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Red wood ants in Bulgaria: distribution and density related to habitat characteristics

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Abstract
The only National Inventory of red wood ants in Bulgaria was carried out about 50 years ago (1970–1973). *Formica rufa* Linnaeus, 1761, *F. pratensis* Retzius, 1783, *F. lugubris* Zetterstedt, 1838 and *F. polyctena* (as *F. polyctena x rufa* hybrid) were found in a current monitoring programme. This study presents data on their current distribution and nest density, and provides more details about the habitat requirements for conservation purposes. Field studies were carried out by the transect method along the main mountainous areas in Bulgaria. We found 256 nests of red wood ants along 172 transects. The most abundant species was *F. lugubris*, followed by *F. rufa* and *F. pratensis*. Among the environmental variables, the elevation, exposure, ecological groups of plants, stone cover, grass cover, canopy cover and forest age appeared as significantly related to the presence and nest density of red wood ants.

Keywords
Balkans, conservation, *Formica rufa* group, habitat preferences, monitoring method

Introduction

Being territorial species, the *Formica rufa* species group, known as red wood ants (RWA), plays a keystone role in the forest ecosystems (Gösswald 1990). Out of thirteen Palaearctic species of *Formica rufa* group, ten are present in Europe: *Formica* (*Formica*) *rufa* Linnaeus, 1761, *F.* (*F.*) *lugubris* Zetterstedt, 1838, *F.* (*F.*) *paralugubris* Seifert, 1996, *F.* (*F.*) *helvetica* Seifert, 2021, *F.* (*F.*) *polyctena* Foerster, 1850, *F.* (*F.*) *pratensis* Retzius, 1783, *F.* (*F.*) *aquilonia* Yarrow, 1955, *F.* (*F.*) *truncorum* Fabricius, 1804, *F.* (*F.*) *dusmeti* Emery, 1909 and *F.* (*F.*) *frontalis* Santschi, 1919 (Seifert 2021). Despite the comprehensive studies of this group (Otto 1960, 1968; Dlussky 1967; Pavan and Ronchetti 1972; Cherix 1977; Gösswald 1989, 1990; Czechowski 1996; Stockan and Robinson 2016), its taxonomy remains unclear (Maeder et al. 2005; Bernasconi et al. 2010, 2011; Korczynska et al. 2010; Seifert 2018, 2021). Detailed information about the distribution and habitat preferences of RWA is much needed to evaluate population changes and to develop conservation and management strategies (Sorvari and Hakkarainen 2007; Freitag et al. 2008, 2016a, b; Dekoninck et al. 2010, 2014; Breen 2014; Chen and Robinson 2014; Vandegehuchte et al. 2017). Research on habitat features in respect of RWA presence and density typically addresses particular species only, and such studies from the Balkans are rather descriptive and scarce (Tsikas et al. 2016; Çamlıtepe and Aksoy 2019).

Nest density estimation of RWA varies among different regions. Risch et al. (2016) summarized data, collected from different European countries and Russia, and reported a maximum of 20 nests/ha, as usually the density is under 5 nests/ha. Nest destruction, air and heavy metal pollution, collection of ant pupae for food for cage birds were reported as the main reasons for low nest densities in Central Europe (Domisch et al. 2005). Additional reasons could also be the variation in climate and differences in habitat characteristics in each country, the different methods of counting the nests and their density, interspecies interactions, etc. RWA often establish their colonies through social parasitism, i.e. the founder-queen uses ready nests of *Formica fusca* (Czechowski et al. 2012), therefore, RWA nest density depends also on the density of their host. Another factor is the presence of their competitors (Savolainen and Vepsäläinen 1988).

Climate, light conditions, productivity and food resource availability seem to be key factors determining the distribution of ant mounds in Finland (Kilpeläinen et al. 2005). According to Vandegehuchte et al. (2017), the RWA abundance in Switzerland depends mainly on the slope aspect, climate, forest structure and conifer abundance but not on the forest fragment size, distance to forest edges, or woody vegetation diversity. Serttaş et al. (2020) reported altitude, aspect, canopy closure, landform, nest substrate and slope as significant habitat variables for *F. rufa* translocation success in Turkey. RWA avoid north-facing slopes and prefer south-, south-west- or west-facing exposure of the slopes (Risch et al. 2016). In the temperate zone, the subalpine *F. lugubris* commonly occurs at a higher elevation of mountainous areas, whereas *Formica rufa* prefers their lower parts (Seifert 2018).

For the UK forest region, Chen and Robinson (2014) reported that in shadier areas the nest size of *F. lugubris* is bigger but the canopy cover had no relation with the number of nests. In Finland *F. rufa* and *F. polyctena* have similar frequencies in an open and closed canopy, while the other RWA prefer mostly open spaces (Punttila and Kilpeläinen 2009).

The presence of conifers is a key factor for RWA existence (Vandegehuchte et al. 2017) but they prefer mixed forests to pure coniferous ones (Rosengren et al. 1979). Domisch et al. (2005) reported a statistically significant difference between nest density in a mature and young boreal forest in Finland.

RWA in Bulgaria have been under protection since 1959 (Izvestiya 1959). The first summarised records about RWA in Bulgaria were published by Otto et al. (1962). A National Inventory of RWA in Bulgaria was carried out in 1970–1973 and the results are given by Bobev (1972, 1973) and Vatov and Bobev (1976). Though these results are currently outdated, they represent a basis for an assessment of the long-term trends in the dynamics of RWA populations in Bulgaria. There is scarce information about the field methodology of the inventory: "Detailed visit of all plantations and discovery of available ant nests" (Bulgarian State Archives). Only a few Forestries keep archive details for registering the nests, fencing, marking and numbering them, filling the field form and collecting samples. In Smolyan Forestry 91 nests had been fenced with wooden cross-beams. It is not clear how exactly the nests have been counted (quadrates, transects or other methods). Detailed, quantitative data about habitat characteristics were not reported.

The last summarized literature data about the findings of RWA in Bulgaria are given by Lapeva-Gjonova et al. (2010). Later, occasional localities were published by Lapeva-Gjonova (2011, 2013), Lapeva-Gjonova and Rücker (2011), Lapeva-Gjonova and Santamaria (2011), Lapeva-Gjonova and Kiran (2012), Lapeva-Gjonova and Ilieff (2012), Antonova et al. (2016) and Lapeva-Gjonova et al. (2021).

The RWA species from Bulgaria – *Formica lugubris*, *F. polyctena*, *F. pratensis*, *F. rufa*, *F. aquilonia* and *F. truncorum* – are considered species of special conservation measures in Europe (IUCN 2021). Except for *F. truncorum*, they all are recognized as Lower Risk /Near Threatened species. All of them are included in CORINE biotopes checklist (Annex 4). In addition, *F. rufa* is protected by the Bulgarian Biodiversity Act (2002), Annex 2 and 3. However, the conservation needs of RWA are underestimated (Sorvari 2016). RWA need proper breeding habitats to support their huge colonies with feeding territory up to a few tens of square kilometres (Savolainen and Vepsäläinen 1988). We need detailed information about their habitats that could advance the conservation policy and management of territories occupied by RWA, thus protecting them more effectively. In monitoring programs, nest density should be used as a dependent variable to assess the population dynamics by quantitative analyses (Delabie et al. 2000). Predictive models describe the relationship between species' distribution and their environment allowing researchers to assess habitat quality by monitoring nests of RWA species (Freitag et al. 2016a).

The present study aims to assess the present distribution, nest density of the red wood ants in Bulgaria, as well as to examine their relationships with environmental variables and to assess the most appropriate habitat characteristics for conservation purposes.

Material and methods

Study area

The study was carried out during a monitoring project between May 2013 and September 2014 in the mountainous areas in Bulgaria. The field studies were conducted in 11 sampling sites in the following mountains: Rila, Pirin, Belasitsa, Vitosha, Osogovo, Western Stara Planina, Central Stara Planina, Eastern Stara Planina, Western Rhodopes, Eastern Rhodopes and Strandza (Fig. 1). In the fieldwork of the recent monitoring project, the efforts were concentrated on the most suitable habitats of RWA in places with registrations of literature data.

Sampling method

For the four ant species, sampling transects were set in areas with appropriate homogeneous biotopes. For a homogeneous biotope, a continuous polygon was taken from

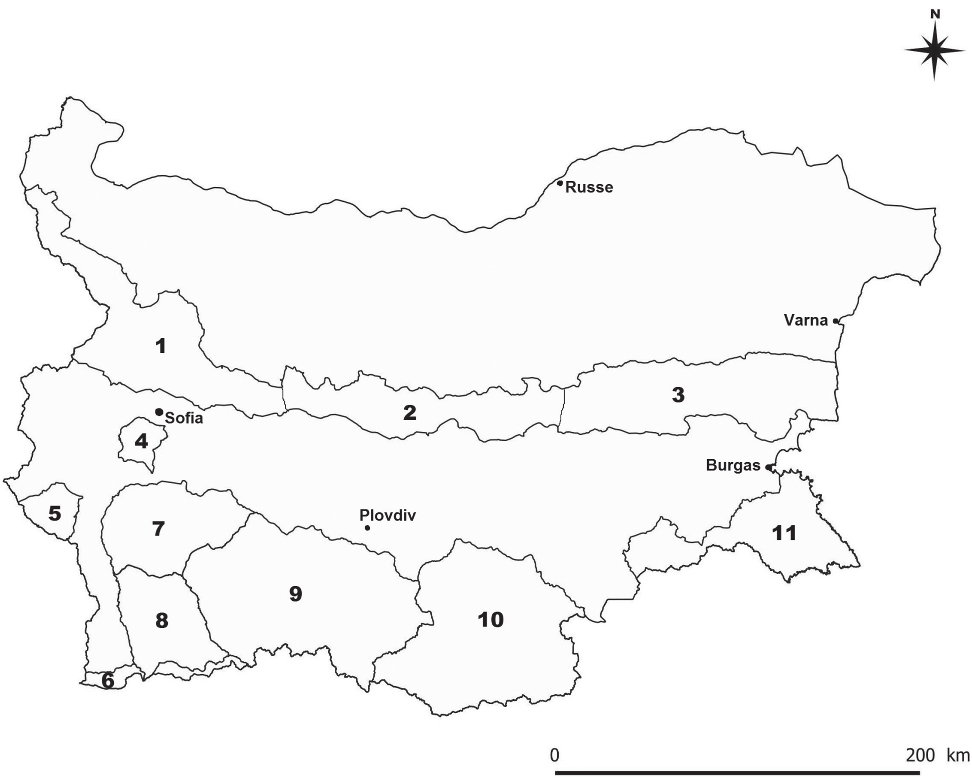


Figure 1. The eleven sampling monitoring sites (mountains): **1** Western Stara Planina, **2** Central Stara Planina, **3** Eastern Stara Planina, **4** Vitosha, **5** Osogovo, **6** Belasitsa, **7** Rila, **8** Pirin, **9** Western Rhodopes, **10** Eastern Rhodopes, **11** Strandza.

the potential habitat of each species, which was falling within a single monitoring area characterised by similar environmental features. Potential habitats are predetermined by mapping patterns or on the terrain. Potential habitat GIS models for each target RWA were made using the "intersect" tool of ArcGIS 10 with polygon vector layers of Bulgarian Forest GIS database: Dominant tree type, Canopy cover <80%, Forest age: young (<50 years), middle (50–100), old (>100 years) and Elevation according to the ecological preferences of each species by literature data for Bulgarian populations (Atanassov and Dlusskij 1992).

According to the Bulgarian landscape characteristics and specifics, we used a combination of sampling methods from other countries in the temperate climatic zone (Cherix 1977; Domisch et al. 2005; Cherix et al. 2007, 2012; Hughes and Broome 2007; Gotelli et al. 2011; Borkin et al. 2012; Zakharov et al. 2013, and Breen 2014).

In each selected monitoring area, a minimum of 8 sampling transects of 250 m and a width of 5 m were examined (i.e. 2 km length in total and 1250 m^2 per transect) across a homogeneous biotope (Borkin et al. 2012). In total, 172 sampling transects (21.5 ha) were selected. Eight to 29 transects were sampled per site (i.e. at least 10 000 m^2) (Pętal and Pisarski 1966). Transects were separated by at least 10 meters to avoid counting nests twice (Leponce et al. 2004). Each transect was visited once per year during the daylight from May to the end of September. In each transect, one GPS point was taken at the beginning of it, one at its end and one point per each localised nest inside the transect (by Garmin MAP 60CS). Thus, the same transects could be used for future monitoring studies.

The separate nests (active and abandoned) were counted, with a diameter greater than 20 cm (Domisch et al. 2005; Zakharov et al. 2013). Each nest was digitally photographed, including the surrounding vegetation within a radius of up to 30 m (Cherix et al. 2007, 2012). At least 10 ant specimens were taken from each nest in 95% ethanol (Bestelmeyer et al. 2000). All the samples were preserved in the collection of V. Antonova at the IBER, Bulgarian Academy of Sciences, Sofia.

The nests' description and environmental variables were filled in a field form (see Suppl. material 1). One field form was completed for each transect.

Nests' description:

1. Nest number: number of nests of each species within one sampling transect (1250 m^2)
2. Nest measurements and identification:
   * Diameter of the nest (± 5 cm) (for further monitoring);
   * Height of the nest (± 5 cm) (vertically from the ground level to the top of the nest, for further monitoring);
   * Active/abandoned: Binary variable (abandoned: for further monitoring) (Domisch et al. 2005); A mound with workers only passing on it was not considered as an active.

* Species. The determination of the species was done in a laboratory using the identification keys of Atanassov and Dlusskij (1992); Czechowski et al. (2012) and Seifert (2018).
* Cluster of colonies (more than one mound per colony): Binary variable (yes/no). It was noted whether the nest was isolated or there was a cluster formed (Hughes and Broome 2007) by observing the workers' behaviour by „transplant experiments": when a worker from one nest being placed near another nest is aggressively attacked by the others, the nests belong to different colonies (Kaspari 2000).

Predictor variables recorded were related to the topography and habitat characteristics of each sampling transect. Data for the first 10 variables were taken in situ as approximate assessment, based on the whole transect range:

1. Elevation: based on GPS current data.
2. Exposure of the slope (based on a compass) 8 variables: N, NW, W, SW, S, SE, E, NE.
3. Slope as degrees in 4 classes: 0–5, 6–15, 16–30, >30 degrees.
4. Ecological groups of plants (adapted for Bulgaria, Lyubenova 2004), defined by dominant plants species in respect of their adaptations to soil and air humidity in 5 categories: xerophytes (low humidity – succulents, *Euphorbia falcata*, *Dianthus petraeus*, *Poa bulbosa*, etc.); meso-xerophytes (drought-tolerant – *Hypericum perforatum*, *Adonis vernalis*, *Potentilla argentea*, *Quercus pubescens*, etc.); mesophytes (moderate humidity – *Bellis perennis*, *Festuca pratensis*, *Medicago arabica*, *Alcea rosea*, etc.); meso-hygrophytes (increased moisture affinity – *Ranunculus sceleratus*, *Carex distans*, *Juncus bufonis*, etc.); hygrophytes (high moisture – mosses, *Caltha palustris*, *Epilobium hirsutum*, *Oxalis acetosella*, etc.).
5. Stone cover as percentage in 5 classes: 0–5%, 6–25%, 26–50%, 51–75%, 76–100%.
6. Habitat type in 7 categories by EUNIS habitat classification (https://www.eea.europa.eu/data-and-maps/data/eunis-habitat-classification, Level 1 and 2; ecotones added): coniferous forest, ecotone of coniferous forest, broadleaved deciduous forest, ecotone of deciduous forest, mixed forest, ecotone of mixed forest, grasslands.
7. Grass cover as a percentage in 4 classes: <25%, 25–49%, 50–74%, 75–100%.
8. Canopy cover as a percentage in 5 classes: <20%, 20–40%, 41–60%, 61–80%, >80%.
9. Undergrowth (shrub) density as a percentage in 4 classes: <5%, 6–25%, 26–50%, 51–100%.
10. Dominant tree species of the forest in 12 categories: *Picea abies*, *Pinus sylvestris*, *Pinus nigra*, *Pinus mugo*, *Pinus peuce*/*heldreichii*, *Pinus* spp. with *Fagus* spp., *Juniperus* spp., *Fagus* spp., *Quercus* spp., *Castanea sativa*, single trees (fruit trees), grass (open habitat). Identification keys according to Delipavlov et al. (2003) were used.
11. Forest mean age as years in 4 classes: 0–50, 51–100, 101–150, >150. The data were taken from the Bulgarian Forest GIS database (www.agrolesproject.com/cgi-bin/agro1?m=med6).

Data analyses

Nest density calculations for each monitoring site were based on the total number of nests of the particular species and the total area of its sampling transects. For each monitoring site, the study surface area (the number of transects) increased proportionally to the area of the appropriate forest habitats for RWA.

Non-parametric statistics were used as our data have not normal distribution and could not be normalised by a transformation. The species represented by < 5 mounds were not included in the statistical analyses as they were too rare. Binomial logistic regression with a logit link function was used for a detailed study of the predictive significance of all independent variables (elevation, exposure, slope, ecological groups of plants, stone cover, grass cover, canopy cover, undergrowth and forest age) for the likelihood of the presence of each species. For all models, we started with a full model and followed a backward stepwise selection procedure to eliminate the effects that were furthest from statistical significance. Only the final models were presented. The categorical variables Habitat and Dominant tree were excluded from the statistical analysis because they consisted of too many levels while there were too few cases relative to the number of levels.

Spearman rank order correlation test was used for searching correlations among nest density of each species and the predictor variables used in the logistic regression analysis (missing data were pairwise deleted). All statistical analyses, except circular-linear correlation tests between exposure and ant species nest density, were performed using JASP 0.14.1. The circular-linear correlation tests and graphs were conducted with ORIANA 3.21.

Results

Distribution and nest density of RWA in Bulgaria

A total of 256 mounds (active and abandoned) were found in 10 of the 11 studied sites and 104 (61%) of the 172 sampling transects (see Suppl. material 2: Table S1). The 229 active nests (89%) belonged to four RWA species: *F. lugubris*, *F. rufa*, *F. pratensis* and *F. polyctena* x *rufa* (Table 1). Abandoned nests were 27 (11%).

The most abundant species was *Formica lugubris* with 100 nests (44%), followed by *F. rufa* with 91 nests (40%), *F. pratensis* with 35 nests (15%) and the hybrid *F. polyctena* x *rufa* with 3 nests (1%). The average nest density of the four species was 11.1 nests/ha. The distribution of the nests is presented on the potential habitats' maps for *F. pratensis* (Fig. 2A), *F. rufa* (Fig. 2B) and *F. lugubris* (Fig. 2C).

The number of the recorded ant nests varied across the studied mountain ranges (Table 1). In Pirin Mt the greatest number of nests (63) was found, followed by Western Rhodopes (47) and Rila (35). In the remaining regions, between 0 and 18 nests were registered in each.

The most abundant population of *F. lugubris* was found in Pirin (43 nests, 14 nests/ha) and Western Rhodope Mts (35 nests, 12 nests/ha); *F. rufa* was most

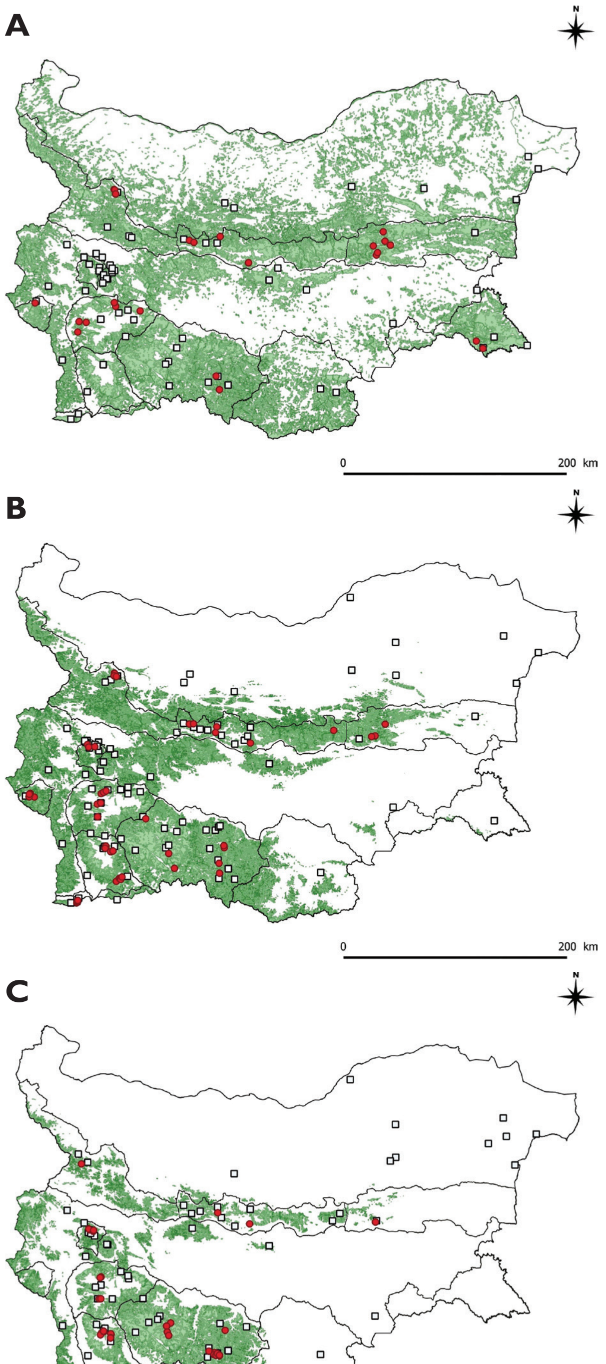


Figure 2. Potential habitats of RWA in Bulgaria. Localities (transects) recorded in the course of the present study are designated (red dots), previous literature data (squares) **A** *Formica pratensis* **B** *Formica rufa* **C** *Formica lugubris*.

Table 1. Number of red wood ants' active nests by monitoring territories in mountainous areas of Bulgaria.

Mountain range	Nest numbers					Activity %	Transects number	Hectares	Nest number/ ha
	Formica rufa	*Formica polyctena x rufa*	*Formica pratensis*	*Formica lugubris*	Total				
Western Rhodopes	11	0	1	35	**47**	92	24	3	16.3
Eastern Rhodopes	0	0	0	0	**0**	-	8	1	0
Vitosha	4	0	0	7	**11**	92	10	1.3	10.8
Osogovo	15	0	1	0	**16**	100	9	1	16
Belasitsa	13	0	0	0	**13**	63	9	1	13
Pirin	17	1	0	43	**61**	87	25	3	21
Rila	15	2	9	9	**35**	94	29	4	8.75
Western Stara Planina	5	0	5	1	**11**	87	9	1	11
Central Stara Planina	8	0	7	3	**18**	95	25	3	6
Eastern Stara Planina	3	0	10	2	**15**	88	16	2	7.5
Strandza	0	0	2	0	**2**	100	8	1	2
Total number	**91**	**3**	**35**	**100**	**229**	**-**	**172**	**-**	**-**
Average						**90**		**21.3**	**11.1**

abundant in Belasitsa (13 nests and 13 nests/ha), Pirin (17 nests, about 5.5 nests/ha), Western Rhodope Mts (11 nests and 3.7 nests/ha), Rila and Osogovo (15 nests and about 3 nests/ha per each); *F. pratensis* – in Eastern Stara Planina (10 nests, about 5 nests/ha) and Rila (9 nests, about 2 nests/ha). Four of the 91 fenced nests of *F. lugubris* in Smolyan Forestry (Western Rhodope Mts) were found (Fig. 3).

Figure 3. One of the four active *Formica lugubris* nests fenced 50 years ago.

Table 2. Results from binomial logistic regression for *F. pratensis*. Only independent variables selected by the backward stepwise procedure are listed. Marked coefficients are significant at $p < 0.05$ level. N = 172.

Parameter	Estimate	Standard Error	Odds Ratio	z	Wald Test		
					Wald Statistic	df	p
(Intercept)	-10.735	4.377	2.176e-5	-2.453	6.016	1	**0.014**
Elevation	-0.002	0.001	0.998	-2.728	7.440	1	**0.006**
Slope	0.735	0.376	2.085	1.956	3.826	1	0.050
Grass cover	2.333	1.041	10.308	2.240	5.019	1	**0.025**

Note: *F. pratensis* presence coded as class 1.

Table 3. Results from binomial logistic regression for *F. rufa*. Only independent variables selected by the backward stepwise procedure are listed. Marked coefficients are significant at $p < 0.05$ level. N = 172.

Parameter	Estimate	Standard Error	Odds Ratio	z	Wald Test		
					Wald Statistic	df	p
(Intercept)	-1.878	0.790	0.153	-2.377	5.651	1	**0.017**
Elevation	0.001	0.001	1.001	2.444	5.973	1	**0.015**
Stone cover	-0.476	0.208	0.621	-2.295	5.267	1	**0.022**
Forest age	0.007	0.005	1.007	1.473	2.171	1	0.141

Note: *F. rufa* presence coded as class 1.

Table 4. Results from binomial logistic regression for *F. lugubris*. Only independent variables selected by the backward stepwise procedure are listed. Marked coefficients are significant at $p < 0.05$ level. N = 172.

Parameter	Estimate	Standard Error	Odds Ratio	z	Wald Test		
					Wald Statistic	df	p
(Intercept)	-32.514	9.220	7.572e-15	-3.526	12.436	1	**< .001**
Elevation	0.019	0.006	1.019	3.313	10.974	1	**< .001**
Slope	1.137	0.613	3.117	1.854	3.439	1	0.064
Ecological groups of plants	1.345	0.842	3.839	1.598	2.555	1	0.110
Stone cover	0.658	0.428	1.931	1.538	2.364	1	0.124
Canopy cover	-1.649	0.696	0.192	-2.370	5.618	1	**0.018**

Note: *F. lugubris* presence coded as class 1.

Formica aquilonia and *F. truncorum* were not found in our samplings during the survey. Similar to RWA *Formica* (*Coptoformica*) *exsecta* and *Formica* (*Raptiformica*) *sanguinea* were recorded in samples from the same monitoring sites.

Factors influencing the presence and density of RWA

The studied species had specific altitudinal distribution, though partly overlapping. *Formica pratensis* was found between 320 and 1173 m, *F. rufa* between 537 and 1650 m, and *F. lugubris* between 1040 and 2240 m. The elevation was a statistically significant predictor of the presence of the three RWA, with a higher probability for *F. pratensis* to be found at a lower elevation, and for *F. rufa* and *F. lugubris* to be present at higher elevations (Table 2–4; Fig. 4A).

Exposure was significantly correlated with the nest density of *F. lugubris* (Table 5) and showed a bidirectional (axial) distribution. The highest nest density corresponded to either NE-E or SW-W nest exposure (Fig. 5). Since the exposure of *F. lugubris* nests

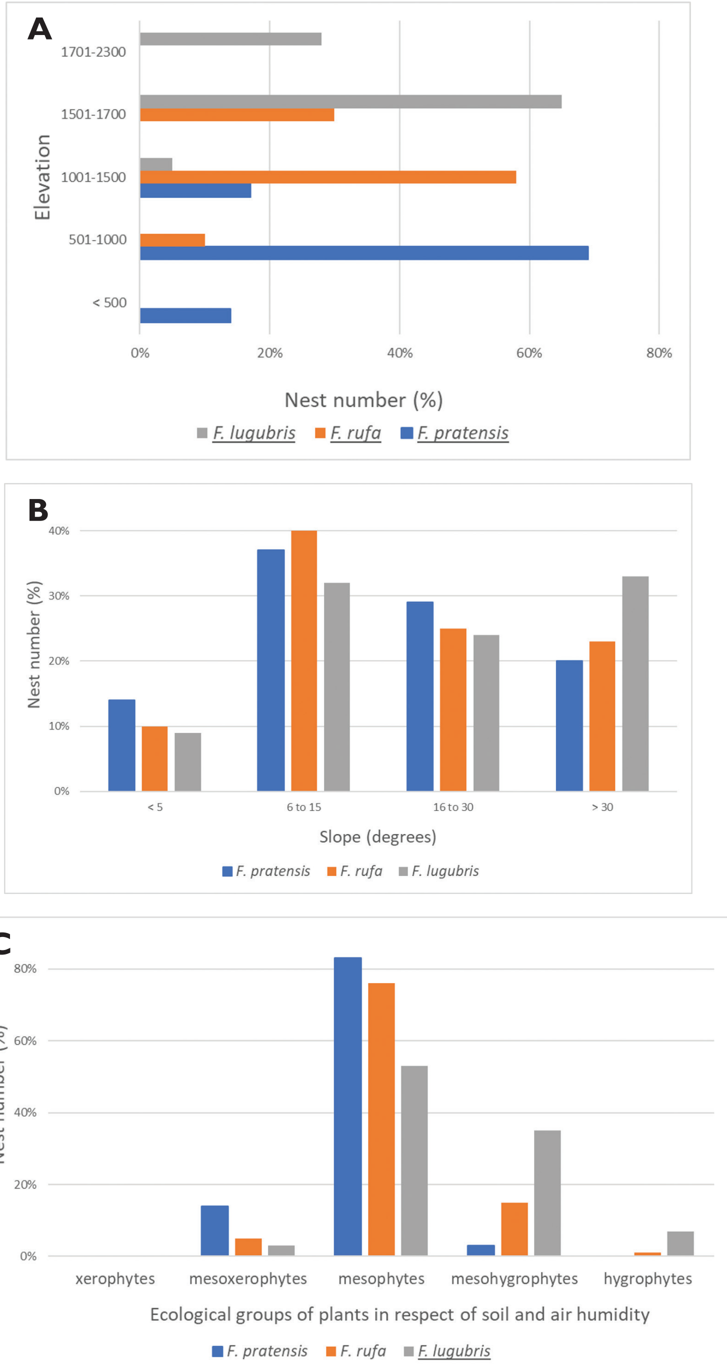
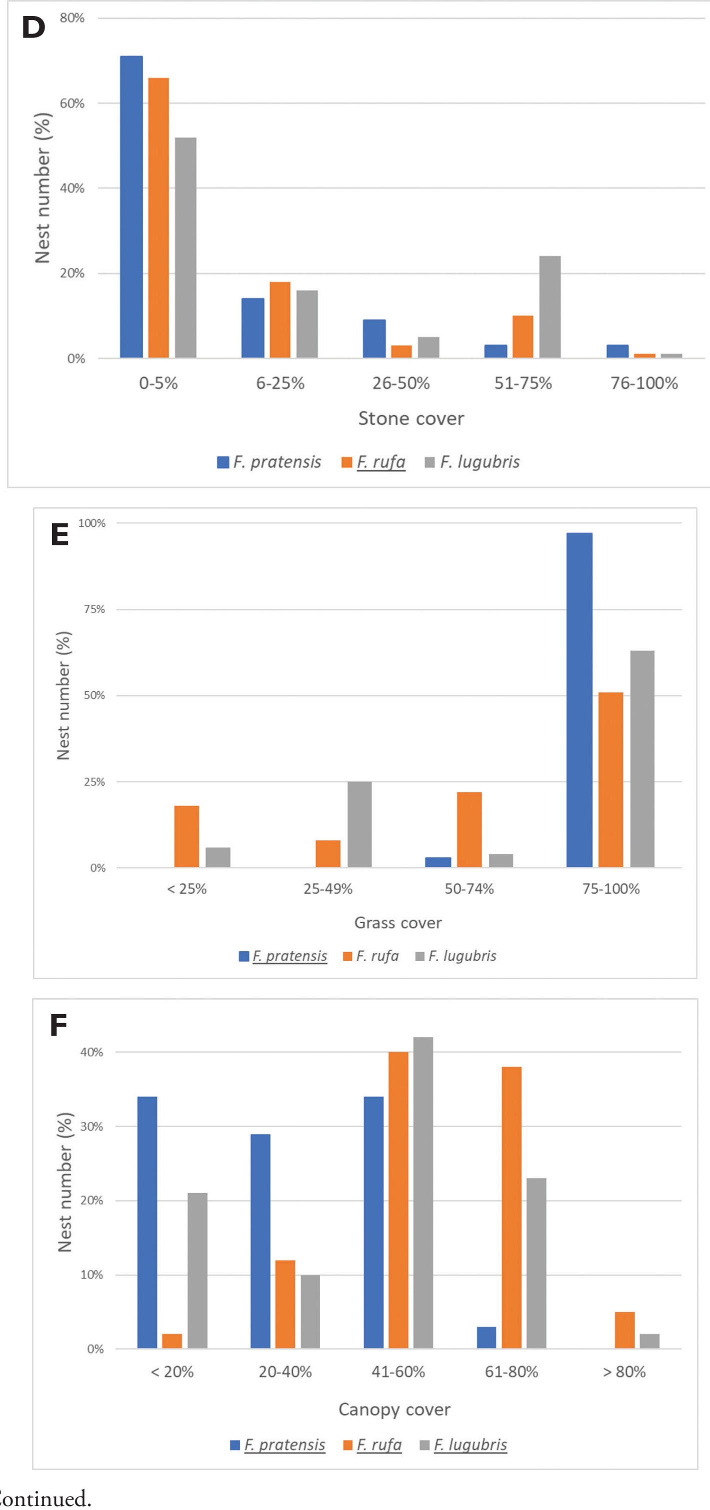


Figure 4. Distribution of RWA by studied variables in percentage by species (*F. pratensis*: N = 35, *F. rufa*: N = 91, *F. lugubris*: N = 100) **A** elevation **B** slope **C** ecological groups of plants **D** stone cover **E** grass cover **F** canopy cover **G** dominant tree **H** forest age (transects with unknown values are excluded). For underlined species the impact is statistically significant.



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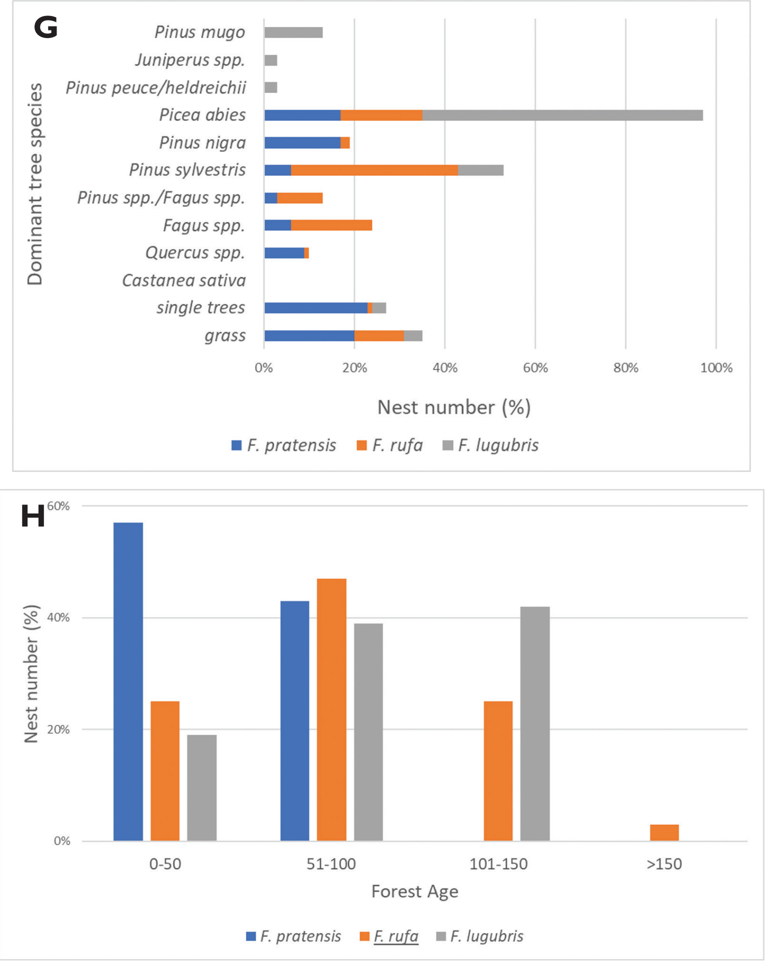

Figure 4. Continued.

was also significantly correlated with elevation, with NE-E exposure corresponding to higher elevations and SW-W exposure to lower elevations ($r = 0.401$, $p = 0.006$, $n = 34$), we suppose that our results reflect rather an interrelationship of exposure with elevation and, perhaps, some other environmental parameters than exposure by itself.

The slope was a marginally significant predictor of *F. pratensis* and *F. lugubris* presence (Table 2, 4; Fig. 4B).

Along a moisture gradient, the ecological groups of plants were significantly correlated with the nest density of *F. lugubris* (Table 5). Most of its nests were found where mesophytes (53%) and meso-hygrophytes (35%) were dominant plants, and nests were rarely recorded in places where meso-xerophytes and xerophytes dominated (Fig. 4C).

All RWA species were most common at the lowest (0–5%) level of stone cover, however, this variable was a significant negative predictor of *F. rufa* presence only, and was negatively correlated with its nest density (Tables 3, 5; Fig. 4D).

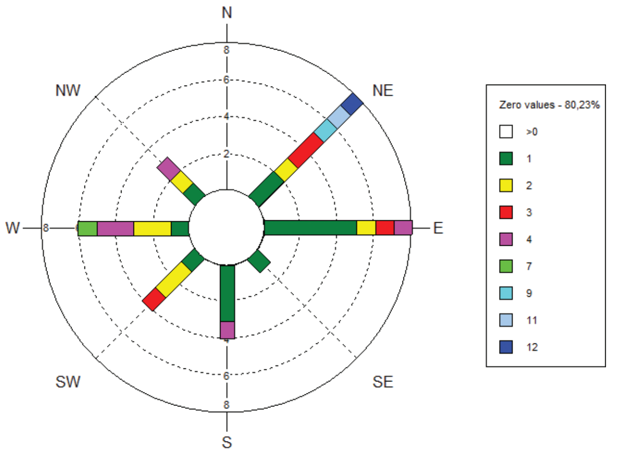

Figure 5. A histogram of *F. lugubris* nest density and exposure. The frequency distribution of the nest number recorded at any particular direction is represented by different colours. For better visualisation, zero values are excluded.

Table 5. Spearman rank order correlations between a number of nests of each ant species and environmental variables. Marked correlations are significant at p < 0.05.

Variable	n	*F. pratensis*		*F. rufa*		*F. lugubris*	
		r(s)	p	r(s)	p	r(s)	p
Elevation	172	**-0.290**	**< .001**	**0.222**	**0.003**	**0.628**	**< .001**
Exposure *	172	0.016	0.955	0.113	0.115	**0.173**	**0.007**
Slope	172	-0.053	0.493	-0.053	0.494	0.011	0.885
Ecological groups of plants	172	-0.077	0.317	0.104	0.173	**0.313**	**< .001**
Stone cover	172	-0.136	0.075	**-0.196**	**0.010**	-0.030	0.700
Grass cover	172	**0.289**	**< .001**	-0.002	0.980	0.135	0.078
Canopy cover	172	**-0.281**	**< .001**	**0.199**	**0.009**	-0.101	0.189
Underwood	172	-0.111	0.146	0.075	0.329	-0.016	0.839
Forest age	128	-0.142	0.110	**0.209**	**0.018**	0.134	0.131

*Circular-linear correlation coefficient, range from 0 to 1.

Most of the nests of examined RWA species were found in habitats with 75–100% grass cover. This variable significantly predicted the presence of *F. pratensis* only and correlated positively with its nest density (Tables 2, 5; Fig. 4E).

Canopy cover negatively predicted the presence of *F. lugubris* (Table 4). Additionally, canopy cover was negatively correlated with the nest density of *F. pratensis* and positively correlated with the nest density of *F. rufa* (Table 5; Fig. 4F). *Formica pratensis* preferred habitats with canopy cover from 0 up to 60% and was lacking in habitats with canopy cover over 80%. *Formica rufa* had peaks of nest numbers between 40 and 80% of the canopy cover as the nests were situated on light spots in forests' interiors. The majority of nests of *F. lugubris* (42%) were found at 40–60% and 21% of them at 0–20% canopy cover.

A great percentage (37%) of the nests of all studied species were found in the habitats of *Picea abies* (Fig. 4G). No RWA were found in *Castanea sativa* forests. In habitats with *Juniperus* sp., *Pinus peuce*, *P. heldreichii* and *P. mugo* we met only *F. lugubris*. In total, 65% of the nests of *F. rufa* were found within or in the vicinity of *P. sylvestris* forests, mixed forests with *Fagus* spp. or deciduous forests of *Fagus* spp.

Concerning the forest age, there was a significant positive correlation with the nest density of *F. rufa* (Table 5). This species was met even in forests older than 160 years, but the highest density was recorded in forests between 70 and 100 years old (Fig. 4H).

None of the species showed a statistically significant correlation with undergrowth density.

Discussion

Species diversity of RWA in Bulgaria at present

Most of the known localities of the particular RWA species (*F. lugubris*, *F. pratensis* and *F. rufa*) were confirmed in our study. The new findings are marked in the Suppl. material 2: Table S1. In all monitoring sites (except Eastern Rhodope Mts), at least one RWA species has been found. *Formica lugubris* was expected (Lapeva-Gjonova et al. 2010) but not found in the transects of Belasitsa and Osogovska Mts. It should be searched at a higher elevation. *Formica rufa* was also expected in Eastern Rhodope Mts and Strandza Mt. In Eastern Rhodope Mts the species should be searched for in the southern parts of the mountain. For Strandza Mt, it has been reported by Bobev (1972); Vatov and Bobev (1976); Wesselinoff (1979) and Vesselinov (1981) and should be searched also at a higher elevation. *Formica pratensis* was not found in our sampling transects on Pirin, Vitosha and Belasitsa Mt but mounds were found in all these mountains out of the transects.

Formica polyctena was not recorded in the course of the present study. Bobev (1972), Keremidchiev et al. (1972) and Vatov and Bobev (1976) reported also that *F. polyctena* is absent from the samples collected during the National Inventory of RWA (1970–1973). According to Bernhard Seifert (pers. comm. 2015), old data reported for *F. polyctena* from Bulgaria (Seifert 2008) are “a misplacement”. The three nests similar to *F. polyctena*, found in this research, were identified as *F. polyctena* x *rufa* and later confirmed by B. Seifert. Stable *F. polyctena* x *rufa* hybrid populations in Europe are known (Seifert 2018, 2021). There is no other recently published record on *F. polyctena* from Bulgaria either. So, we could accept Seifert’s opinion about the old findings: “The earlier findings of *F. polyctena* in Bulgaria, some 50 years ago, I suppose more likely to represent a misidentification rather than indicating a dying out process”.

Formica aquilonia was not found during our survey. If this species occurs in the country as reported for Rila Mt. at Zavrachitsa hut (Wesselinoff 1973) and Western Predbalkan at Belogradchik (Atanassov and Dlusskij 1992), its presence is probably very scarce. The samples identified as “*F. aquilonia*”, collected in 1982 from Bulgaria and preserved in the Senckenberg Museum of Natural History Görlitz, were kindly checked by B. Seifert in 2015 with modern taxonomic methods and were found to be seta-reduced *F. lugubris* (Pirin Mt) and seta-reduced *F. pratensis* (Rhodope Mts).

Table 6. Average nest density of RWA according to the National Inventory (1970–1973) and the recent survey (2013–2014). The non-target species *F. exsecta* and *F. sanguinea* were included in the percentage calculations for the sake of comparability with the National Inventory (1970–73) where these species have been initially included.

Regions grouped by RWA richness and abundance	Data from the National Inventory (1970–1973): Bobev (1972, 1973); Vatov and Bobev (1976): nest density and proportions	This study (2013–2014): nest density and proportions
Rich region	Central and Western Rhodopes: 0.3–0.6 nests/ha;	Pirin, Western Rhodopes (previous Central+Western Rhodopes), Osogovo: 16–21 nests/ha.
	F. rufa 47%; *F. lugubris* 42%; *F. pratensis* 10% and *F. exsecta* 0.5%.	*F. lugubris* 63%; *F. rufa* 35%; *F. pratensis* 1.6%; others (*F. polyctena x rufa* and non-target species) 0.4%.
Middle rich region	Rila and Pirin: 0.06 nests/ha.	Belasitsa, Western Stara Planina, Vitosha: 10–13 nests/ha.
	F. rufa 37%; *F. lugubris* 37%; *F. pratensis* 22%; and *F. exsecta* 4%.	*F. rufa* 63%; *F. lugubris* 23%; *F. pratensis* 13.9%; non-target species 0.1%.
Middle poor region	Western and Central Stara Planina, Sredna Gora, Eastern Rhodopes and the mountains in West-SouthWest Bulgaria (Kraishte, Malashevska, Ograzden, Belasitsa): 0.01 nests/ha.	Rila, Central and Eastern Stara Planina: from 6 to 9 nests/ha.
	F. pratensis 49%; *F. rufa* 45%; *F. lugubris* 3% and *F. sanguinea* 3%	*F. pratensis* 38%; *F. rufa* 38%; *F. lugubris* 20%; others (*F. polyctena x rufa* and non-target species) 1%.
Poor region	Danube plain, Strandza and Eastern Stara Planina: 0.006/ha.	Strandza, Eastern Rhodopes: up to 2 nests/ha.
	Dominant species: *F. pratensis* 79%; *F. rufa* 20% and non- target species 1%.	Dominant species: *F. pratensis*

Nest densities and habitat requirements

The average nest density (11.1 nests/ha) is greatly increased compared to that calculated at the National Inventory 1970–1973 (0.1 nests/ ha) given by Bobev (1972, 1973) and Vatov and Bobev (1976) (see Table 6). The reasons are the different sampling methodology, the difference in the calculation of the nest density, and the concentration of efforts for the studied species in the most suitable habitats during the fieldwork of this project.

Regarding the field's methodology of the Inventory, there is scarce information in the Bulgarian State Archives and it is difficult to compare the results with confidence. Nevertheless, the confirmation of the localities and the new findings are extremely valuable from the conservation viewpoint.

As seen from the comparative Table 6, Western Rhodope Mts were the richest of the RWA region in 1970 and still, they are. The majority of the nests in the 1970s and nowadays are of *F. lugubris* and *F. rufa*. In our study, *F. lugubris* was the dominant species in Pirin Mt. Middle rich/poor regions in both projects remain Western and Central Stara Planina, Rila and Belasitsa Mts. There the species' proportions remained approximately the same, although *F. lugubris* decreased at the expense of *F. rufa* and *F. pratensis*. In the poor regions (Strandza and Eastern Rhodope Mts) the dominance of *F. pratensis* was confirmed again in our study.

The nest density of *F. rufa* and *F. lugubris* in our survey is many times larger in the forest boundary area (in a strip with a width of up to 10 m) or small-size forest fragments with respectively higher solar radiation. Similar are the observations made by Punttila et al. (1994), Punttila (1996), Underwood and Fisher (2006), Babik et al. (2009), Crist (2009) and Chen and Robinson (2014) for RWA in the cool temperate forests in Europe. The density of the *Formica rufa* group is higher in deforested strips as they prefer nesting in sun-warmed places and use the forest interior as foraging area (Babik et al. 2009). In Western Poland, the nest density decreases towards the centre of clear-cuts although the influencing factors are not clear (Żmihorski 2010). The mounds in young forests and clear-cuttings are smaller and flatter, caused by the splitting of the large mounds into smaller colonies (Rosengren and Pamilo 1978; Domisch et al. 2005). During our survey, nests of *Formica fusca* (the host species of RWA) were often found in grassy runs or shrubs at the forest edges.

In our study, *F. lugubris*, *F. rufa* and *F. pratensis* were found at altitudes corresponding to the previously reported altitude from Bulgaria by Atanassov and Dlusskij (1992). In Switzerland, the great abundance of *F. lugubris* was at about 1000 m (Cherix et al. 2012) while in Bulgaria was higher, between 1500 and 1800 m. In Switzerland, *F. rufa* and *F. polyctena* were found at lower elevations up to 800 m (Vandegehuchte et al. 2017) similar to their optimal range in Bulgaria. *Formica polyctena* x *rufa* hybrid was found between 1200 and 1410 m, as the elevation preference for *F. polyctena* was up to 1200 m (Atanassov and Dlusskij 1992). In other European countries, almost every species of RWA occurs at lower altitudes than in Bulgarian mountains, which is the expected phenomenon of shifting up of altitudinal preferences of mountainous species in the southern part of their ranges.

In the present study, the nest density of *F. pratensis* and *F. rufa* was significantly influenced by the canopy cover. There was an example of shading effect: Wesselinov (1968) published an article about the high diversity of RWA for Parangalitza Biosphere Reserve in the Rila National Park. As a reserve since 1933 (Darzhaven vestnik 1933) with about 300–400 years old trees of *Picea abies*, the wood clearance, removing the old fallen woods and felling had been strongly forbidden. The consequences nowadays are that as the forest interior is almost closed (over 80% canopy cover), the RWA are only to be found in the vicinity of the forest, around the roads and the alpine meadows at present. A similar situation was observed in other old reserves and RWA populations there were decreasing. Our observations confirmed the conclusions of other authors (Collingwood 1979; Punttila et al. 1994; Dekoninck et al. 2010; Tsikas et al. 2016; Serttaş 2020) that RWA populations decline with increasing the canopy closure and a little intervention as forest thinning for light spots would be a support for the RWA existence.

In Bulgaria, the mounds of *F. lugubris* were mostly located on north-east-facing slopes, similarly to the same species in Switzerland, found mainly at an eastern aspect (Freitag et al. 2016b; Vandegehuchte et al. 2017). In southern Norway, it was strongly orientated to the South (Hill et al. 2018).

The positive correlation of grass cover to both *F. pratensis* presence and nest density was expected because this species is an open habitat specialist (Seifert 2018). This variable did not show a statistically significant impact on the abundance of other RWA as well as in West Poland for *F. rufa* and *F. polyctena* (Żmihorski 2010). According to Sorvari (2016), the higher ground vegetation benefits RWA as it plays a protection and feeding role.

The dominant tree species associations, where the RWA species occur, were *Picea abies*, *Pinus sylvestris*, *Abies alba*, young *Juniperus* spp. communities (according to Atanasov and Dlusskji 1992) and also *Pinus mugo*, *P. heldreichii* and *P. peuce* communities (according to our study).

The conservation needs of the RWA are underestimated (Sorvari 2016). For a viable colony, these territorial species need at least a few hectares of stable habitat with enough food resources to maintain sexual forms for reproduction. The loss, shading, drying/flooding, disturbance or destruction of their habitat are their major threats (Dekoninck 2010). Not only human activities but changes in climatic conditions and natural enemies (as pathogens) may cause a decline of a colony. As RWA play a keystone role in the ecosystems (Dlussky 1967), the reduction or extinction of their populations may have a huge effect on the local and even global ecosystems. Therefore, studying the most suitable habitat characteristics in detail, and conducting regular monitoring studies on RWA populations are of primary importance.

Conclusions

- The recent proportions of RWA species in Bulgaria remain similar to those in the National Inventory of RWA (1970–1973): most abundant is *F. lugubris* followed by *F. rufa*, *F. pratensis* and the hybrid *Formica polyctena* x *rufa*. The existence of *F. polyctena* and *F. aquilonia* in Bulgaria is probably doubtful and should be a matter of further investigation.
- Western Rhodope Mts were the richest of the RWA region in 1970–1973 and still are at present, with successful examples of nests that have survived over 50 years.
- The GIS predictive models of RWAs' potential habitats are useful for optimising their area for conservation purposes.
- As habitat characteristics of primary importance for the distribution and density of the three RWA, we identified elevation and canopy cover; exposure and ecological groups of plants were important for *F. lugubris*, stone cover and forest age – for *F. rufa*, and grass cover – for *F. pratensis*. However, the slope was marginally significant for the presence of *F. pratensis* and *F. lugubris* only, and undergrowth density was not related to the ecological demands of neither of the three RWA species.
- The optimal habitat conditions for the distribution of the four RWA species in Bulgaria are: elevation between 500–2000 m (lower parts occupied by *F. pratensis*, middle – by *F. rufa* and upper – by *F. lugubris*); mesophyte coniferous, mixed and deciduous forests with canopy cover between 30 and 80% and their ecotones; lack of stone cover on the surface; the presence of forest meadows and clearings/rides.

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Supplementary material 1

Supplementary Field Form

Authors: Vera Antonova

Data type: Empty field form (excel file)

Explanation note: Field form for each transect with lines for General information of the locality, Habitat data, Threats, Nests' description, GPS points.

Link: https://doi.org/10.3897/jhr.85.61431.suppl1

Supplementary material 2

Table S1

Authors: Vera Antonova, Martin P. Marinov

Data type: Sample transects, localities, environmental variables, number of nests (excel file)

Explanation note: The locality, environmental variables and number of red wood ants nests are given per each sample transect (172). The new localities per each species are marked.

Link: https://doi.org/10.3897/jhr.85.61431.suppl2