibericum* ($p = 0.8098$). Running all six workers of this sample simultaneously in a 4-class LDA as wild-cards, the mean posterior probabilities are 0.1933 for *T. darioi* sp.n., 0.6283 for *T. ibericum*, 0.1784 for *T. magnum* and 0.0000 for *T. nigerrimum*. The identification as *T. ibericum* appears extremely doubtful as this species was never found in Italy or neighboring countries. To make the case even more complicated, the mtDNA sequence of TLa24 belongs to a clade of 33 haplotypes 32 of which are associated with clear *T. magnum* phenotypes (Fig. 6). We investigated ten workers of this colony which all had identical haplotypes. In the absence of unambiguous information we hypothesize that the problematic phenotype of TLa24 workers was generated by a hybridization event of *T. darioi* sp.n. with *T. magnum* that took place so recently that a purging of nuclear DNA could not happen or remained incomplete. This differs from the situation in the nine cases of phenotype-mtDNA mismatches discussed under Results of DNA analysis (see below). A recent hybridization appears plausible in sample TLa24 as *T. darioi* sp.n. and *T. magnum* occur sympatrically in the Lazio region.

NUMOBAT clustering: males form four separate clades and the type-based name allocation is convincing

The primary type specimen of *Tapinoma ibericum* and the lectotype of *T. magnum* are single males apparently not collected from nests but during swarming. In contrast to the strong differences of male genital morphology to any species outside the complex, there are no obvious inter-

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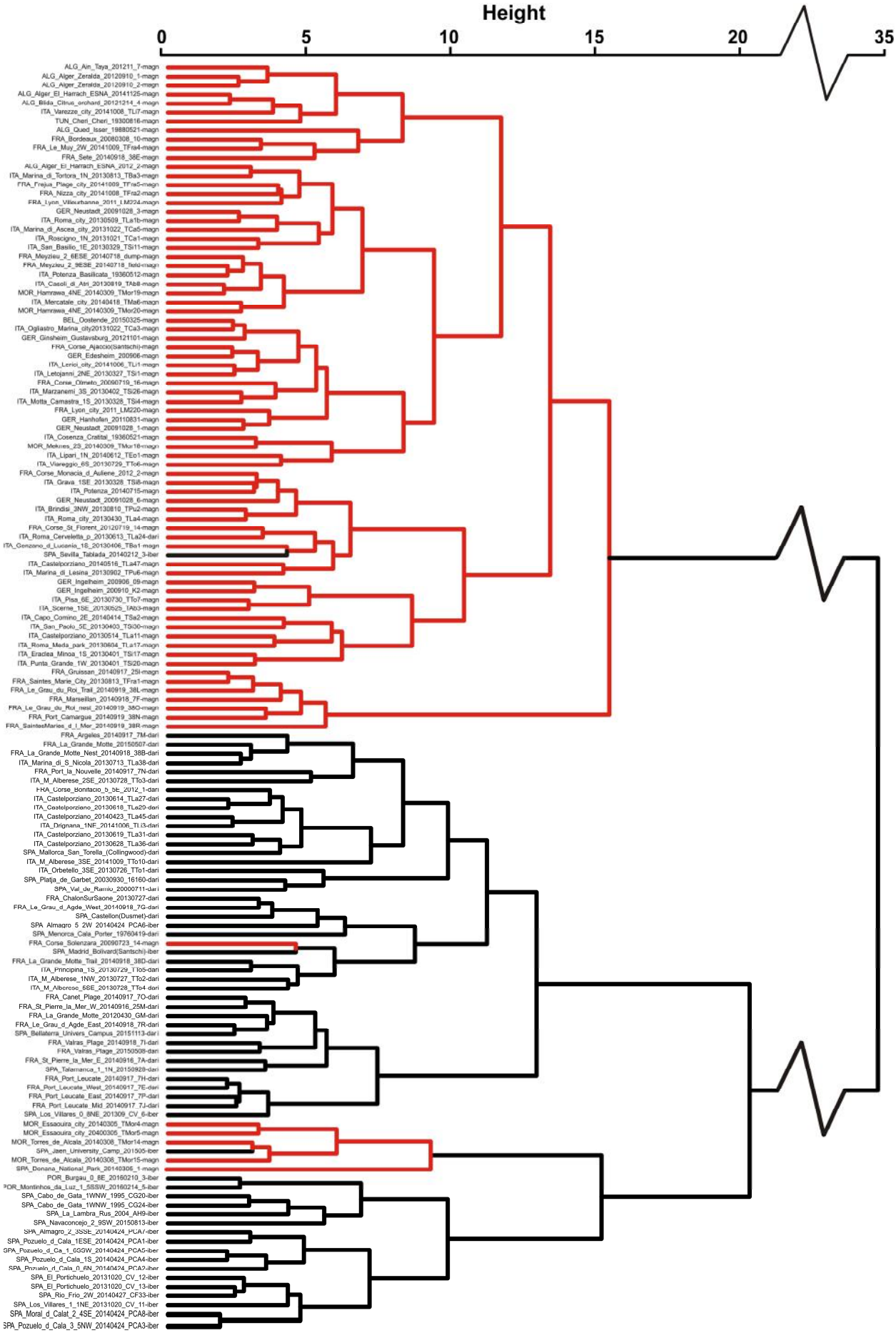
Fig. 3: NC-Ward dendrogram of worker nest samples of *Tapinoma magnum* (red branches) and of *T.darioi* sp.n. plus *T. ibericum* (black branches) considering 15 NUMOBAT characters unselectively. The classification error relative to the controlling LDA is here as large as 5.0% whereas it is only 0.7% both in NC-NMDS-K-means and NC-K-means clustering.


Height
0
5
10
15
20
35
ALG_Ain_Taya_201211_7-magn
ALG_Alger_Zeralda_20120910_1-magn
ALG_Alger_Zeralda_20120910_2-magn
ALG_Alger_El Harrach_ESNA_20141125-magn
ALG_Blida_Citrus_orchard_20121214_4-magn
ITA_Varezze_city_20141008_TLi7-magn
TUN_Cheri_Cheri_19300816-magn
ALG_Oued_Isser_19880521-magn
FRA_Bordeaux_20080308_10-magn
FRA_Le_Muy_2W_20141009_TFra4-magn
FRA_Sete_20140918_38E-magn
ALG_Alger_El_Harrach_ESNA_2012_2-magn
ITA_Marina_di_Tortora_1N_20130813_TBa3-magn
FRA_Frejus_Plage_city_20141009_TFra5-magn
FRA_Nizza_city_20141008_TFra2-magn
FRA_Lyon_Villeurbanne_2011_LM224-magn
GER_Neustadt_20091028_3-magn
ITA_Roma_city_20130509_TLa1b-magn
ITA_Marina_di_Ascea_city_20131022_TCa5-magn
ITA_Roscigno_1N_20131021_TCa1-magn
ITA_San_Basilio_1E_20130329_TSi11-magn
FRA_Meyzieu_2_6ESE_20140718_dump-magn
FRA_Meyzieu_2_9ESE_20140718_field-magn
ITA_Potenza_Basilicata_19360512-magn
ITA_Casoli_di_Atri_20130819_TAb8-magn
MOR_Hamrawa_4NE_20140309_TMor19-magn
ITA_Mercatale_city_20140418_TMa6-magn
MOR_Hamrawa_4NE_20140309_TMor20-magn
BEL_Oostende_20150325-magn
ITA_Ogliastro_Marina_city20131022_TCa3-magn
GER_Ginsheim_Gustavsburg_20121101-magn
FRA_Corse_Ajaccio(Santschi)-magn
GER_Edesheim_200906-magn
ITA_Lerici_city_20141006_TLi1-magn
ITA_Letojanni_2NE_20130327_TSi1-magn
FRA_Corse_Olmeto_20090719_16-magn
ITA_Marzanemi_3S_20130402_TSi26-magn
ITA_Motta_Camastra_1S_20130328_TSi4-magn
FRA_Lyon_city_2011_LM220-magn
GER_Hanhofen_20110831-magn
GER_Neustadt_20091028_1-magn
ITA_Cosenza_Cratiti_19360521-magn
MOR_Meknes_2S_20140309_TMor18-magn
ITA_Lipari_1N_20140612_TEo1-magn
ITA_Viareggio_6S_20130729_TTo6-magn
FRA_Corse_Monacia_d_Auliene_2012_2-magn
ITA_Grava_1SE_20130328_TSi8-magn
ITA_Potenza_20140715-magn
GER_Neustadt_20091028_6-magn
ITA_Brindisi_3NW_20130810_TPu2-magn
ITA_Roma_city_20130430_TLa4-magn
FRA_Corse_St_Florent_20120719_14-magn
ITA_Roma_Cervelletta_p_20130613_TLa24-dari
ITA_Genzano_d_Lucania_1S_20130406_TBa1-magn
SPA_Sevilla_Tablada_20140212_3-iber
ITA_Castelporziano_20140516_TLa47-magn
ITA_Marina_di_Lesina_20130902_TPu6-magn
GER_Ingelheim_200906_09-magn
GER_Ingelheim_200910_K2-magn
ITA_Pisa_6E_20130730_TTo7-magn
ITA_Scerne_1SE_20130525_TAb3-magn
ITA_Capo_Comino_2E_20140414_TSa2-magn
ITA_San_Paolo_5E_20130403_TSi30-magn
ITA_Castelporziano_20130514_TLa11-magn
ITA_Roma_Meda_park_20130604_TLa17-magn
ITA_Eraclea_Minoa_1S_20130401_TSi17-magn
ITA_Punta_Grande_1W_20130401_TSi20-magn
FRA_Gruissan_20140917_25I-magn
FRA_Saintes_Marie_City_20130813_TFra1-magn
FRA_Le_Grau_du_Roi_Trail_20140919_38L-magn
FRA_Marseillan_20140918_7F-magn
FRA_Le_Grau_du_Roi_nest_20140919_38O-magn
FRA_Port_Camargue_20140919_38N-magn
FRA_SaintesMaries_d_l_Mer_20140919_38R-magn
FRA_Argeles_20140917_7M-dari
FRA_La_Grande_Motte_20150507-dari
FRA_La_Grande_Motte_Nest_20140918_38B-dari
ITA_Marina_di_S_Nicola_20130713_TLa38-dari
FRA_Port_la_Nouvelle_20140917_7N-dari
ITA_M_Alberese_2SE_20130728_TTo3-dari
FRA_Corse_Bonifacio_5_5E_2012_1-dari
ITA_Castelporziano_20130614_TLa27-dari
ITA_Castelporziano_20130618_TLa29-dari
ITA_Castelporziano_20140423_TLa45-dari
ITA_Orbetello_1NE_20141006_TLi3-dari
ITA_Castelporziano_20130619_TLa31-dari
ITA_Castelporziano_20130628_TLa36-dari
SPA_Mallorca_San_Torella_(Collingwood)-dari
ITA_M_Alberese_3SE_20141009_TTo10-dari
ITA_Orbetello_3SE_20130726_TTo1-dari
SPA_Platja_de_Garbet_20030930_16160-dari
SPA_Val_de_Ranilo_20000711-dari
FRA_ChalonSurSaone_20130727-dari
FRA_Le_Grau_d_Agde_West_20140918_7G-dari
SPA_Castellon(Dusmet)-dari
SPA_Almagro_5_2W_20140424_PCA6-iber
SPA_Menorca_Cala_Porter_19760419-dari
FRA_Corse_Solenzara_20090723_14-magn
SPA_Madrid_Bolivard(Santschi)-iber
FRA_La_Grande_Motte_Trail_20140918_38D-dari
ITA_Principina_1S_20130729_TTo5-dari
ITA_M_Alberese_1NW_20130727_TTo2-dari
ITA_M_Alberese_6SE_20130728_TTo4-dari
FRA_Canet_Plage_20140917_7O-dari
FRA_St_Pierre_la_Mer_W_20140916_25M-dari
FRA_La_Grande_Motte_20120430_GM-dari
FRA_Le_Grau_d_Agde_East_20140918_7R-dari
SPA_Bellaterra_Univers_Campus_20151113-dari
FRA_Valras_Plage_20140918_7I-dari
FRA_Valras_Plage_20150508-dari
FRA_St_Pierre_la_Mer_E_20140916_7A-dari
SPA_Talamanca_1_1N_20150928-dari
FRA_Port_Leucate_20140917_7H-dari
FRA_Port_Leucate_West_20140917_7E-dari
FRA_Port_Leucate_East_20140917_7F-dari
FRA_Port_Leucate_Mid_20140917_7J-dari
SPA_Los_Villares_0_8NE_201309_CV_6-iber
MOR_Essaouira_city_20140305_TMor4-magn
MOR_Essaouira_city_20400305_TMor5-magn
MOR_Torres_de_Alcala_20140308_TMor14-magn
SPA_Jaen_University_Camp_201505-iber
MOR_Torres_de_Alcala_20140308_TMor15-magn
SPA_Donana_National_Park_20140305_1-magn
POR_Burgau_0_8E_20160210_3-iber
POR_Montinhos_da_Luz_1_5SSW_20160214_5-iber
SPA_Cabo_de_Gata_1WNW_1995_CG20-iber
SPA_Cabo_de_Gata_1WNW_1995_CG24-iber
SPA_La_Lambra_Rus_2004_AH9-iber
SPA_Navaconcejo_2_9SW_20150813-iber
SPA_Almagro_2_3SSE_20140424_PCA7-iber
SPA_Pozuelo_d_Cala_1ESE_20140424_PCA1-iber
SPA_Pozuelo_d_Ca_1_6SSW_20140424_PCA5-iber
SPA_Pozuelo_d_Cala_1S_20140424_PCA4-iber
SPA_Pozuelo_d_Cala_0_6N_20140424_PCA2-iber
SPA_El_Portichuelo_20131020_CV_12-iber
SPA_El_Portichuelo_20131020_CV_13-iber
SPA_Rio_Frio_2W_20140427_CF33-iber
SPA_Los_Villares_1_1NE_20131020_CV_11-iber
SPA_Moral_d_Calat_2_4SE_20140424_PCA8-iber
SPA_Pozuelo_d_Cala_3_5NW_20140424_PCA3-iber

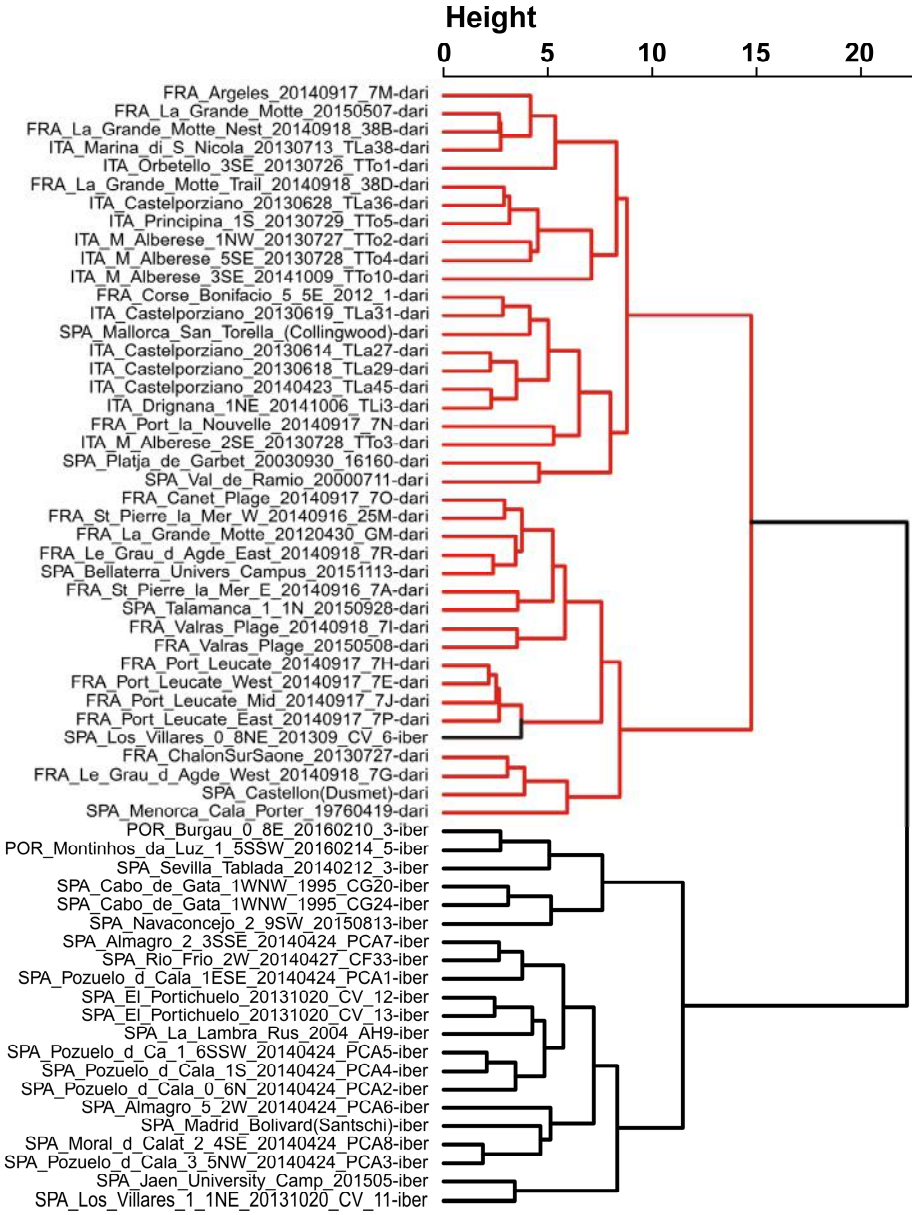


Fig. 4: NC-Ward dendrogram of worker nest samples of *Tapinoma darioi* sp.n. (red branches) and *T. ibericum* (black branches) considering 15 NUMOBAT characters. The classification error relative to the controlling LDA is here 1.4% whereas it is 0% and 6.6% in NC-NMDS-K-means and NC-K-means clustering, respectively.

specific differences within the *T. nigerrimum* complex. This did not allow an assessment by simple eye inspection and made a combined NUMOBAT analysis of both non-genitalic and genitalic characters inevitable. NC-clustering allows computing single-specimen samples under the condition that a good fraction of the material contains multiple-specimen samples. Investigated was a total of 42 samples including eight samples of *T. darioi* sp.n., nine of *T. ibericum*, 16 of *T. magnum*, and nine of *T. nigerrimum*. Nine samples contained only a single specimen. 40 male samples were worker-associated nest samples. The classifications between the exploratory data analyses NC-Ward, NC-K-means and NMDS-K-means clustering differed in only one sample which was run as a wild-card in the controlling 4-class LDA. Due to the small sample size in the smallest class, with only 18 males available in *T. ibericum*, a character-reduced LDA was run. It considered the seven characters EL / CS, Fu2 / CS, CS, CL / CW, ML / CS, ALPH, and dAN. The agreement of the LDA classifications with NC-Ward (Fig. 5), NC-K-means and NMDS-K-means clustering was 100, 97.6 and 97.6% respectively. The only misclassified sample was a single-male sample TSi26 from Marzaniemi / Sicily. This was a dwarf male from a safely determined *T. magnum* nest (p = 0.9987 in workers).

The classification error in 101 individual males was 3.0%. The type specimens had the following posterior probabilities in the 4-class LDA : 0.996 and 0.999 in paratypes of *Tapinoma darioi* sp.n., 1.000 in the type of *T. ibericum*, 0.945 and 1.000 in males from the neotype nest of *T. nigerrimum*, and 0.829 in the lectotype of *T. magnum*. The next probable classification in the lectotype of *T. magnum* was *T. ibericum* with p = 0.171. Considering the clusters with the *T. magnum* and *T. ibericum* types only and running the type specimens as wild-cards in a character-reduced LDA using EL / CS, ALPH, CL / CW, dAN / CS, and ExCly / CS, we got clear results. The male lectotype of *T. magnum* is allocated with p = 1.000 to the cluster in

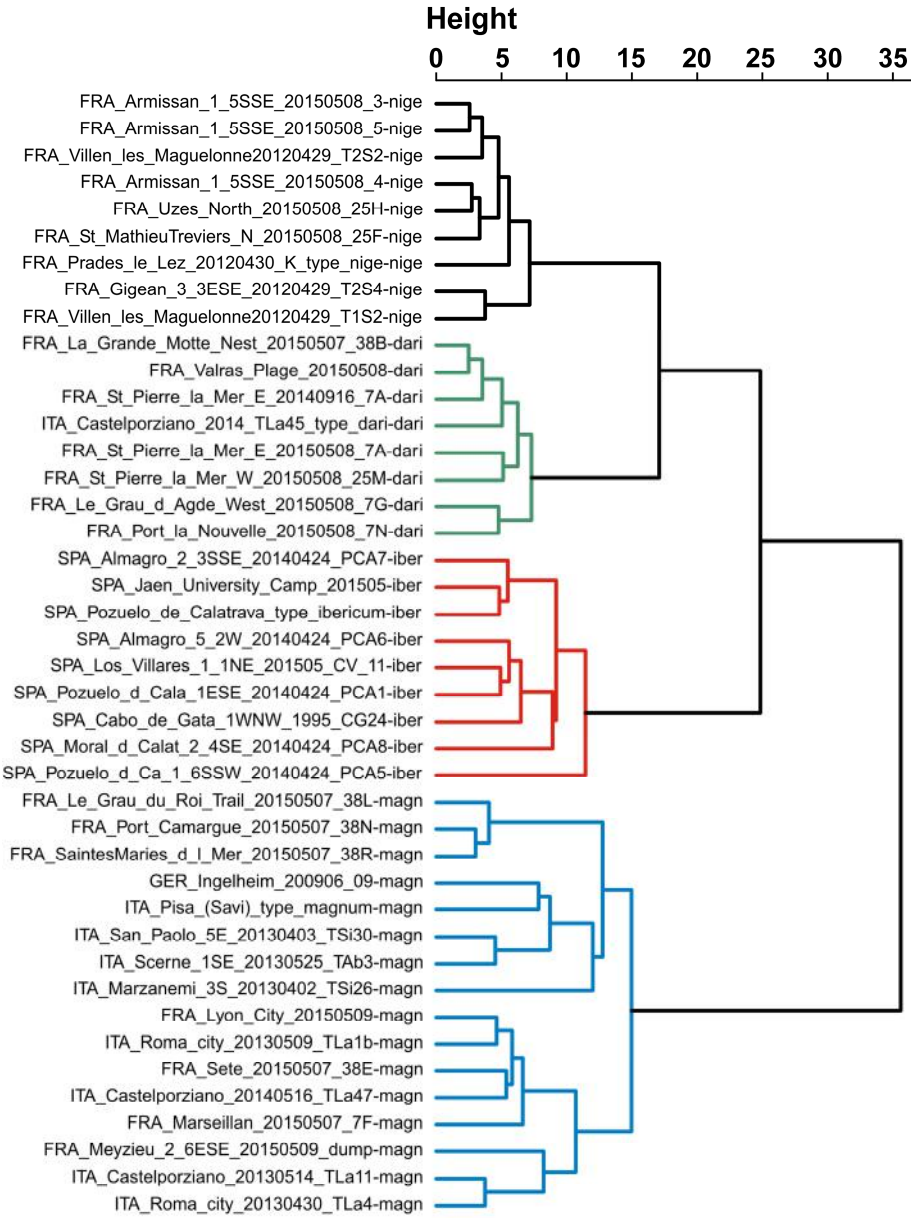


Fig. 5: NC-Ward dendrogram of male nest samples of *Tapinoma nigerrimum* (black branches), *T. darioi* sp.n. (green), *T. ibericum* (red) and *T. magnum* (blue) considering seven NUMOBAT characters.

which all males were collected from nests belonging to worker cluster 1 and the type of *T. ibericum* is allocated with p = 1.000 to the clade in which all males were associated with cluster 2b workers. Apart from the unbiased nature of wild-card runs in the types, the risk of this LDA to confirm a wrong prejudice also in the non-type samples is low because the number of specimens in the smallest class (*T. ibericum*) is 3.6fold larger than the number of characters considered. An accessory argument that the male lectotype of *T. magnum* cannot belong to worker cluster 2b is the complete absence of *T. ibericum* from Italy and the neighbouring countries. Complementarily, it is unlikely that the type specimen of *T. ibericum* belongs to worker cluster 1 (*T. magnum*) because no worker samples of this cluster are known within a radius of 300 km around the type locality Pozuelo de Calatrava.

The conclusion from these analyses is that we have a clear type-based designation of the taxonomic name in all four species of the *Tapinoma nigerrimum* complex.

NUMOBAT clustering: gynes

Exploratory data analyses of gynes are strongly affected by lack of material in *Tapinoma ibericum* and *T. nigerrimum* with only four and five specimens respectively being available. Therefore, we reduced the analysis to assessing the position of the type specimens by a LDA in which the classification of the gynes was determined by those of the associated workers. Wild-card runs in a character-reduced 4-class LDA confirmed the cluster allocation of any type specimen. Posterior probabilities were 0.9993 and 0.9995 in the paratypes of *T. darioi* sp.n., 1.000 in the paralectotype gyne of *T. magnum*, 1.000 and 0.990 in the paratypes of *T. nigerrimum*.

Recommendations for phenotypical species delimitation in workers

Unfortunately, the investigated character system does not offer a simple and safe method for phenotypical species

Tab. 1: RAV-corrected morphological data of four species of the *Tapinoma nigerrimum* species complex and of *T. simrothi* KRAUSSE, 1911. Given are nest sample means in the arrangement arithmetic mean ± standard deviation [lower extreme, upper extreme]; n = number of nest samples; *n* printed in *italics* is the number of nest samples in which PoOc / CL data were available. The means of some diagnostic characters are printed in heavy font.

	T. simrothi (n = 61), *n = 61*	***T. magnum*** (n = 79), *n = 23*	***T. darioi*** (n = 40), *n = 25*	***T. ibericum*** (n = 23), *n = 8*	***T. nigerrimum*** (n = 19), *n = 10*
CS	844 ± 67 [688, 1033]	945 ± 81 [734, 1220]	870 ± 90 [685, 1074]	990 ± 75 [811, 1106]	1003 ± 88 [807, 1116]
CL / CW_{900}	1.080 ± 0.015 [1.049, 1.123]	1.065 ± 0.015 [1.037, 1.102]	1.043 ± 0.016 [1.014, 1.072]	1.060 ± 0.012 [1.031, 1.079]	1.039 ± 0.010 [1.022, 1.060]
SL / CS_{900}	0.948 ± 0.017 [0.901, 0.980]	0.982 ± 0.015 [0.944, 1.044]	0.963 ± 0.012 [0.929, 0.985]	0.972 ± 0.016 [0.936, 1.000]	0.956 ± 0.016 [0.910, 0.977]
ExOcc / CS_{900} [%]	1.50 ± 0.46 [0.52, 2.77]	1.50 ± 0.48 [0.48, 2.67]	2.07 ± 0.48 [0.90, 3.32]	2.16 ± 0.45 [1.04, 2.91]	2.23 ± 0.34 [1.47, 3.00]
ExCly / CS_{900} [%]	**12.34** ± 0.93 [10.25,1 4.42]	**8.92** ± 0.67 [7.14, 11.07]	9.80 ± 0.60 [8.64, 11.72]	10.37 ± 0.60 [8.30, 11.13]	10.21 ± 0.75 [8.95, 11.56]
ExClyW / CS_{900} [%]	5.71 ± 0.61 [4.39, 7.15]	6.67 ± 0.57 [5.43, 8.14]	6.02 ± 0.59 [4.53, 7.08]	6.68 ± 0.47 [5.76, 7.57]	6.77 ± 0.35 [6.31, 7.54]
ExCly / $ExClyW_{900}$ [%]	**2.18** ± 0.21 [1.75, 2.72]	**1.347** ± 0.140 [1.037, 1.917]	1.647 ± 0.202 [1.375, 2.235]	**1.562** ± 0.142 [1.303, 1.850]	1.515 ± 0.135 [1.290, 1.815]
$nExCly_{900}$	8.11 ± 3.10 [1.7, 15.2]	13.01 ± 3.10 [4.9, 20.1]	13.14 ± 2.82 [6.0, 19.0]	15.31 ± 3.03 [9.1, 23.9]	13.97 ± 3.56 [8.7, 22.1]
dAN / CS_{900}	0.290 ± 0.005 [0.282, 0.302]	0.309 ± 0.007 [0.292, 0.325]	0.301 ± 0.006 [0.289, 0.312]	0.293 ± 0.006 [0.286, 0.306]	0.299 ± 0.004 [0.291, 0.305]
PoOc / CL_{900}	0.394 ± 0.007 [0.374,0.410]	0.380 ± 0.009 [0.364, 0.395]	0.379 ± 0.008 [0.365, 0.400]	0.375 ± 0.006 [0.366, 0.381]	0.384 ± 0.007 [0.370, 0.395]
EL / CS_{900}	0.251 ± 0.006 [0.236, 0.265]	**0.260** ± 0.006 [0.246, 0.274]	0.254 ± 0.006 [0.236, 0.265]	0.255 ± 0.006 [0.243, 0.264]	**0.235** ± 0.004 [0.229, 0.240]
MpGr / CS_{900} [%]	3.36 ± 0.67 [1.92, 5.16]	3.95 ± 0.49 [2.86, 5.41]	2.63 ± 0.44 [1.73, 3.82]	2.94 ± 0.33 [2.11, 3.43]	3.34 ± 0.54 [2.06, 3.86]
MW / CS_{900}	0.641 ± 0.016 [0.610, 0.678]	0.643 ± 0.014 [0.621, 0.688]	0.632 ± 0.014 [0.609, 0.662]	0.627 ± 0.013 [0.596, 0.649]	**0.615** ± 0.010 [0.601, 0.642]
ML / CS_{900}	1.287 ± 0.026 [1.237, 1.336]	1.300 ± 0.023 [1.244, 1.382]	1.279 ± 0.024 [1.223, 1.334]	1.284 ± 0.022 [1.251, 1.326]	**1.230** ± 0.017 [1.196, 1.259]
Fu2 / CS_{900} [%]	13.41 ± 0.36 [12.76, 14.56]	14.93 ± 0.35 [14.10, 16.08]	14.56 ± 0.26 [14.04, 15.08]	14.23 ± 0.38 [13.62, 14.97]	13.81 ± 0.27 [13.39, 14.42]
$IFu2_{900}$	**1.689** ± 0.047 [1.609, 1.802]	1.922 ± 0.069 [1.770, 2.082]	1.961 ± 0.033 [1.876, 2.056]	1.871 ± 0.058 [1.740, 1.986]	**1.806** ± 0.052 [1.741, 1.947]

delimitation in the *Tapinoma nigerrimum* complex and repeating the methods performed here is a challenge for unexperienced investigators. Precise recording of NUMOBAT characters requires some training and a good optical equipment as many diagnostic characters are microstructures. Most important is careful consideration of character definitions, to avoid several types of measuring errors as they were described by SEIFERT (2002) and use of a stereomicroscope with a resolution of at least 650 lines / mm (or a numeric aperture of about 0.22) and a final magnification of at least 250 ×. Given that, a safe delimitation of all four cryptic species still requires almost the complete set of characters. We provide the primary NUMOBAT data of the four species and 553 worker specimens in Appendix S3. The file gives the absolute measurements in millimeters plus the seta count, the identification, nest sample number and collecting data. Users can investigate their own specimens and run them as wild-cards in an LDA using the classifications provided by the file. The standard file provides correct classifications in 95.8% of specimens. Therefore, we recommend investigation of three workers to increase the identification success above 99%.

Tentative determinations can be tried using Table 1. It shows the RAV-corrected data as they are found in a medium-sized specimen with CS = 900 µm. The table also provides data for the relatively large-sized, supercolonial and sympatric species *Tapinoma simrothi* KRAUSSE, 1911. *Tapinoma nigerrimum*, as the least difficult case, is characterized by a short length of eye, mesosoma, and 2nd funiculus segment. *Tapinoma magnum* shows the smallest depth-to-width ratio of clypeal excision of all species. *Tapinoma simrothi* shows the largest depth-to-width ratio of clypeal excision and the smallest length-to-width ratio of second funiculus segment. Giving a recommendation for

a simple delimitation of *T. darioi* sp.n. and *T. ibericum* is impossible.

Results of the mtDNA analysis

A total of 655 bp were aligned, with 140 variable sites (singleton variable: 27; parsimony informative sites: 113) resulting in 62 distinct haplotypes. The best-fit model selected in jModeltest according to the Bayesian Information Criterion was the HKY + I + G; this model was used in ML and BI inference, while parameters' values were estimated during the analyses. Parallel NUMOBAT and mtDNA investigation was performed in 122 nest samples. Figure 6 shows the topology of the phylogenetic tree for these samples obtained with Maximum Likelihood and the bootstrap values and posterior probabilities are given for each node. The phylogenetic tree shows four major clades. Three of these (A: *Tapinoma ibericum*, B: *T. darioi* sp.n., and C: *T. nigerrimum*) were well supported by both ML (81 - 98) and BI (0.99 - 1), and one (D: *T. magnum*) was poorly supported using ML (26) and unresolved with BI. NUMOBAT data and mtDNA haplotypes provided congruent classifications in 92.6% of the samples. No disagreements between NUMOBAT and mtDNA occurred in *T. ibericum* (13 samples), *T. magnum* (62 samples), and *T. nigerrimum* (11 samples). However, nine samples (= 30%) of *T. darioi* sp.n. were placed in the COI tree within the *T. magnum* branch. These nine samples, marked by single asterisks in Figure 6, were found along the shore line of the Mediterranean Sea from Marina di Alberese (42.642° N, 11.054° E) northwest to Port la Nouvelle (43.007° N, 3.057° E). Considering *T. darioi* sp.n. against *T. magnum* and using 15 morphological characters in a 2-class LDA, each of these conflicting samples showed a clear NUMOBAT classification as *T. darioi* sp.n. with $p > 0.965$ (Tab. 2). Furthermore, there was no difference of the position between the conflicting and congruent samples along the discriminant vector separating from *T. magnum*. A consideration of characters one by one (Tab. 3) does also not give any suggestion for a significant deviation from the mean of the typical population.

The methodology of the study does not provide data that can clearly explain the reasons for the mismatches between the NUMOBAT tree and mitochondrial gene trees in these nine samples. A multitude of complicated and mixed scenarios is possible in such a complex social and genetic system but disscussing all of these would distract from the main purpose of the paper which is advance in taxonomy. We consider here only four possible explanations with increasing rank of probability: (a) nuclear mitochondrial pseudogenes (NUMTs), (b) gene silencing, (c) incomplete lineage sorting, and (d) hybridization in the younger evolutionary history with subsequent unidirectional genomic purging.

After inspecting the sequences of the nine problematic samples for stop codons in amino acid translation the involvement of NUMTs appears unlikely but is not completely excluded. Explanation (b), gene silencing or nucleolar dominance is a process observed in interspecific hybrids. It basically means that the ribosomal RNA (rRNA) genes inherited from one parent are transcribed but the rRNA genes derived from the other progenitor remain silent (PICKAARD 2000). This poorly understood, obviously epigenetic phenomenon is largely known from many gen-

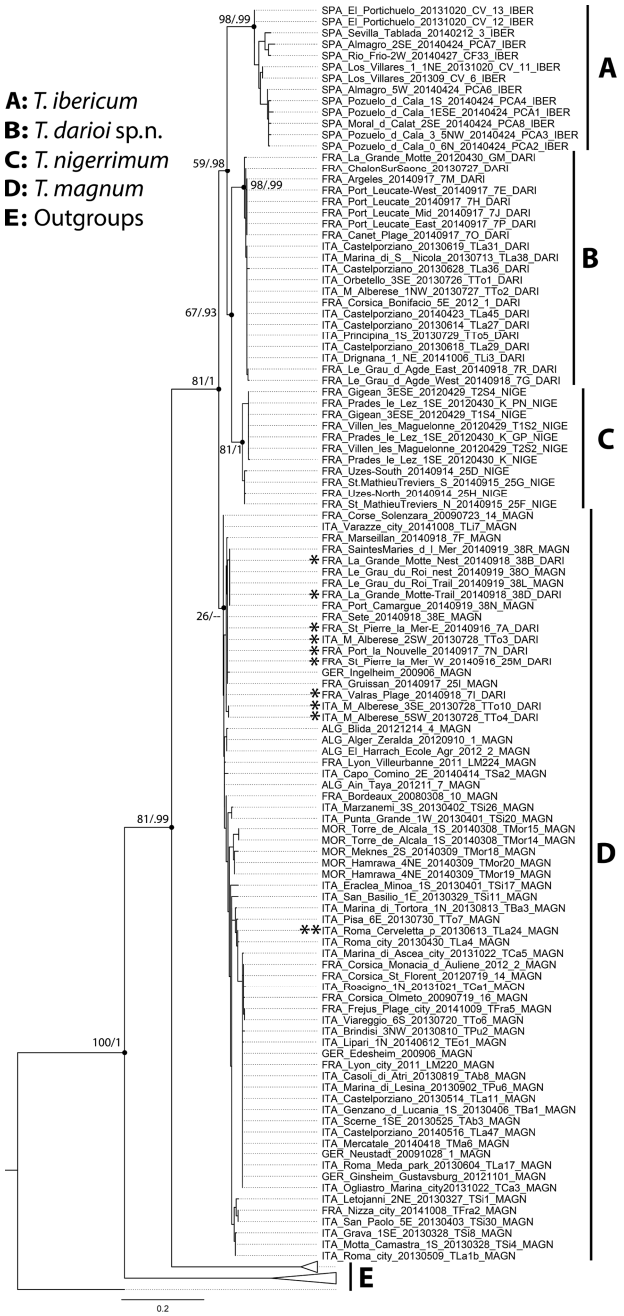


Fig. 6: Phylogenetic tree based on 655 bp of the COI gene built with only the samples subjected to NUMOBAT and using the topology of the Maximum Likelihood method. Numbers at each node significant for our purpose are bootstrap values (left) and posterior probabilities (right). Samples clearly classified by NUMOBAT as *Tapinoma darioi* sp.n. but showing haplotypes of *T. magnum* are marked with a single asterisk. Two asterisks mark the problematic *T. magnum* sample TLa24 (see main text) with supposed very recent introgression of *T. darioi* genes. Outgroups were collapsed: upper triangle – three samples of *T. simrothi*, lower triangle – six samples of *T. erraticum*, *T. madeirense* and *T. subboreale*, lower line – *T. sessile*.

era of plants but has also been observed in *Xenopus* frogs (HONJO & REEDER 1973), *Drosophila* flies (DURICA & KRIDER 1978, TWEEDIE & al. 1999), marine copepodes (FLOWERS & BURTON 2006), or became known as X-

Tab. 2: Position of worker nest sample means of *Tapinoma darioi* sp.n. within the vector space of a linear discriminant analysis separating from *T. magnum*. Shown are data of a discriminant calculated under consideration of all 15 morphological characters and the corresponding posterior probabilities. n = number of nest sample means.

	Samples with mismatch of NUMOBAT and mtDNA (n = 9)	**ANOVA F, p**	**Samples with congruence of NUMOBAT and mtDNA** (n = 21)
Discriminant D(15)	-2.324 ± 0.634 [-3.282, -1.441]	0.357, 0.555	-2.478 ± 0.652 [-3.872, -0.954]
Posterior probability	0.9915 ± 0.0140 [0.9656, 1.0000]	0.050, 0.824	0.9885 ± 0.0381 [0.8250, 1.0000]

Tab. 3: Nest sample means of RAV-corrected NUMOBAT characters in *Tapinoma magnum* and of *T. darioi* sp.n. with mismatching and congruent mtDNA.

	T. magnum (n = 62)	**ANOVA F, p**	***T. darioi* mismatching** (n = 9)	**ANOVA F, p**	***T. darioi* congruent** (n = 21)
CS [µm]	943 ± 84	4.79, 0.032	878 ± 76	1.84, n.s.	835 ± 80
CL / CW_{900}	1.064 ± 0.015	11.03, 0.001	1.047 ± 0.014	1.58, n.s.	1.039 ± 0.016
SL / CS_{900}	0.980 ± 0.013	19.02, 0.000	0.960 ± 0.015	0.93, n.s.	0.965 ± 0.011
$ExOcc / CS_{900}$ [%]	1.45 ± 0.48	8.37, 0.005	1.96 ± 0.60	2.84, n.s.	2.26 ± 0.38
$ExCly / CS_{900}$ [%]	8.79 ± 0.57	43.60, 0.000	10.17 ± 0.70	7.59, 0.010	9.64 ± 0.37
$ExClyW / CS_{900}$ [%]	6.61 ± 0.58	6.12, 0.016	6.09 ± 0.60	0.02, n.s.	6.06 ± 0.58
$ExCly / ExClyW_{900}$ [%]	1.341 ± 0.143	42.69, 0.000	1.675 ± 0.144	1.10, n.s.	1.606 ± 0.172
$nExCly_{900}$	13.20 ± 3.13	0.17, n.s.	12.73 ± 3.58	0.01, n.s.	12.84 ± 2.32
dAN / CS_{900}	0.308 ± 0.007	4.17, 0.045	0.303 ± 0.007	2.73, n.s.	0.299 ± 0.005
EL / CS_{900}	0.260 ± 0.006	7.84, 0.007	0.254 ± 0.008	0.90, n.s.	0.256 ± 0.005
$MpGr / CS_{900}$ [%]	3.92 ± 0.51	45.18, 0.000	2.69 ± 0.48	0.31, n.s.	2.59 ± 0.43
MW / CS_{900}	0.644 ± 0.014	5.77, 0.019	0.632 ± 0.014	0.04, n.s.	0.631 ± 0.012
ML / CS_{900}	1.299 ± 0.022	5.76, 0.019	1.279 ± 0.027	0.92, n.s.	1.287 ± 0.015
$Fu2 / CS_{900}$ [%]	14.87 ± 0.32	2.28, n.s.	14.71 ± 0.20	3.81, n.s.	14.51 ± 0.28
$IFu2_{900}$	1.913 ± 0.064	9.31, 0.003	1.980 ± 0.041	4.78, 0.037	1.952 ± 0.028

chromosome inactivation in the somatic cells of female mammals (KAY & al. 1994, WILLARD 1996, HEARD & al. 1997). If gene silencing is observed in a species pair, it is found in only a fraction of F1 hybrids and is reversible in the next generation (NAVASHIN 1934, PREUSS & PIKAARD 2007). Gene silencing has been attributed to transcription factor competition and seems to occur only at a specific time of early development when nuclear dominance is first established (NEVES & al. 1995). Furthermore it has been demonstrated in *Arabidopsis thaliana* × *A. arenosa* hybrids that the direction of nucleolar dominance can be reversed (CHEN & al. 1998). Considering this currently available information on gene silencing in F1 hybrids and its unstable behaviour in the next generations, it appears unlikely that gene silencing can generate worker populations of consistently pure phenotypes in nine colonies of supercolonial ants each containing hundreds or thousands of reproductive females.

The fully undisturbed *Tapinoma darioi* sp.n. phenotypes in these conflicting samples suggest them not to represent F1 hybrids and probably also not backcrosses of hybrids with a parental species. This conclusion is derived from knowledge about character expression in some 35 different interspecific hybrid combinations studied in the ant genera *Acanthomyops*, *Formica*, *Lasius*, *Leptothorax*, *Messor*, *Myrmica*, *Myrmoxenus*, and *Temnothorax* (BAGHERIAN YAZDI & al. 2012, BUSCHINGER 1972, JESSEN & KLINKRICHT 1990, SEIFERT 1984b, 1991, 1999, 2006, SEIFERT & al. 2010, STEINER & al. 2011, WING 1968). These studies showed that an average of about 50% of characters are intermediate, nearly the same number fluctuates strongly in the direction of one or the other parental species and very few, perhaps some 3%, show mean values even exceeding the character expression in parental species.

Accordingly, explanations (c) – incomplete lineage sorting – or (d) – rather recent hybridization with subsequent unidirectional genomic purging of nuDNA – appear as the most likely evolutionary histories. The latter hypothesis seems to have the strongest support by our data. Firstly, we have examples of exactly the same mtDNA sequence in clear *Tapinoma darioi* sp.n. and *T. magnum* supercolonies: The phenotypically clear *T. magnum* supercolony from La Grande Motte and the phenotypically clear *T. darioi* sp.n. supercolony from Le Grau du Roi (10 km away) were both sampled twice and coincided in having haplotype h026, and the *T. magnum* supercolony from Sète coincided with the *T. darioi* sp.n. supercolony from Saint-Pierre-la-Mer (55 km distant) in having haplotype h031. Secondly,

Tab. 4: Mean (minimum-maximum) genetic K2p distances in % among four major clades of the *Tapinoma nigerrimum* complex and within each clade (diagonal in heavy type).

	T. darioi	***T. nigerrimum***	***T. magnum***	***T. ibericum***
T. darioi sp.n.	**0.4 (0.0 - 0.5)**			
T. nigerrimum	2.0 (1.8 - 2.9)	**0.4 (0.0 - 0.9)**		
T. magnum	1.8 (1.2 - 2.4)	2.4 (2.8 - 2.9)	**0.8 (0.0 - 1.6)**	
T. ibericum	3.6 (3.0 - 4.6)	4.0 (3.1 - 4.4)	3.8 (2.7 - 4.8)	**1.3 (0.0 - 2.0)**

the geographic distribution of *T. darioi* sp.n. samples – two small areas in France and one in Italy with conflicting samples are separated by areas not showing conflicting samples (Fig. 7b) – is also suggesting more recent, independent events of mtDNA introgression. A mosaic-like distribution should be lost after very long time intervals. At least we can risk to say that these events took place long after the separation of the *T. magnum* and *T. darioi* sp.n. main clades 1.5 (1.0 - 2.0) Ma before present – this estimate is based on 1.8% genetic distance and a mean Protostomian substitution rate of 1.23% per million years according to the K2p model (WILKE & al. 2009). In contrast to this, the mean K2p distance between the mismatching *T. darioi* sp.n. samples and the *T. magnum* samples sharing the same branch of 0.29% would correspond to 0.23 (0.13 - 0.32) Ma.

Apart from the methodological problems with such datings (see TAKAHATA 2007 and references therein, WILKE & al. 2009), we may expect hybridizations to have occurred independently at different places and different times from mid Pleistocene up to the Holocene. Regarding recent or very recent hybridization events we have to consider anthropogenous introduction of species into areas where other species were already established. Introduction of alien species is one of the most frequent causes for hybridization because there is an increased likelyhood that species that never met before did not develop sufficient pre- or postzygotic isolation mechanisms. Hybridization of introduced with autochthonous species is a constantly growing issue not only for species conservation (e.g., BEHM & al. 2010, DREIJERS & al. 2013, MUNOZ-FUENTES & al. 2007, SEIFERT 2006). The main way to introduce *Tapinoma* species most probably was transport of potted plants and trees over the Mediterranean. This should have happened already during the time of the Roman Empire. Much later, a real wave of shipping such materials to newly founded tourist and recreation areas at the Mediterranean coast of France and Italy started in the middle of the 19th century. One may speculate, for instance, that the highly invasive *Tapinoma magnum* has been introduced from Africa to Italy and South France, resulting in occasional hybridization with the autochthonous *T. darioi* sp.n.

Hybridization between ant species is frequent on the per-species level: 18% of 178 Central European ant species are known to hybridize (SEIFERT 1999, KULMUNI & al. 2010, SEIFERT & al. 2010, STEINER & al. 2011, SEIFERT 2013). The estimated mean hybridization frequency in the Central European ant fauna on the per-individual level is about 1% – or about 2% if all cases with social cleptogamy are included (SEIFERT 1999, 2006, 2013). Accordingly, hybridization should represent a significant factor in ant evolution. In the case reported here, *Tapinoma magnum* gynes obviously have been mated by *T. darioi* sp.n. males and the backcrossing of the hybrid gynes and of the next generations of their female offspring was preferentially (or always) with *T. darioi* sp.n. males. Asymmetric backcrossing and introgression is a normal and well-known post-hybridization scenario in bisexual organisms (e.g., WIRTZ 1999, TIFFIN & al. 2001, GARCIA-VAZQUEZ & al. 2004). This may be caused by prezygotic mechanisms such as asymmetric mate acceptance / recognition (e.g., DEVIS & al. 1997, SAETHER & al. 2007, SCHEFFLER & al. 1996, WIRTZ 1999), asymmetric pollination (e.g., FIELD & al. 2011, NATALIS & WESELINGH 2012), gamete selection (O'RAND 1988, SHIRT & ANGUS 1992), population structure and density (BETTLES & al. 2005), or, as special scenario in ants, by a combination of intranidal mating, queen dominance and polygyny (SEIFERT 2010, SEIFERT & al. 2010). Asymmetric postzygotic mechanisms are largely explained by Dobrzhansky-Muller incompatibilities (e.g., ABBOTT & al. 2013, CAUWELLIER & al. 2012).

Table 4 shows mean K2p substitution rates among the four clades ranging from 1.8% to 4.0%. Using the mean Protostomian substitution rate of 1.23% per million years according to the K2p model (WILKE & al. 2009), species-splitting took place from late Pliocene to early Pleistocene (3.3 - 1.5 Ma). This suggests that the last phase of speciation could have been accelerated by isolation in glacial refuges. Between-clade K2p distances of mtDNA are clearly larger than within-clade distances. The latter are 0.4% in *Tapinoma darioi* sp.n., 0.4% in *T. nigerrimum*, 0.8% in *T. magnum*, and 1.3% in *T. ibericum*. The rather strong within-clade differentiation of mtDNA haplotypes in the latter species does not find any correlate in the NUMOBAT data or zoogeography and is unlikely to have taxonomic significance.

The development of only weak morphological differences during such long divergence times appears remarkable. A possible explanation for this low variation is a strong selective burden on many of the investigated NUMOBAT characters in connection with weak interspecific differences in utilization of space and food sources. An adaptive value of taxonomically informative characters such as length of scape and funiculus segments or eye size is immediately intelligible but, surprisingly, this is also given in depth of clypeal excision (SEIFERT 2016).

The mtDNA haplotypes suggest that invasive populations of *Tapinoma magnum* established north of the Alps originate from different source populations in Italy and France. The German populations have the haplotypes h038 and h057. Haplotype h057 is the most abundant sequence

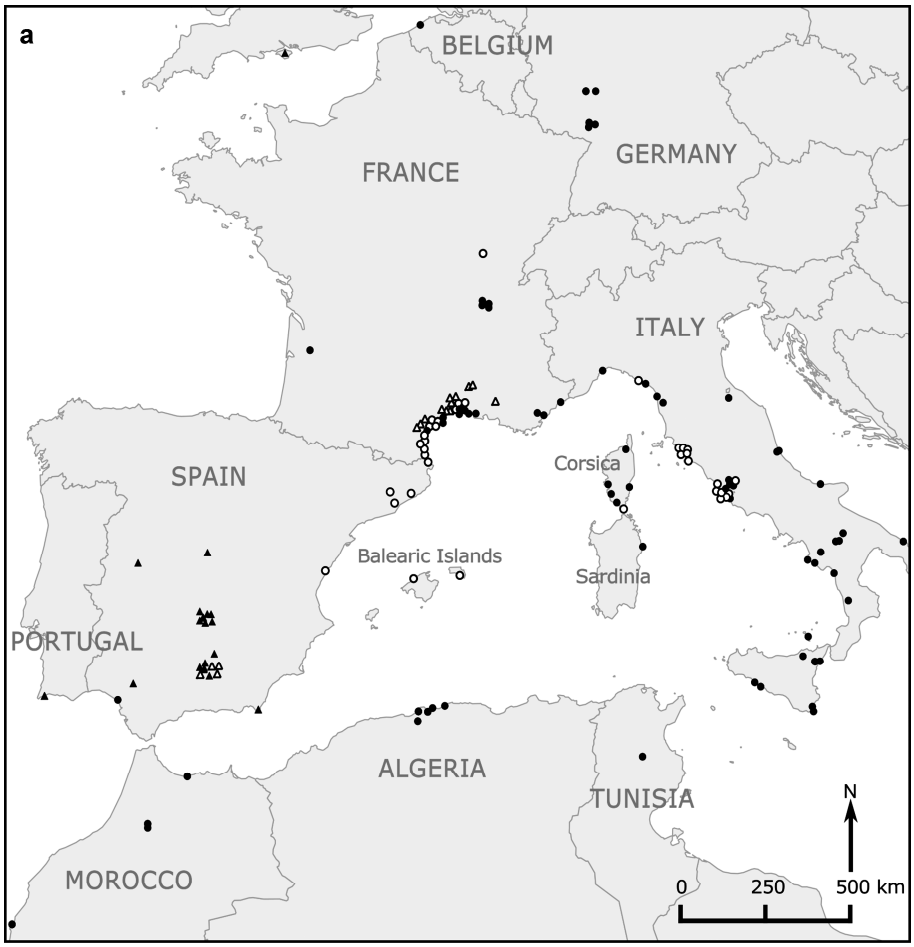


Fig. 7a: Map of all collecting sites of the NUMOBAT study. White discs = *Tapinoma darioi* sp.n. black discs = *T. magnum*, white triangles = *T. nigerrimum*, black triangles = *T. ibericum*. For a close-up view of heavily sampled areas see Fig. 7b.

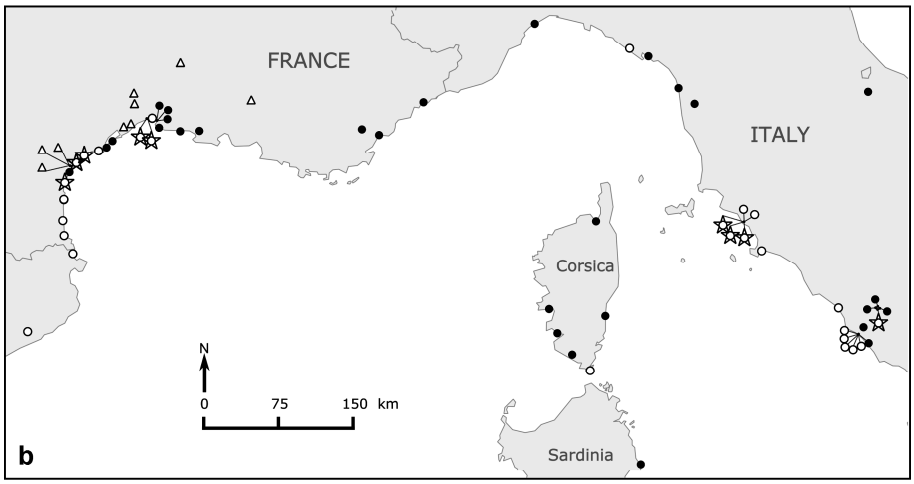


Fig. 7b: Map of samples used in the NUMOBAT study, showing the intensively sampled areas of France and Italy, as well as the *Tapinoma dario* sp.n. samples clustering with *T. magnum* in the mtDNA phylogenetic tree. Asterisk = *T. darioi* sp.n. with *T. magnum* COI; white discs = *T. darioi* sp.n., black discs = *T. magnum*, white triangles = *T. nigerrimum*.

in all NUMOBATed samples: It is found in 16 Italian, three French, and three German populations. The number of introduction events to Germany is certainly larger than three and is connected with transport of potted plant material. In the extreme, enormous amounts of soil in the cubic meter range are transferred in within the root system of old olive trees sold by Mediterranean farmers to garden centers in Germany.

Comments on geographic distribution and biology of the four species

The geographic distribution of the four cryptic species shown by Figure 7a provides an incomplete picture but some general conclusions may be derived. The most unknown aspects are the upper limits of altitudinal distribution in the Mediterranean region and occurrence in natural or semi-natural habitats. The phylogeography revealed by species occurrence and mtDNA phylogeny remains unclear in many respects. The present distribution is expected to deviate from the natural situation, mostly because of anthropogenous translocation of at least three species one of which is highly invasive. *Tapinoma nigerrimum* is spread across France and Spain. Yet, it has been impossible to retrieve DNA from the Spanish *T. nigerrimum* samples, which would have shed some light on the homogeneity of the species across the Pyrenees, and the position of its likely glacial refuge. Use

Tab. 5: Frequency of the *Tapinoma nigerrimum* complex species along the coast line of Mediterranean Sea compared to inland areas. Given are p values of Fisher's exact test in a 2 × 2 table. Test 1 compares the observations in a particular species against a homogenous distribution of equal total sample size. Test 2 compares the observation in a particular species against the sum of observations in the other three species. The data of *T. ibericum* are probably misleading (see main text).

	Along shore line ≤ 4 km from sea	**Inland areas > 4 km from sea**	**Test 1: against homogenous distribution**	**Test 2: against sum of the other three species**
T. magnum	44	35	0.5793	0.2682
T. darioi sp.n.	32	7	0.0054	0.0000
T. nigerrimum	2	17	0.0185	0.0001
T. ibericum	(3)	(19)	(0.0217)	(0.0002)
sampling spots	81	78		

of appropriate nuDNA markers is required to elucidate phylogeography and invasive dynamics within the *T. nigerrimum* species complex. Regarding information on biology published in the past under the name "*Tapinoma nigerrimum*", a post hoc allocation of the valid taxonomic names was sometimes possible using geographic information and some other cases could be cleared up by NUMOBAT investigation of material sent to the senior author. This paper gives only fragmentary information on the biology of these ants – we publish this paper with the hope that some Mediterranean researchers are prompted to do in-depth studies revealing specific traits of the cryptic species and their interspecific relations.

Comments on distribution and biology of *Tapinoma magnum*

The supercolonial *Tapinoma magnum* has by far the widest distribution among the four species. It is found in North Africa from Morocco east to Tunisia, over entire Italy, in Corsica, Sardinia, and southern France. It showed the strongest invasive potential of the three supercolonial species and was anthropogenically introduced to nine sites in Germany, Belgium, and the Netherlands (HELLER 2011, DEKONINCK & al. 2015, plus data given in this paper) which are all situated north of 48° N. *Tapinoma magnum* became here a pest species with strong local impacts. The beachheads for the German introductions were garden centers and tree nurseries and for the Belgian introduction probably the harbor of Oostende. The frost resistance and low foraging temperatures are remarkable for a Mediterranean ant. Colonies survived in Germany a 14-days frost period with mean air temperatures of -6.6 °C and an absolute minimum of -15 °C without any visible damage (DEKONINCK & al. 2015). The German populations of *T. magnum* showed the last activity in late December at air temperatures of 8 °C and resumed activity after snowmelt in January during a cloudy day and mean and maximum air temperatures of 10.6 and 12.2 °C (DEKONINCK & al. 2015; G. Heller, pers. comm.).

In the Mediterranean area, *Tapinoma magnum* is particularly abundant in open unstable or degraded areas with significant to very strong anthropogenic influence and a weakly developed tree layer. It is more abundant on sandy soils and significantly rarer on rock. If reports from South France (BERNARD 1968, 1983) should largely refer to *T. magnum*, it shows a quite developed tolerance against flooding, occurs in high numbers on irrigated clay soils in areas of market gardening and is found even in swampy habitats. In contrast to *T. darioi* sp.n., there is no preference of coastal habitats in *T. magnum*: 44 samples were taken along the shore line within a maximum distance of 4 km from sea and 35 samples inland more than 4 km from sea (Tab. 5). This is not significantly different from a homogenous distribution (Fisher's exact test p = 0.5793). *Tapinoma magnum* seems to be absent from Iberia except for a beachhead in southernmost Spain. The rarity in Spain is somewhat surprising considering the strong invasive potential of *T. magnum.* The dominance of the supercolonial *T. ibericum* in southern Iberia and of the supercolonial *T. darioi* sp.n. in northern Spain probably will have hampered a colonization of these areas by *T. magnum.* We got the general impression that syntopic occurrence of the three supercolonial species is exceptional.

According to geography of collecting sites (we did not get samples from the authors), the investigations of BLIGHT & al. (2010) can be referred with a fair probability to *Tapinoma magnum*, but *T. darioi* sp.n. cannot be excluded. BLIGHT & al. (2010) showed that their *Tapinoma* ants limited the spread of the invasive Argentine Ant, *Linepithema humile* (MAYR, 1866) in Corsica and southern France. In space and food competition assays the *Tapinoma* species of BLIGHT & al. (2010) was more efficient than *Linepithema* in both interference and exploitative competition, clearly superior in direct fighting, dominated food in 100% of the replicates after one hour, and invaded *Linepithema* nests while the reverse was never observed. Such isolated laboratory investigations do not necessarily tell us who is the final winner in a particular outdoor confrontation because this is determined not only by basic fighting and recruiting properties but also by ecological adaptation and demographic factors. Strong intraspecific aggressivity between polydomous colonies of *T. magnum* in gardener's centres of Germany suggests either repeated introductions to the same site or secondary colony splitting – artificially isolated colony fragments developed a significant aggressivity between former nest mates already six months after separation (G. Heller, pers. comm.). Protection of vine and citrus mealybug colonies by *T. magnum* (a posteriori determined by geographic indication) significantly reduced the effect of several parasitoids and predators with the exception of adult Coccinellidae (MANSOUR & al. 2012). In south

Italy, *T. magnum* caused direct damage in horseradish cultures by injuring of plants and licking of phloem sap (D. Battaglia, pers comm., sample Potenza-2014.07.15).

The nests are subterranean and often very extended, frequently reaching to a depth of 1 m. Nest entrances typically develop to big crater-like domes of ejected soil particles. When occurring in sand dunes (a typical habitat is the back of coastal sand dunes closest to sea) they excavate and maintain long-lasting trails in the sand which are V-shaped in section and up to 5 cm deep. The biggest supercolonies stretch over areas of one hectare or more, should number > 20 million workers and show a permanent exchange of broods between the nests. Single nest spots may contain up to 350 queens. Alates occurred in Italy, Germany, and the Netherlands 10 May ± 26 d [2 April - 17 June] n = 9 (arithmetic mean ± standard deviation [earliest, latest] number of observations). This is apparently the main period but there is an observation of alates in Algeria in August / September. Swarming occurred in May and June, not earlier than 2 - 3 weeks after eclosion from pupae. Most mated gynes stay in or near to the home colony seeking adoption in conspecific nests but their big bodies and well-developed flight muscles should indicate a basically good potency for dispersal flight and independent colony foundation.

Comments on distribution and biology of *Tapinoma darioi* sp.n.

Tapinoma darioi sp.n. is found in northeast Spain, the Balearic islands, southern France, Corsica, and northwest Italy south to Rome in an area delimited by 39.8° N, 44.2° N, 0.1° W, and 12.4° E. In contrast to *T. magnum*, there is a clear preference for coastal areas: 32 samples were taken along the shore line within a maximum distance of 4 km from beach and seven samples more than 4 km behind shore. This is significantly different from a homogenous distribution over shoreline and inland (Fisher's exact test p = 0.0054). With 16 sequences belonging to 10 haplotypes, *T. darioi* sp.n. is more diverse in southern France than in Italy where 11 investigated sequences belonged to only two haplotypes. This suggests a radiation center of this species in southern France. A disjunct, northern population at Chalon-sur-Saone (46.78° N, 4.86° E) and the introduction to Wageningen (51.98° N, 5.67° E) indicate a tramp species potential.

Nest construction and the build-up of an impressive network of deeply excavated trails in coastal dune habitats is the same as in *Tapinoma magnum*. The information on biology given by XERDA & al. (1989) could be referred to *T. darioi* sp.n. by geographic indication. Foraging in northeast Spain is diurnal from February to May and crepuscular and nocturnal from June to November. Very extensive trophobiosis with aphids occurs both on high trees and in the herb layer – honey dew accounts for probably > 80% of total food mass. Consumption of nectar, elaiosomes of diverse myrmecochorous plants and of fallen sweet fruits is less important. Carnivory of insects, snails and spiders is significant but probably more a collecting of carcasses than active hunting. Predation of sexuals of ants alighting after nuptial flight may be intensive. After discovery of very large food items, a mass recruitment starts. The item is disintegrated and the cut-off pieces are retrieved by single workers. Cooperative transport of items is so far unknown. XERDA & al. (1989) also observed a tool use: Small twigs or pebbles are dropped into liquid food and retrieved after impregnation. The phenology of sexual production is poorly studied but apparently comparable to the situation in *T. magnum*: 22 May ± 20 d [23 April - 23 June] n = 12. There is one observation of alates from France 16 September 2014. One flight observed in Wageningen / Netherlands took place 9:23 h ST on 31 May 2014 (11:00 h CEST according to NOORDIJK (2016), adjusted to solar time ST). Foraging time and temperatures of the Wageningen population are comparable to observations in German *T. magnum*: last activity was in late December at air temperatures of 3 °C (cloudy day) or -2 °C (sunny day) and activity was resumed by the end of February at 6 °C (NOORDIJK 2016).

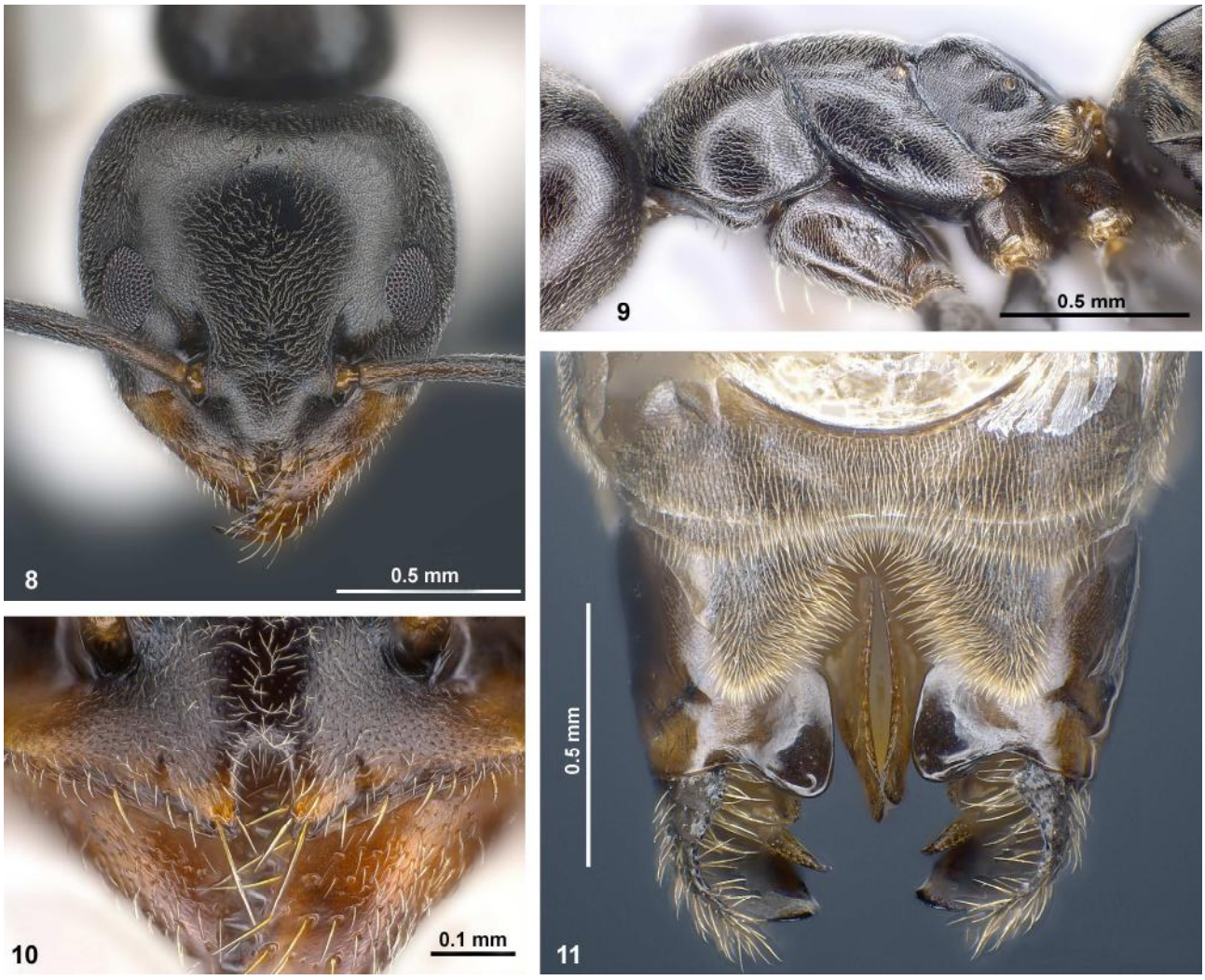
Supercolonies have the same extension as in *Tapinoma magnum* and probably also the same demography and competitive power: a very large supercolony at La Grande Motte, being more than 200 m long, had occupied in 2012 just the area covered by a huge colony of *Linepithema humile* ten years ago.

Comments on distribution and biology of *Tapinoma ibericum*

There is almost nothing known on the biology of this species except for the fact that it may form large supercolonies. *Tapinoma ibericum* apparently has a weaker tramp species potential than *T. magnum.* Only one anthropogenous introduction is known so far: A mature polydomous colony was found in the Ventnor Botanic Garden on the Isle of Wight in 2016 which was probably founded during the import of several large plants from southern Spain some ten years ago (C. Pope, pers. comm.). Apart from this finding, *T. ibericum* seems to be restricted to its native range in Iberia south of 41° N – an area probably not exceeding 250,000 km^2. It is unknown if *T. ibericum* is abundant along the shore line of southern Iberia because there was no systematic sampling at coastal sites. Accordingly, the data in Table 5 are likely to be misleading. *Tapinoma ibericum* and *T. darioi* sp.n. are the only species of the complex apparently not showing a broader geographic range overlap. The phenology of sexual production, as derived from our poor data, seems to be comparable to the situation in the other species: 15 May ± 28 d [24 April - 15 June] n = 5. Low foraging temperatures are apparently also typical for this species: according to observation of the senior author in Portugal 14 February 2016, it was the only ant species showing surface activity at air temperatures of 10 °C.

Comments on distribution and biology of *Tapinoma nigerrimum*

Tapinoma nigerrimum differs from the other three species in frequently showing monodomous, not very large colonies which behave aggressively to each other. Polydomous colonies seem to exist but true supercoloniality or an invasive potential are not confirmed so far. Furthermore, there seems to be some trend in *T. nigerrimum* of selecting more natural or semi-natural habitats – without avoiding habitats with anthropogenous impact. The other three species, in contrast, are clearly more abundant in sites with strong anthropogenous pressure. *T. nigerrimum*, furthermore, shows a clear avoidance of coastal areas: Only two samples were



Figs. 8 - 11: *Tapinoma darioi* sp.n. (8 - 10) Holotype worker. (8) Head in dorsal aspect; pubescence partially abraded. (9) Mesosoma in lateral aspect. (10) Clypeus in dorsofrontal aspect; pubescence partially abraded. (11) Paratype male from the holotype's nest: genital in ventral aspect.

taken along the shore line within a maximum distance of 4 km from beach but 17 samples more than 4 km inland from shore. This is significantly different from a homogenous distribution over shore line and inland ($p = 0.0185$). As the sampling schedule of this study strongly underrecorded inland areas and thus the main habitats of *T. nigerrimum*, the relative rarity and disjunct distribution suggested by this paper are probably no real traits. Alates were observed: 4 May ± 10 d [29 April - 21 May] n = 5.

Description of *Tapinoma darioi* sp.n. (Figs. 8 - 11)

Etymology. The new species is dedicated to Dario D'Eustaccio who has been a major protagonist in this project but did not experience its completion due to his tragic death.

Type material. See above under "Type Material".

Description of worker (Figs. 8 - 10; Tab. 1; numeric data given in the description were obtained from 137 examined worker individuals of any body size, are primary data without removal of allometric variance and are arranged in the sequence arithmetic mean ± standard deviation [minimum, maximum]): Significantly smaller than the other three species of the *Tapinoma nigerrimum* complex; CW 859 ± 158 [559, 1189] µm. Depth-to-width ratio of clypeal excision and length-to-width ratio of second funiculus segment on average larger than in the other species: ExCly / ExClyW 1.609 ± 0.265 [1.115, 2.289] and IFu2 1.948 ± 0.114 [1.679, 2.289]. Mandibles with a large apical, a less large subapical, and 9 - 11 smaller teeth homogenously distributed over the whole masticatory margin. Whole head (except for clypeus) and dorsum of mesosoma always without standing setae. Long standing setae are present on the fourth gaster tergite (rarely beginning at the 2nd), on all gaster sternites, the ventral surfaces of hind and middle coxae and the frontal and caudal face of fore coxae. Anterior margin of clypeus with several setae; the two longest and strongest are inserted at anterior corners of clypeal excision (Fig. 10). All body surfaces with the exception of mandibles covered by an appressed, rather dense pubescence (Figs. 8, 9); mean distance of insertion points of pubescence hairs on central vertex of a major worker 8.5 µm. Pubescence on frontomedian vertex and clypeus more subdecumbent with 6 to 19 pubescence hairs protruding at a few micron across margin of clypeal excision. Whole body dark to blackish brown except for red-

dish brown or yellowish mandibles, anterior clypeus, and distal and proximal ends of tibiae in many major workers.

Description of male (Fig. 11): On average smaller than in the other three species of the *Tapinoma nigerrimum* complex. Data of 29 individuals: CW 1.028 ± 31 [972, 1121] µm, CL / CW 0.908 ± 0.021 [0.871, 0.961], SL / CS 0.957 ± 0.026 [0.901, 1.023], ExClyW / CS 6.83 ± 1.06 [4.15, 9.39] %, ExClyW / CS 6.96 ± 0.89 [4.27, 8.83] %, dAN / CS 0.286 ± 0.008 [0.265, 0.302], EL / CS 0.322 ± 0.009 [0.300, 0.338], ML / CS 1.847 ± 0.059 [1.727, 1.976], Fu2 / CS 25.44 ± 0.72 [23.86, 27.35] %, IFu2 2.692 ± 0.129 [2.387, 2.910], ExBasi / CS 6.62 ± 0.93 [4.68, 8.93] %, WSPL / CS 23.65 ± 3.14 [16.5, 29.9] %, ALPH 60.0 ± 6.7 [44, 71] °. The genital shows in ventral aspect a very broad basimere and a broad blade-like harpago (Fig. 11) which separates very well from members of other species complexes. However, genital morphology does not offer eye-catching differences to the other species of the *T. nigerrimum* complex. A sufficiently clear species delimitation is only possible by multivariate analyses which consider both genital and extragenital characters.

Final conclusions and recommendations for further research

Congruent results of NUMOBAT investigations in all three castes and characteristic geographic distribution patterns provide evidence for the existence of at least four cryptic species in the *Tapinoma nigerrimum* complex. This view is supported by full congruence of mtDNA and NUMOBAT clustering in three species. The mismatch of mtDNA with phenotype in three local populations of *T. darioi* sp.n. is credibly explained by taking-over *T. magnum* matrilines after hybridization events in the younger evolutionary history.

Future research should investigate the special zoology and autecology of these interesting species and study interspecific relations under syntopic or sympatric occurrence in contact zones. We are far from really understanding the species. There is insufficient knowledge of the mating scenarios of each species, on the relation between intranidal mating and swarming flight, inbreeding and outbreeding, on the frequency of single-queen (flight) dispersal and colony foundation relative to dispersal by colony fission. Answering these questions will also allow a better assessment of the invasive potential. Larger segments of geographic distribution are also unknown. A web-based distribution map of "*T. nigerrimum*" in Iberia (http://www.hormigas.org/xEspecies/Tapinoma%20nigerrimum.htm), for example, shows plenty of collecting points along the southern Spanish shore line. Do these points refer to *T. ibericum* or to another species?

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References

ABBOTT, R., ALBACH, D., ANSELL, S., ARNTZEN, J.W., BAIRD, S.J.E, BIERNE, N., BOUGHMAN, J., BRELSFORD, A., BUERKLE, C.A., BUGGS, R., BUTLIN, R.K., DIECKMANN, U., EROUKHMANOFF, F., GRILL, A., CAHAN, S.H., HERMANSEN, J.S., HEWITT, G., HUDSON, A.G., JIGGINS, C., JONES, J., KELLER, B., MACZEWSKI, T., MALLET, J., MARTINEZ-RODRIGUEZ, P., MÖST, M., MULLEN, S., NICHOLS, R., NOLTE, A.W., PARISOD, C., PFENNIG, K., RICE, A.M., RITCHIE, M.G., SEIFERT, B., SMADJA, C.M., STELKENS, R., SZYMURA, J.M., VÄINÖLÄ, R., WOLF, J.B.W. & ZINNER, D. 2013: Hybridization and speciation. – Journal of Evolutionary Biology 26: 229-246.

BAGHERIAN YAZDI, A., MÜNCH, W. & SEIFERT, B. 2012: A first demonstration of interspecific hybridization in *Myrmica* ants by geometric morphometrics (Hymenoptera: Formicidae). – Myrmecological News 17: 121-131.

BEHM, J.E., IVES, A.R. & BOUGMAN, J.W. 2010: Breakdown in postmating isolation and the collapse of a species pair through hybridization. – The American Naturalist 175: 11-26.

BERNARD, F. 1968: Les fourmis d'Europe occidentale et septentrionale. – Faune de l'Europe et du Bassin Méditerranéen 3, Masson et Cie Èditeurs, Paris, 411 pp.

BERNARD, F. 1983: Les fourmis et leur millieu en France méditerranéenne. – Encyclopédie Entomologique 16, Lechevalier, Paris, 149 pp.

BETTLES, C.M., DOCKER, M.F., DUFOUR, B. & HEATH, D.D. 2005: Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. – Journal of Evolutionary Biology 18: 1220-1233.

BLIGHT, O., PROVOST, E., RENUCCI, M., TIRARD, A. & ORGEAS, J. 2010: A native ant armed to limit the spread of the Argentine ant. – Biological Invasions 12: 3785-3793.

BUSCHINGER, A. 1972: Kreuzung zweier sozialparasitischer Ameisenarten, *Doronomyrmex pacis* KUTTER und *Leptothorax kutteri* BUSCHINGER (Hymenoptera: Formicidae). – Zoologischer Anzeiger 189: 169-179.

CAUWELIER, E., GILBEY, J., JONES, C.S., NOBLE, L.R. & VERSPOOR, E. 2012: Asymmetrical viability in backcrosses between highly divergent populations of Atlantic salmon (*Salmo salar*): implications for conservation. – Conservation Genetics 13: 1665-1669.

CASQUET, J., THEBAUD, C. & GILLESPIE, R.G. 2012: Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. – Molecular Ecology Resources 12: 136-141.

CHEN, Z.J., COMAI, L. & PIKAARD, C.S. 1998: Gene dosage and stochastic effects determine the severity and direction of uniparental rRNA gene silencing (nucleolar dominance) in *Arabidopsis* allopolyploids. – Proceedings of the National Academy of Sciences of the United States of America 95: 14891-14896.

DEKONINCK, W., PARMENTIER, T. & SEIFERT, B. 2015: First records of a supercolonial species of the *Tapinoma nigerrimum* complex in Belgium. – Bulletin de la Société royale belge d'Entomologie / Bulletin van de Koninklijke Belgische Vereniging voor Entomologie 151: 206-209.

DEVIS, N., AIELLO, A., MALLET, J., POMIANKOWSKI, A. & SILBERGLIED, R.E. 1997: Speciation in two neotropical butterflies: extending Haldane's rule. – Proceedings of the Royal Society B-Biological Sciences 264: 845-851.

DREIJERS, E., REISE, H. & HUTCHINSON, J.M.C. 2013: Mating of the slugs *Arion lusitanicus* auct. non MABILLE and *A. rufus*

(L.): Different genitalia and mating behaviours are incomplete barriers to interspecific sperm exchange. – Journal of Molluscan Studies 79: 51-63.

DURICA, D.S. & KRIDER, H.M. 1978: Studies on the ribosomal RNA cistrons in *Drosophila* hybrids. II. Heterochromatic regions mediating nucleolar dominance. – Genetics 89: 37-64.

FIELD, D.L., AYRE, D.J., WHELAN, R.J. & YOUNG, A.G. 2011: Patterns of hybridization and asymmetrical gene flow in hybrid zones of the rare *Eucalyptus aggregata* and common *E. rubida*. – Heredity 106: 841-853.

FLOWERS, J.M. & BURTON, R.S. 2006: Ribosomal RNA gene silencing in interpopulation hybrids of *Tigriopus californicus*: nucleolar dominance in the absence of intergenic spacer subrepeats. – Genetics 173: 1479-1486.

GARCIA-VAZQUEZ, E., PEREZ, J., AYLLON, F., MARTINEZ, J.L., GLISE, S. & BEALL, E. 2004: Asymmetry of post-F1 interspecific reproductive barriers among brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). – Aquaculture 234: 77-84.

GOUY, M., GUINDON, S. & GASCUEL, O. 2010: SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. – Molecular Biology and Evolution 27: 221-224.

GUINDON, S., DUFAYARD, J.F., LEFORT, V., ANISIMOVA, M., HORDIJK, W. & GASCUEL, O. 2010: New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. – Systematic Biology 59: 307-321.

HABERMEHL, G. 1976: Gifttiere und ihre Waffen. – Springer Berlin-Heidelberg-New York, NY. 126 pp.

HEARD, E., CLERC, P. & AVNER, P. 1997: X-chromosome inactivation in mammals. – Annual Review of Genetics 31: 571-610.

HEBERT, P.D.N., PENTON, E.H., BURNS, J.M., JANZEN, D.H. & HALLWACHS, W. 2004: Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. – Proceedings of the National Academy of Sciences of the United States of America 101: 14812-14817.

HELLER, G. 2011: *Pheidole pallidula* (NYLANDER, 1849) und *Tapinoma nigerrimum* (NYLANDER, 1886) (Hymenoptera: Formicidae), etablierte Neozoa in Rheinland-Pfalz. – Mainzer naturwissenschaftliches Archiv 48: 273-281.

HONJO, T. & REEDER, R.H. 1973: Preferential transcription of *Xenopus laevis* ribosomal RNA in interspecies hybrids between *Xenopus laevis* and *Xenopus mulleri*. – Journal of Molecular Biology 80: 217-228.

HUELSENBECK, J.P. & RONQUIST, F. 2001: MRBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics 17: 754-755.

JESSEN, K. & KLINRICHT, M. 1990: Hybridization in the social parasitic ant genus *Epimyrma* (Hymenoptera: Formicidae). – Insectes Sociaux 37: 273-293.

JOSEPH, L., WILKE, T., TEN HAVE, J. & CHESSER, R.T. 2006: Implications of mitochondrial DNA polyphyly in two ecologically undifferentiated but morphologically distinct migratory birds, the Masked and Whitebrowed Woodswallows *Artamus* spp. of inland Australia. – Journal of Avian Biology 37: 625-636.

KAY, G.F., BARTON, S.C., SURANI, M.A. & RASTAN, S. 1994: Imprinting and X chromosome counting mechanisms determine Xist expression in early mouse development. – Cell 77: 639-650.

KULMUNI, J., SEIFERT, B. & PAMILO, P. (2010): Segregation distortion causes large-scale differences between male and female genomes in hybrid ants. – Proceedings of the National Academy of Sciences of the United States of America 107: 7371-7376.

KUTTER, H. 1977: Hymenoptera-Formicidae. In: SAUTER, W. (Eds.): Fauna Insecta Helvetica 6. – Schweizerische Entomologische Gesellschaft, Zürich, 298 pp.

LACHENBRUCH, P. & MICKEY, M. 1968: Estimation of error rates in discriminant analysis. – Technometrics 10: 1-11.

LESAFFRE, E., WILLEMS, J.L. & ALBERT, A. 1989: Estimation of error rate in multiple group logistic discrimination. The approximate leaving-one-out method. – Communication in Statistics: Theory and Methods 18: 2989-3007.

LIBRADO, P. & ROZAS, J. 2009: DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. – Bioinformatics 25: 1451-1452.

MANSOUR, R., SUMA, P., MAZZEO, G., LA PERGOLA, A., PAPPALARDO, V., GRISSA LEBDI, K. & RUSSO, A. 2012: Interactions between the ant *Tapinoma nigerrimum* (Hymenoptera: Formicidae) and the main natural enemies of the vine and citrus mealybugs (Hemiptera: Pseudococcidae). – Biocontrol Science and Technology 22: 527-537.

MUÑOZ-FUENTES, V., VILÀ, C., GREEN, A.J., NEGRO, J.J. & SORENSON, M.D. 2007: Hybridization between white-headed ducks and introduced ruddy ducks in Spain. – Molecular Ecology 16: 629-638.

NATALIS, L.C. & WESSELINGH, R.A. 2012: Post-polination barriers and their role in assymmetric hybridization of *Rhinanthus* (Orobranchaceae). – American Journal of Botany 99: 1847-1856.

NAVASHIN, M. 1934: Chromosomal alterations caused by hybridization and their bearing upon certain general genetic problems. – Cytologia 5: 169-203.

NEVES, N., HESLOP-HARRISON, J.S. & VIEGAS, W. 1995: rRNA gene activity and control of expression mediated by methylation and imprinting during embryo development in wheat × rye hybrids. – Theoretical and Applied Genetics 91: 529-533.

NILSEN, G., BORGAN, O., LIESTOEL, K. & LINGJAERDE, O.C. 2013: Identifying clusters in genomics data by recursive partitioning. – Statistical Applications in Genetics and Molecular Biology 12: 637-652.

NOORDIJK, J. 2016: Leefwijze van *Tapinoma nigerrimum* (Hymenoptera: Formicidae), een nieuwe exotische mier in Nederland. – Entomologische Berichten 76: 86-93.

O'RAND, M.G. 1988: Sperm-egg recognition and barriers to interspecific fertilization. – Gamete Research 19: 315-328.

PIKAARD, C.S. 2000: Nucleolar dominance: uniparental gene silencing on a multi-megabase scale in genetic hybrids. – Plant Molecular Biology 43: 163-177.

POSADA, D. 2008: jModelTest: phylogenetic model averaging. – Molecular Biology and Evolution 25: 1253-1256.

PREUSS, S. & PIKAARD, C. S. 2007: rRNA gene silencing and nucleolar dominance: insights into a chromosome-scale epigenetic on/off switch. – Biochimica et Biophysica Acta 1769: 383-392.

RAMBAUT, A. & DRUMMOND, A.J. 2007: Tracer v1. 4: MCMC trace analyses tool. – <http://tree.bio.ed.ac.uk/software/tracer/>, retrieved 4 January 2012.

R DEVELOPMENT CORE TEAM (2012): R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria. – <http://www.R-project.org/>, retrieved December 2012.

RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D. L., DARLING, A., HÖHNA, S., LARGET, B., LIU, L., SUCHARD, M.A. & HUELSENBECK, J.P. 2012: MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. – Systematic Biology 61: 539-542.

SÆTHER, S.A., SÆTRE, G.-P., BORGE, T., WILEY, C., SVEDIN, N., ANDERSSON, G., VEEN, T., HAAVIE, J., SERVEDIO, M., BURES, S., KRAL, M., HJERNQUIST, M., GUSTAFSSON, L, TRÄFF, J. & QVARNSTRÖM, A. 2007: Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers. – Science 318: 95-97.

SEIFERT, B. 1984a: A method for differentiation of the female castes of *Tapinoma ambiguum* EMERY and *Tapinoma erraticum* (LATR.) and remarks on their distribution in Europe north of the Mediterranean Region. – Faunistische Abhandlungen des Museums für Tierkunde Dresden 11: 151-155.

SEIFERT, B. 1984b: Nachweis einer im Freiland aufgetretenen Bastardierung von *Leptothorax nigriceps* MAYR und *Leptothorax unifasciatus* (LATR.) mittels einer multiplen Diskriminanzanalyse. – Abhandlungen und Berichte des Naturkundemuseums Görlitz 58: 1-8.

SEIFERT, B. 1991: The phenotypes of the *Formica rufa* complex in East Germany. – Abhandlungen und Berichte des Naturkundemuseums Görlitz 65: 1-27.

SEIFERT, B. 1999: Interspecific hybridisations in natural populations of ants by example of a regional fauna (Hymenoptera: Formicidae). – Insectes Sociaux 46: 45-52.

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: Social cleptogamy in the ant subgenus *Chthonolasius* – survival as a minority. – Abhandlungen und Berichte des Naturkundemuseums Görlitz 77: 251-276.

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. 2010: Intranidal mating, gyne polymorphism, polygyny, and supercoloniality as factors for sympatric and parapatric speciation in ants. – Ecological Entomology 35 (Suppl. 1): 33-40.

SEIFERT, B. 2012: Clarifying naming and identification of the outdoor species of the ant genus *Tapinoma* FÖRSTER, 1850 (Hymenoptera: Formicidae) in Europe north of the Mediterranean region with description of a new species. – Myrmecological News 16: 139-147.

SEIFERT, B. 2013: Keynote – Frequency and evolutionary significance of hybridization in ants. In: SCHLICK-STEINER, B.C., ARTHOFER, W. & STEINER, F.M. (Eds.): Abstract Book of the 5th Central European Workshop of Myrmecology. – CEWM 2013 Organising Committee, Innsbruck, Austria, p. 41.

SEIFERT, B. 2014: A pragmatic species concept applicable to all eukaryotic organisms independent from their mode of reproduction or evolutionary history. – Soil Organisms 86: 85-93.

SEIFERT, B. 2016: Clypeal excision in *Tapinoma* FÖRSTER, 1850 ants is adaptive (Hymenoptera: Formicidae). – Contributions to Entomology 66: 1-5.

SEIFERT, B. & CSŐSZ, S. 2015: *Temnothorax crasecundus* sp. n. – a cryptic Eurocaucasian ant species (Hymenoptera, Formicidae) discovered by Nest Centroid Clustering. – Zookeys 479: 37-64.

SEIFERT, B., KULMUNI, J., PAMILO, P. 2010: Independent hybrid populations of *Formica polyctena X rufa* wood ants (Hymenoptera: Formicidae) abound under conditions of forest fragmentation. – Evolutionary Ecology 24: 1219-1237.

SEIFERT, B., RITZ, M. & CSŐSZ, S. 2014: Application of Exploratory Data Analyses opens a new perspective in morphology-based alpha-taxonomy of eusocial organisms. – Myrmecological News 19: 1-15.

SHIRT, D.B. & ANGUS, R.B. 1992: A revision of the nearctic water beetles related to *Potamonectes depressus* FABRICIUS (Coleoptera, Dityscidae). – Coleopterologists' Bulletin 46: 109-141.

SRIVATHSAN, A. & MEIER, R. 2012: On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. – Cladistics 28: 190-194.

STEINER, F.M., SEIFERT, B., GRASSO, D.A., LE MOLI, F., ARTHOFER, W., STAUFFER, C., CROZIER, R.H. & SCHLICK-STEINER, B.C. 2011: Mixed colonies and hybridisation of *Messor* harvester ant species (Hymenoptera: Formicidae). – Organisms, Diversity & Evolution 11: 107-134.

TAKAHATA, N. 2007: Molecular clock: an anti-neo-Darwinian legacy. – Genetics 176: 1-6.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013: MEGA6: molecular evolutionary genetics analysis version 6.0. – Molecular Biology and Evolution 30: 2725-2729.

TIFFIN, P., OLSON, M.S. & MOYLE, L.C. 2001: Asymmetrical crossing barriers in angiosperms. – Proceedings of the Royal Society B-Biological Sciences 268: 861-867.

TWEEDIE, S., NG, H.H., BARLOW, A.L., TURNER, B.M., HENDRICH, B. & BIRD, A. 1999: Vestiges of a DNA methylation system in *Drosophila melanogaster*? – Nature Genetics 23: 389-390.

WILLARD, H.F. 1996: X chromosome inactivation, XIST, and the pursuit of the X-inactivation center. – Cell 86: 5-7.

WILKE, T., SCHULTHEISS, R. & ALBRECHT, C. 2009: As time goes by: a simple fool's guide to molecular clock approaches in invertebrates. – American Mallacological Bulletin 27: 25-45.

WING, M.W. 1968: Taxonomic revision of the Nearctic genus *Acanthomyops* (Hymenoptera: Formicidae). – Cornell University Agricultural Experiment Station Memoir 405: 1-173.

WIRTZ, P. 1999: Mother species – father species: unidirectional hybridization in animals with female choice. – Animal Behaviour 58: 1-12.

XERDA, X., RETANA, J., BOSCH, J. & ALSINA, A. 1989: Exploitation of food resources by the ant *Tapinoma nigerrimum* (Hymenoptera: Formicidae). – Acta Oecologica 10: 419-429.

YOSHIMURA, M. & FISHER, B.L. 2011: A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): key to genera of the subfamily Dolichoderinae. – Zootaxa 2794: 1-34.