CB005.2.12.012 "Joint initiatives for the conservation of zonal European red wood ants facing the danger of extinction"

S C I E N T I F I C R E P O R T

Under the contract NO CB005.2.12.012/085-PP2-3 / LOT 1 for „Field and laboratory studies for the needs of the project CB005.2.12.012 „Joint Initiatives for the Conservation of Zonal European Red Wood Ants Facing the Danger of Extinction"

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Content

I. Background

Introducing red wood ants

Red wood ants are typical inhabitants of forest ecosystems in the temperate zone of the Northern Hemisphere. In the scientific literature they are known as the species from the group *Formica rufa* and subgenus *Formica* sensu stricto. Their popular name comes from the characteristic coloration of the body - red and brown-black and their characteristic habitats, where they build their nests. Most red wood ants build underground nests in the soil and have a dome that in some cases reaches 2 meters in height and is inhabited by thousands to tens of thousands of individuals. Often individual nests of one species may be associated with active transport by individuals between them, covering 2.5 km^2. Preferred habitats are sunlit places in the forests.

Like other eusocial insects, the members of the ant colony belong to three main castes - workers, queens and males. Workers are the most numerous part of individuals who are female by sex, but their reproductive systems are underdeveloped and the functions they perform in the colony are related to feeding, building and maintaining the nest structure, caring for the queen and preimaginal stages (eggs, larvae, pupae). Males develop from unfertilized eggs, participate only in reproduction and do not live long after that. Males and females are usually winged, while workers are wingless. Queens can be one (monogynous) or several in the nest (polygynous) depending on the species and their main role is related to the reproduction and laying of eggs. They live the longest - up to 20 years, depending on the species, during which time they lay millions of eggs.

Two weeks after laying larvae hatch, which pass through the 4 stages of development, then pupate. After two weeks of development, adults hatch from the pupa.

The division of labor between female workers is so evolutionarily entrenched that many species have a pronounced polymorphism, a caste system in which individuals who perform different activities differ from one another. Ants have a complex communication system that facilitates the activities of the colony and ensures the identification between its members and their distinction from alien species. The cooperation between the individuals of an anthill is so close that many authors treat the colony as a "superorganism."

Environmental role and importance of the red wood ants

Red wood ants, due to their social organization of life, ubiquity and large numbers of individuals in the colonies, play an important role in ecosystems by modeling the environment and establishing various relationships with other organisms.

As a result of the nesting activity of ants, concentration and accelerated decomposition of organic residues, aeration and improvement of the water regime, soil structure, changes in the

mechanical and chemical composition of the soil are provided: enrichment of soil with humus and elements important for plants (phosphorus, nitrogen, potassium, magnesium, etc.) in forms accessible to them. A specific environment is created in the nests, which differs significantly from the environment and contributes to the faster decomposition and humification of the plant residues in the nest, the soil biological activity increases. This is due to the higher temperature of the nest than that of the surrounding soil and the development of rich microflora in the anthill - bacteria, fungi, actinomycetes. Thus, the degradation increases tenfold.

Ant nests are places of high soil fertility. The influence of red wood ant nests older than 10 years and a diameter greater than 1 m is able to provide a significant impact on the growth not only directly of local grasses and shrubs, but also of trees far enough away from the nest. The roots of the trees extend below the soil surface by several meters. The closest to the zone of influence of the nest, thrive, using the accumulated nutrients under the ant nest. Rapid growth of pine trees has been observed in areas where there are a large number of ant nests. They are much taller and thicker around the them.

The significant role of red forest ants in nature is also determined by the trophic connections with other organisms. Due to the relatively constant and safe conditions and the rich nutrient base, a large number of other organisms, called myrmecophiles, live in the nests of ants and most of them are not found outside the their nests. Among them are various species of blue-green algae, fungi, roundworms, arthropods - woodlice, arachnids, centipedes and insects (it is assumed that only myrmecophilous beetles are 10 thousand species).

All red wood ants are polyphagous predators, and the importance of predation for prey populations depends on the size of the colonies and their density. During the mass reproduction of forest pests, they make up 90% or more of their food. Their advantages as bioregulators are determined by:

1. Territoriality;
2. Effectiveness of impact on the main pests in a certain forest massif;
3. Duration of exposure to pests during the seasons;
4. Density stability for several years regardless of the number of pests;
5. High density of individuals in a certain area;
6. Multifunctional positive role in biocenoses
7. Compatibility with the action of other factors regulating the number of pests.

Ants ensure the preservation of plantations from damage by pests such as *Lymantria monacha* (nun moth), *L. dispar* (European gypsy moth), *Panolis flammea* (pine beauty), *Bupalus piniaria* (pine looper), *Operophtera brumata* (winter moth), *Neodiprion sertifer* (the European pine sawfly), *Tortrix viridana* (oak leafroller) and many others.

Red wood ants use trophobiosis to remove excess honeydew from aphids, which is an essential part of their diet, thus supplementing their protein diet with sugars. Many species of aphids cause serious damage to deciduous trees. By collecting their sweet excreta, red wood ants reduce the indirect damage caused by the honeydew released on the plants, which clogs the pores through which the plants perform gas exchange.

On the other hand, the numerous nests of forest ants represent a rich food base for a number of vertebrates important for the ecosystem or of economic importance - songbirds, forest hens, many amphibians and reptiles, badgers, foxes, bears and others. They are especially important in periods when other foods are missing or severely restricted. The amount of ants eaten by birds is about 10% of the total value of natural mortality in ants. It is known that their nests are used for winter nights of wild boars. An interesting phenomenon is the use of wood ant nests by some species of birds to remove their external parasites, burying in them and under the strong action of the released formic acid.

Identification

Determining the species affiliation of red wood ants is a huge challenge, including for taxonomists in this group. This requires serious knowledge of the morphology of the species, the distinction characters used, the availability of modern microscopic technique with high optical parameters (sometimes up to 150x), which is needed for the study, morphometric measurements and counting of meristic traits. Most often in the modern specialized literature are used characters such as length and width of the head, length of the longest hair from the occipital end of the head, number of hairs on the dorsal part of the tibia of the last pair of legs.

Red wood ants in Bulgaria – species composition, description, ecology and distribution

The red wood ants, which are known to be found in Bulgaria, are representatives of 5 closely related species from the group *Formica rufa.*

1. *Formica aquilonia* Yarrow, 1955 (the Scottish wood ant) (fig. 1, 2)

Short description: According to Collingwood (1979), the body is 4.0-8.5 mm long, bicoloured with dark markings on head and promesonotum varying in size and intensity. Occipital margin with at least a few (4-12) short erect or suberect hairs, mostly aroun the occipital corners; mesosomal dorsum with abundant erect hairs; workers relatively small in size (Czechowski *et al*. 2012).

Distribution: Boreomontane species, distributed mainly in the coniferous forests of northern Europe and Siberia. It is found from the Eastern Alps to Siberia and from northern Italy to northern

Norway. This is the most common species in the Palearctic mountains (Czechowski *et al*. 2012). In Bulgaria it is known only from two localities - Rila Mountain, Zavrachitsa locality, 1900-2000 m and Belogradchik (Atanassov & Dlusskij, 1992).

Ecological preferences: It inhabits more shady places than other species, where it builds its nests (polygynous), often interconnected in a polycalic system. They establish their nests through temporary social parasitism in nests of other species of the genus Serviformica or by dividing polygynous colonies (Czechowski *et al*. 2012). The nuptial flight is from May to July.

Fig. 1. General view of *Formica aquilonia* (Antweb).

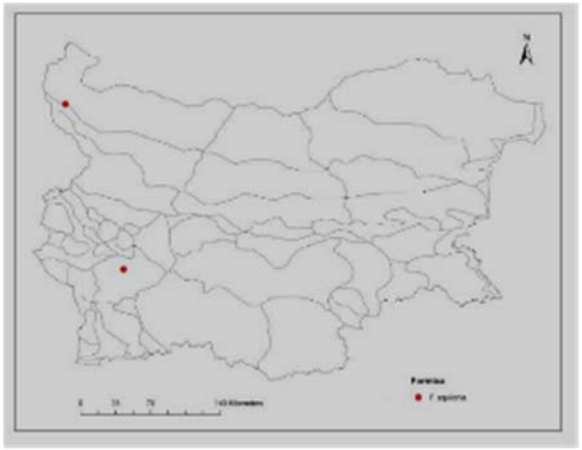


Fig. 2. Localities of *Formica aquilonia* in Bulgaria.

2. *Formica lugubris* Zetterstedt, 1838 (hairy wood ant) (fig. 3, 4)

Short description: Body size is between 6.5 to 9 mm. The dark grey or brown patch on promesonotal dorsum weaker and with diffuse margins; frons not completely matt, but a narrow light-reflecting band extends posteriorly along the frontal furrow (Czechowski et al. 2012). Occiput with a thick fringe of hairs extending forward over area between ocelli and sides of head and laterally round to the eyes (Collingwood, 1979).

Distribution: Boreomontane, Euro-Siberian species. In Bulgaria it is widespread between 900-2300 meters above sea level, known from the Eastern Danube Plain, Predbalkan, Stara Planina, Viskyar, Lyulin, Verila, Vitosha, Plana, Sredna Gora, Sakar, Belasitsa, Slavyanka, Sandanski, Rila, Pirin , Rhodopes, Northern Black Sea coast (Lapeva-Gjonova *et al*. 2010).

Ecological preferences: The species is characteristic of beech, mixed and coniferous forests. Nests are built in open places - meadows, forest edges, near streams and roadside habitats (Atanassov & Dlusskij, 1992). The nests are monogynous and less often polygynous, most often 1 meter wide and about 60 centimeters high. The new nests are established by temporary parasitism in the nests of ants of the subgenus *Serviformica*, and in the polygynous ones - by division. The number of workers is from 100,000 to 200,000. A significant part of the food is honeydew, separated from aphids. Winged individuals are observed from June to September (Atanassov & Dlusskij, 1992).

Fig. 2. General view of *Formica lugubris* (Antweb).

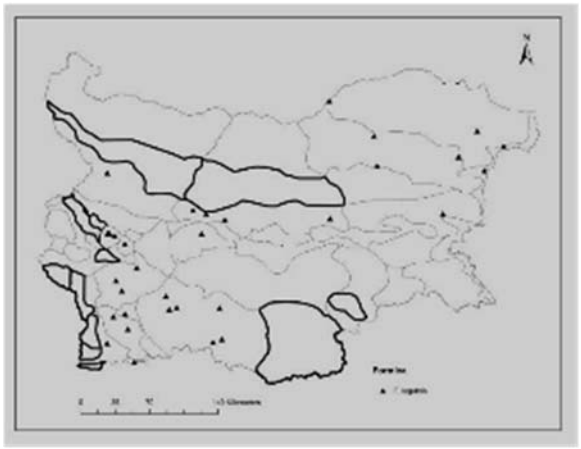


Fig. 3. Localities of *Formica lugubris* in Bulgaria.

3. *Formica polyctena* Förster, 1850 (small red wood ant) (fig. 5, 6)

Short description: Workers on average relatively small – 4,5-7,2 см. Each segment of mesosomal dorsum without or with less than 6 standing hairs; ventral surface of head without or, at most, with a few short suberect hairs (Czechowski *et al*. 2012).

Distribution: North Palearctic species (Czechowski *et al.* 2012). In Bulgaria it is found at altitudes up to 1200 m. It is found in the Danube plain, Eastern Stara Planina, Golo Bardo, Rila and Pirin mountains. (Atanassov & Dlusskij, 1992, Lapeva-Gjonova *et al.* 2020).

Ecological preferences: Inhabits mixed, deciduous and spruce forests, sometimes in the more denser parts of forests compared to *Formica rufa*. The above-ground part of the nests is made of small plant remains, 5-6 in a polycalycal system. The nests are large, polygynous, reaching up to 3 meters in diameter (Czechowski *et al.* 2012), inhabited by a huge number of individuals. New nests are based on temporary social parasitism or by division, as in other species in the group. The nuptial flight is relatively earlier – May-June (Czechowski *et al*. 2012).

Fig. 4. General view of *Formica polyctena* (Antweb).

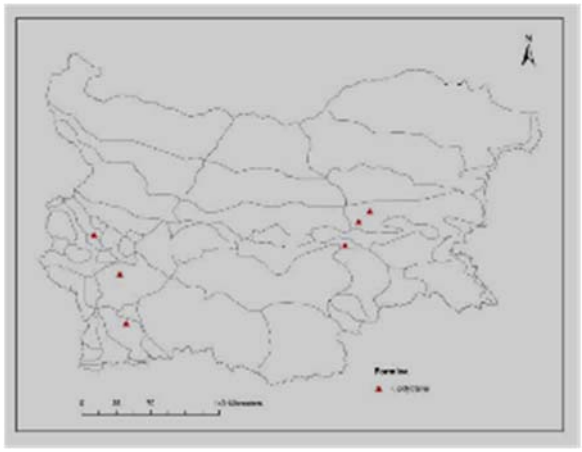


Fig. 5 Localities of *Formica polyctena* in Bulgaria.

4. *Formica pratensis* Retzius, 1783 (European red forest ant, meadow ant) (fig. 6, 7)

Short description: The body is 4.0-9.0 mm long, covered with dense adjacent and rarely long erect hairs; black large patch on promesonotal dorsum with sharp margins; frons completely matt (Czechowski *et al*. 2012). The species is closest morphologically to *Formica lugubris*.

Distribution: Danube Plain, Predbalkan, Stara Planina, Verila Mountain, Viskyar Mountain, Zemen gorge, Sofia plain: Sofia, Lyulin Mountain, Vitosha, Plana, Rose Valley, Sredna Gora, Lozenska Mountain, along the Tundzha River, Strandzha, Osogovo Mountain, Belasitsa, Sandansko-Petrich valley, Rila, Pirin, Mesta river valley, Western Rhodopes, Eastern Rhodopes, Northern Black Sea Coast, Southern Black Sea Coast (Atanassov & Dlusskij, 1992, Lapeva-Gjonova *et al*. 2020).

Fig. 6. General view of *Formica pratensis* (Antweb).

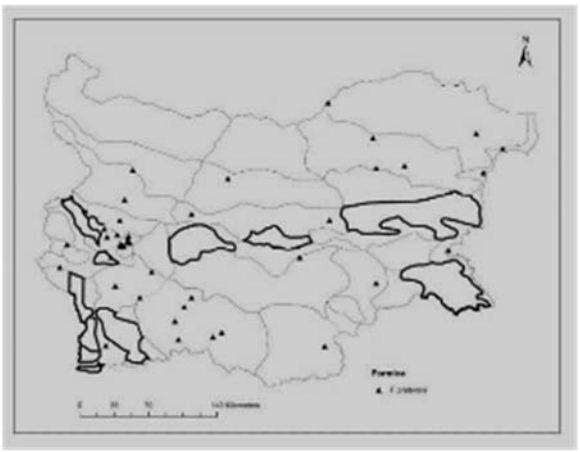


Fig. 7. Localities of *Formica pratensis* in Bulgaria.

Ecological preferences: The species builds its nests on dry, sunny forest edges, young plantations, meadows, roadside biotopes. The dome is flat or steep, of plant debris, mixed with soil, measuring 1.20-2.80 in diameter and an average height of 0.40-0.80. As a rule, the inner cone consists only of twigs and leaves. The nests can be mono- and polydomes. Their main food is other arthropods and honeydew secreted by aphids. This species is considered to have the most strong trophobiont relations with aphids compared to other species of the subgenus. The nuptial flight is twice a year - in May-June and August-September. The colonies are monogenous - with one egg-laying queen in the nest.

5. *Formica rufa* Linnaeus, 1761 (red wood ant) (fig. 8, 9)

Short description: According to Atanassov & Dlusskij (1992), the body is matte bicolor, 4.0-9.0 mm long; pronotum often with small, black-brown patch which does not reach promesonotal suture. Each segment of mesosomal dorsum usually with at least 6 standing hairs; ventral surface of head with long erect hairs (Czechowski *et al*. 2012). The species is closest morphologically to *Formica polyctena*.

Disribution: In Bulgaria it is found up to 2000 m above sea level. In the mountains, most often 600-1600 m. The species has been reported from the Danube Plain, Predbalkan, Stara Planina,

Viskyar Mountain, Verila, Zemen gorge, Sofia Field, Lyulin Mountain, Vitosha, Plana Mountain, Sredna Gora, Lozenska Mountain, Tundzha, Strandzha, Belasitsa, Petrich, Sandanski river valleys,. Rila, Pirin, Slavyanka, Western Rhodopes, Eastern Rhodopes, Northern Black Sea Coast (Atanassov & Dlusskij, 1992, Lapeva-Gjonova *et al.* 2020).

Environmental preferences: The species prefers mixed, oak, beech and coniferous forests. It builds domes of plant remains with a diameter of up to 1.5 m and a height of 1.2 m. The nests are inhabited by a large number of individuals - up to 400,000.

Fig. 8. General view of *Formica rufa* (Antweb).

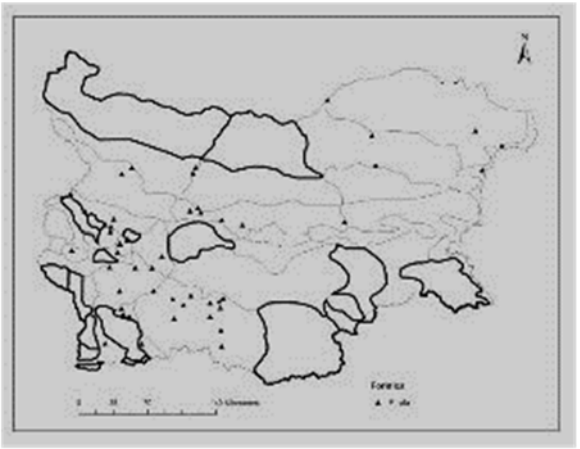


Fig. 9. Localities of *Formica rufa* in Bulgaria.

Conservation status of red wood ants in Bulgaria

The conservation of populations of rare species and those with a significant effect on the stability of ecosystems, such as wood ants, has led to 141 species out of a total of nearly 14,000 in the world to be included in the Red List of the International Union for Conservation of Nature (IUCN) - the largest conservation organization in the world. The species on this list have been pre-assessed by experts, which includes the state of the populations and habitats of the species at the global and regional levels. The assessments are prepared according to approved criteria, and the data on the species are updated over a certain period of time. The most significant indicators are the area of distribution and the area of occupation.

All five species of the group of red wood ants found in Bulgaria are included in the IUCN Red List in the category of almost endangered (Near Threatened – NT): “Taxa which do not qualify for Conservation Dependent, but which are close to qualifying for Vulnerable”.

Due to their multifunctional role in ecosystems, red wood ants can be identified as key to their functioning. Therefore, any change in their status and distribution affects not only their survival but also all related organisms. (Stockan & Robinson, 2016).

Red wood ant (*Formica rufa*) is a species under protection according to the Bulgarian Biodiversity Act (2002) (protected on the entire territory of the country). It is included in in the Annex 2 and Annex 3 (the Bulgarian Biodiversity Act, 2002). Article 38 (3) explicitly states that the "destruction, damage or relocation of nests" of the animal species listed in Annex №3 of the the Bulgarian Biodiversity Act is prohibited.

Red wood ants have been assessed as extremely suitable objects in monitoring programs. Three of them - *Formica lugubris*, *Formica pratensis* and *Formica rufa* are included in the National System for Monitoring of Biological Diversity in Bulgaria and monitoring methodology and field forms have been developed for them (http://eea.government.bg/bg/bio/nsmbr) (Antonova, 2014).

II. Design of initial work plan

Target region

The target area of the present project is Strandzha Mountain, located mainly in the Burgas region (fig. 10). Strandzha is a border mountain in southeastern Bulgaria and European Turkey. To the north it extends to the Burgas lowland, to the northeast and east to the Black Sea, to the southwest to the East Thracian plain. Its length from northwest to southeast is about 125 km and its width reaches 65 km. The total area of the mountain is about 10,000 sq. km, of which about 35% on the Bulgarian territory.

Strandzha is a relatively low mountain. Its relief is characterized by scattered hills and rounded ridges, bounded by deeply incised, gorge-like river valleys. About 38% of the territory is 0-200 m above sea level, 60% - 200-600 m and about 2% over 600 m. The most widespread are the territories with altitude between 200 and 300 m. The highest peak in the territory on the mountain in the Bulgarian part is Gradishte peak - 710 m.

The climate of the region is formed under the continental influence from the west and north, the Black Sea influence from the east and the Mediterranean from the south. In general, the Strandzha climate has a transitional-Mediterranean character. The specific climate of Strandzha is the reason why the vegetation has features that distinguish it from the European plant formations and bring it closer to the flora of the Caucasus. The forests (83072.9 ha) in Strandzha are a remnant of the Tertiary vegetation, preserved due to its remoteness from the Quaternary glaciations, mild winter, high rainfall and humidity.

Fig. 10. View from Strandzha near Petrova niva locality (photo: A. Gjonova).

Most of the mountain area has been declared a nature park. In 1995 it was declared a National Park (Nature Protection Act) "in order to protect the long-term unique nature of the catchments of the Veleka and Rezovska rivers and to ensure sustainable socio-economic development of the region", and since 2000 it has been re-categorized as Nature park (Protected Areas Act). The area of the park is 1161 km^2, which is about 1% of the country's territory. In 1933 the first reserve of Bulgaria - Silkosia was declared here. On the territory of Strandzha there are four more reserves: Uzunbodjak, Sredoka, Tisovitsa and Vitinovo, as well as a number of protected areas and natural landmarks. The territory of the park is included in various international programs - European project "CORINE-biotops", "Natura 2000" and others. This is the only Bulgarian territory of the five priority areas for protection in Central and Eastern Europe. The second largest bird migration route in Europe - Via Pontica - passes over Strandzha. On the territory of Strandzha Nature Park there are a number of protected plant and animal species, which makes Strandzha an extremely important place for nature protection.

The characteristics of the region have been used data from Velev et al. (1997), Gusev et al., (2002), Gusev and Tsonev (2011), Gusev and Tsonev (2014), Mihneva and Petranov (2001), Ninov (2002), Simeonova-Chorbadjiyska (2013).

Target species and preliminary studies

The object of study in the present project is the European red wood ant *Formica pratensis* Retzius, 1783 (Hymenoptera, Formicidae) on the territory of the cross-border mountain Strandzha. This is the only species of the group of red wood ants that is certainly found in the target area. In this regard, all available information on the distribution and ecological preferences of the species was examined in order to conduct an effective field study.

A review of the scientific literature in connection with the distribution of red wood ants in Strandzha found that two species - *Formica rufa* and *Formica pratensis* were reported to occur in the area without giving any specific localities (Bobev 1972, Vatov and Bobev 1976, Wesselinoff 1979, Vesselinov 1981). Perhaps their reports are due to the probability to find these species, given the vast area of the mountain, mainly occupied by forests, although the species *Formica rufa* generally prefers significantly higher places. The lack of specific data on forest ants from Strandzha Mountain for nearly 130 years of research on myrmecofauna in Bulgaria, including in this area shows that these species are not common.

The only specific data on the species Formica pratensis in the Bulgarian part of the Strandzha Mountains were found in the publication by Lapeva-Gjonova & Kiran (2012), in which only two localities are marked (Fig. 11) – the surroundings of the town of Malko Tarnovo (N41°58'28" E27°31'40") and the village of Rezovo (N41°59'01" E28°01'41"), despite intensive studies of ant

fauna over a period of 3 years (2009-2011) under the project № ДО 02-159/16.12.0 to the Research Fund (Ministry of Education and Science). This indicates that the species is relatively rare in the area and further studies are needed to establish its distribution and appropriate living conditions. The lack of data from a longer period in previous studies does not allow at this stage to outline trends in the state of the population of the species and emphasizes the need for long-term monitoring.

Fig.11. Localities of the European red forest ant Formica pratensis according to literature data - the surroundings of the town of Malko Tarnovo and the village of Rezovo (Lapeva-Gjonova & Kiran (2012).

In the European part of Turkey and the Turkish part of Strandzha, there are significantly more data on the distribution of the species Formica pratensis contained in the publications by Aras & Aktaç 1990, Aktaç et al. 1998, Radchenko 2007, Lapeva-Gjonova & Kiran (2012) and Çamlitepe & Aksoy (2019). During the inventory and monitoring study (2012-2015) in the last study a total of 340 localities in the area were visited and a total of 89 nests were found in 48 locations. Twenty of the identified nests out of a total of 89 died during the study period. Çamlitepe & Aksoy (2019) use up-to-date information from the localities on the basis of which the species has been assessed as vulnerable in the Thracian part of the country according to IUCN (criteria B1b - i. distribution area, ii. occupied area, iii. area, range and / or habitat quality, (iv) the number of localities or subpopulations). For this purpose, the following were measured:

1. Extent of occurrence (EOO) – the territory bounded by the shortest continuous mental limit, which may be delineated to cover all known or presumed sites of the modern distribution of the taxon, to the exclusion of cases of vagrancy.

2. Area of Ocupancy (AOO) – the area of the 'distribution area' that is occupied by the taxon, excluding cases of vagrant. This measure reflects the fact that a taxon does not normally occupy the entire area of its distribution area, which may contain inappropriate and unoccupied habitats.

Planning of the study

Using the data on the preferred habitats of the species in the target and from other regions in Bulgaria, known from the literature and by the personal field experience of the experts, the places for visits to Strandzha were planned in advance. In order to maximally cover the territory and search for suitable habitats, the selected places for visiting are located in the four plant-climatic areas of the region:

1. Northwestern arid region - with the highest average monthly and annual temperatures, the highest amount of precipitation with a pronounced Mediterranean character, with the maximum amount of precipitation (mostly rain) in winter and minimum - in summer (August) and high humidity.

2. High area (east of the line Slivarovo-Gramatikovo-Tisovitsa) - with higher rainfall than those along the coast, especially snow. The maximum is in winter and there is a second small maximum in spring. Frequent summer and spring fogs are common. Although temperatures are relatively lower than the coastal zone, the region's climate is milder, especially in winter and early spring due to the terrain (Papia Peak), which protects the area from cold air currents. The breezes entering the valleys of Veleka and Rezovska are the reason for the high humidity.

3. Area of Pontic rhododendron (west of Slivarovo: Malko Tarnovo-Moryanski region) - with the lowest average monthly and annual temperatures, with the lowest absolute minimum and highest maximum measured temperatures. The amount of winter precipitation is the highest for Strandzha, and the snow cover lasts twice as long as in the coastal zone.

4. Coastal area. To the northwest of the high area and the line Stoilovo-Vizitsa there is an increased, gradual influence of the continental climate. The winter maximum of precipitation reflects the Mediterranean influence, but the pronounced second summer maximum and the tendency of equalization of the monthly curve of precipitation, as well as their general decrease, are an indicator for strengthening of the drier character. Lower rainfall in autumn and winter has an adverse effect on soil moisture storage and water runoff.

The ID-maps (field forms) (fig.12) for mapping and description of the nests have been prepared, which have been agreed between the Bulgarian and Turkish team, so that the collected data can be compared and serve as a basis for future monitoring of the state of the species.

Date:	Nest №	Locality №	Locality name:			GPS:			
Nest features:									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
Habitat features/Threats:									

Fig. 12. Identification card for ant nests and habitats.

Mandatory information collected in these cards includes:

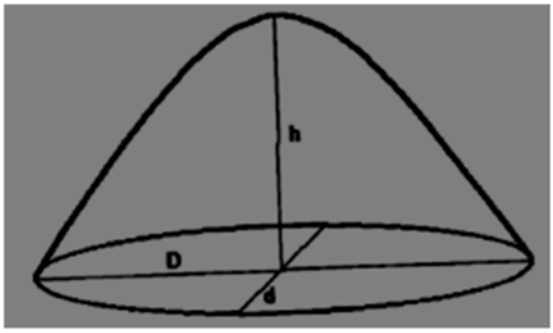
1. Location data - name, geographical coordinates, altitude, date of establishment. This information is needed for subsequent nest localization and tracking. The presence of seasonal changes in the size and shape of the nests will be able to be reported as the surveys are conducted at different times of the year.

2. Nest data - includes a general description, as well as recording various characteristics that reflect the age and activity of the nests. The dimensions of the above-ground part of the nest are measured with a tape measure and the small and large diameter and height are measured on the dome part. (fig. 13). The volume of the nest is calculated by the formula: $V= 2/3 \times \pi \times D/2 \times d/2 \times h$, where D is the large diameter, d - small diameter and h – the height.

The marking of monodomy (M) or polydomy nest (P) depends on whether a nest is connected to another nest by continuous transport of workers between them.

3. Habitat data and assessment of possible threats. This information will allow tracking the changes of the locality over time and the possibility to take measures for their protection.

Fig. 13. Measurements of the small and large diameter of the nest.



$$V = 2/3 \times \pi \times D/2 \times d/2 \times h$$

III. Field work

Identification of nest inventory and distributional patterns

During the study period, two field studies of the Bulgarian and Turkish teams were conducted in July and August in the Bulgarian part of the target area. 30 different localities were visited (fig. 14), where transects 250 meters long were carried out to search for nests of the European red wood ant (*Formica pratensis*). The list of visited places and the starting points of the transects, distributed in the four plant-climatic areas of the region are the following:

Northwestern arid region

the surroundings of the town of Sredets - 42°20'2.32"N 27°11'10.02"E

the surroundings of the village of Bliznak – 42°11'45.80"N 27°19'33.89"E

the village of Bliznak - 42°10'3.00"N 27°18'32.04"E

the surroundings of the village of Bliznak - 42°10'19.00"N 27°18'34.00"E

Petrova Niva locality - 42° 4'2.83"N 27°31'34.47"E

Petrova Niva locality 2 - 42° 4'59.43"N 27°29'37.46"E

the surroundings of the village of Zvezdets - 42° 6'20.84"N 27°25'20.32"E

the surroundings of the village of Slivarovo - 41°58'16.19"N 27°39'30.11"E

the surroundings of the village of Golyamo Bukovo - 42°10'52.41"N 27°13'26.01"E

the surroundings of the village of Belevren - 42° 7'28.66"N 27°11'13.76"E

the surroundings of the village of Granichar - 42° 8'7.87"N 27°14'34.80"E

the surroundings of the village of Stoilovo - 42° 1'53.23"N 27°30'42.85"E

High area

the surroundings of the town of Malko Tarnovo – 41°59'18.33"N 27°32'33.68"E

the surroundings of the town of Malko Tarnovo 2 – 41°59'18.60"N 27°32'34.44"E

the surroundings of the town of Malko Tarnovo 3 - 41°59'24.60"N 27°32'45.42"E

Kachul locality - 42° 1'39.14"N 27°37'18.09"E

the surroundings of the village of Gramatikovo - 42° 1'22.34"N 27°40'18.65"E

the surroundings of the town of Malko Tarnovo, Propada locality 1 - 41°58'54.00"N 27°29'32.00"E

the surroundings of the town of Malko Tarnovo, Propada locality 2 - 41°59'2.68"N 27°29'58.34"E

the surroundings of the town of Malko Tarnovo – 41°58'21.87"N 27°29'57.76"E

the surroundings of the town of Malko Tarnovo - 41°58'30.96"N 27°30'24.60"E

the surroundings of the village of Brashlyan, Kalenik locality 1 - 42° 2'42.47"N 27°26'24.07"E

the surroundings of the village of Brashlyan, Kalenik locality 2 - 42° 2'13.39"N 27°26'41.27"E

the surroundings of the village of Brashlyan, St. Panteleimon - 42° 2'44.09"N 27°26'1.25"E

Area of Pontic rhododendron

the surroundings of the village of Kosti - 42° 3'30.22"N 27°45'51.30"E

Coastal region

the village of Rezovo - 41°59'0.65"N 28° 1'43.53"E

the surroundings of the town of Ahtopol - 42° 6'32.31"N 27°54'54.31"E

the surroundings of the town of Ahtopol 2 - 42° 5'29.99"N 27°56'42.95"E

the surroundings of the village of Sinemorets - 42° 3'0.76"N 27°57'58.34"E

Fig. 14. Map of the visited places in Strandzha for searching nests of *Formica pratensis.*

Nests (8 in number) of the European red forest ant were found in 4 of the visited localities (fig. 15):

1. the surroundings of the town of Malko Tarnovo (41°59'18.33"N 27°32'33.68"E) – 5 nests

2. the surroundings of the town of Malko Tarnovo 2 (41°59'18.60"N 27°32'34.44"E) – 1 nest

3. the surroundings of the village of Brashlyan, St. Panteleimon (42°2'44.09"N 27°26'1.25"E) – 1 nest

4. the surroundings of the village of Bliznak (42°10'3.00"N 27°18'32.04"E) – 1 nest

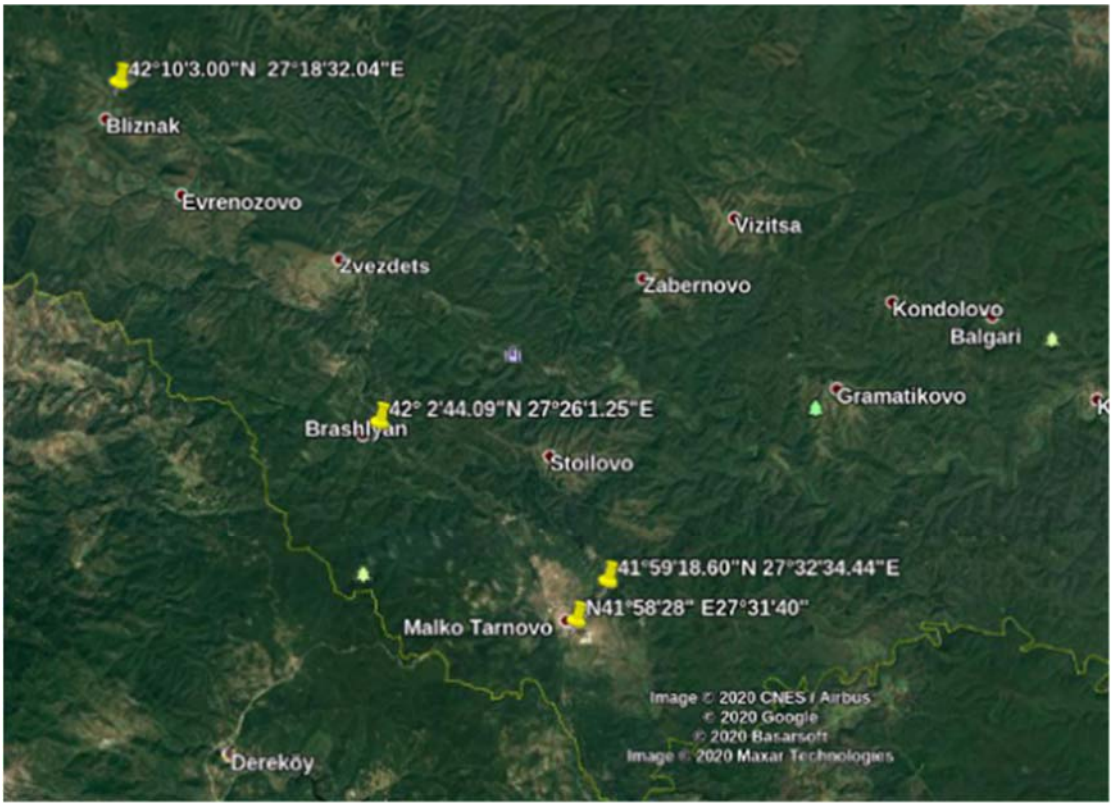


Fig. 15. Map of the four localities in which the nests of *F. pratensis*

Nest ecology

A brief description and characteristic have been made for each of the identified localities of the European red wood ant nests.

Fig. 16. Locality 1.

The first habitat (41°59'18.33"N 27°32'33.68"E, 317 meters above sea level) is located on the outskirts of the urbanized area of Malko Tarnovo. It is a vast pasture near a small water channel and woody vegetation around it (figs. 16, 17, 18). The place is a reclaimed tailings pond. In part of the territory a process of construction of a photovoltaic park has started, which in Figure 16 can be seen behind the built fence. This locality was visited and the discovery of the target species was most likely expected, as in the article by Lapeva-Gjonova & Kiran (2010) one of the two known nests on the territory of Strandzha Mountain was noted there. The first nest was found in the same place.

Fig. 17, 18. General view from locality 1.

The nest (fig. 18) is well masked behind a bushy wild pear (*Pyrus* sp.) and a willow (*Salix* sp.). The measured indicators of the nest show that this is the largest of all found nests. The dome is flat, which is typical for nests located in relatively bright and warm places and consists of dry, plant parts, fused with soil. Active transport of workers from ants to the wetland was observed, where abundant food from small invertebrates, mainly insects, is found. In addition, on the branches of wild pear and willow, around the nest was noted trophobiosis with aphids, which is very characteristic of this species of red wood ant. No other nest was found to be connected to it, so it was marked as monodomic. In fig. 19 the data from the ID-card are presented.

Fig. 18. View of the nest № 1, locality 1.

Date: 20.07.2020	Nest № 1	Locality № 1	Locality name: Malko Tarnovo, tailings pond			GPS: 41.9896 27.5249			
Nest features: nest hidden by a bush of *Pyrus* and *Salix*									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
115	125	56	421,28	flatted	soil, plant dry stems	M	N/A	317	N/A
Habitat features/Threats: Habitat is a pasture, near water small marsh. Threats: deforestation, construction of a photovoltaic park nearby.									

Fig. 19. ID-card fro nest №1.

Four more nests were found on the territory of locality 1 (fig. 20-23), but all of them were significantly smaller in size and located on the more open parts of the pasture. Most of them were found visually when their above-ground domes were discovered, but also by the presence of trophobiotic workers with aphids on shrubby plant species near their nests. All 5 nests from locality 1 are located in the more peripheral parts of the pasture, in close proximity to the wetland with the presence of woody vegetation. This confirms the ecological preferences of this species for warm and sunny places, but close to trees or forests. Of the five established nests, two are polydomic - connected between each-other.

All nests were photographed (fig. 20-23) and for each of them an identification card (fig. 24-27) was filled in with data on the locality, characteristics and parameters of the nest and the identified potential threats to the habitat of the species.

Fig. 20. View of the nest № 2, locality 1.

Date: 20.07.2020	Nest № 2	Locality № 1	Locality name: Malko Tarnovo, tailings pond			GPS: 41.9907 27.5251			
Nest features: nest hidden by a bush of *Salix* and *Cichorium intybus*; trophobiosis with aphids on the *Salix*									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
68	71	40	101,06	dommed	soil, plant live and dry stems	P	9	318	N/A
Habitat features/Threats: Habitat is a pasture, near water canal. Threats: deforestation, construction of a photovoltaic park nearby.									

Fig. 21. ID-card for nest №2.

Fig. 22. View of the nest № 3, locality 1.

Date: 20.07.2020	Nest № 3	Locality № 1	Locality name: Malko Tarnovo, tailings pond			GPS: 41.9912 27.5252			
Nest features: nest on an open pasture, no trees nearby									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
98	105	48	258,48	dommed	soil, plant live and dry stems	M	N/A	317	N/A
Habitat features/Threats: Habitat is a pasture, near water canal. Threats: deforestation, construction of a photovoltaic park nearby.									

Fig. 23. ID-card for nest №3.

Fig. 24. View of the nest № 4, locality 1.

Date: 20.07.2020	Nest № 4	Locality № 1	Locality name: Malko Tarnovo, tailings pond			GPS: 41.9914 27.5249			
Nest features: nest on an open pasture, no trees nearby									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
50	55	35	50,37	dommed	soil, plant live and dry stems	M	N/A	316	N/A
Habitat features/Threats: Habitat is a pasture, near water canal. Threats: deforestation, construction of a photovoltaic park nearby.									

Fig. 25. ID-card for nest №4.

Fig. 26. View of the nest № 5, locality 1.

Date: 21.07.2020	Nest № 5	Locality № 1	Locality name: Malko Tarnovo, tailings pond			GPS: 41.9893 27.5240			
Nest features: low, small nest on an open pasture, no trees nearby									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
49	56	28	40,21	dommed	soil, plant dry stems	M	N/A	319	N/A
Habitat features/Threats: Habitat is a pasture, near is a marsh. Threats: deforestation									

Fig. 27. ID-card for nest №5.

The second locality, where only one nest of the European red wood ant (*Formica pratensis*) was found, is located northeast of the town of Malko Tarnovo. (41°59'18.60"N 27°32'34.44"E, 382 m above sea level). Despite a thorough survey of the area during both field surveys conducted in July and July, no other nests with a diameter of 500 m were found. The nest is located near the asphalt road Malko Tarnovo - Tsarevo (fig. 28, 29) in an artificially green sparse, pine forest. Due to the peripheral location of the nest in the forest, the place is well lit. About 0.5 m from the nest passes a forest path, which further provides higher light and heat to reach the nest. There were 3 main paths of ant workers walking around the nest in search of food. Near the established nest (fig. 30), at a distance of about 3 meters, nest material was found up to a stump, probably from another nest destroyed and then abandoned (fig. 31). The nest found in locality 2 is characterized by relatively small size, which can be seen from the recorded parameters (Fig. 32).

Fig. 28. Locality 2.

Fig. 29. General vew from locality 2.

Fig. 30. The ant nest from locaity 2.

Fig. 31. View from an abandoned nest from a locality 2.

Date: 21.07.2020	Nest № 6	Locality № 2	Locality name: along the main road between Malko Tarnovo and Tsarevo			GPS: 41.9885 27.5429			
Nest features: small nest next to the border of a sparse pine forest, next to an asphalt road									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
38	40	20	15,90	flatted	soil, plant dry stems	M	N/A	382	N/A
Habitat features/Threats: Habitat is near a road, artificial pine forest,. Threats: deforestation, traffic									

Fig. 32. ID-card for the nest from locality 2.

The third locality (fig. 33), where another nest of Formica pratensis was found, is located in the vicinity of the village of Brashlyan, near the church of St. Panteleimon (42°2'44.09"N 27°26'1.25"E, 826 m above sea level). It was discovered during the field work in August, and information about it was received by an employee of Strandzha Park. During the site visit, the ant workers were first found, forming a trail to search for food nearby, crossing the other side of a forest road. The nest is located in a meadow (fig. 34), near a wooded area with a wetter ravine. The nest itself has an exceptionally flat dome (fig. 35), it is possible that it was partially destroyed if haymaking was carried out at this place earlier in the year. The field form from the nest in locality 3 is shown in Figure 36.

Fig. 33. Locality 3.

Fig. 34. General view from locality 3.

Fig. 35. View from the nest from a locality 3.

Date: 21.07.2020	Nest № 6	Locality № 2	Locality name: along the main road between Malko Tarnovo and Tsarevo			GPS: 41.9885 27.5429			
Nest features: small nest next to the border of a sparse pine forest, next to an asphalt road									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
38	40	20	15,90	flatted	soil, plant dry stems	M	N/A	382	N/A
Habitat features/Threats: Habitat is near a road, artificial pine forest,. Threats: deforestation, traffic									

Fig. 36. ID-card of the nest of locality 3.

The fourth locality (fig. 37) with one nest of Formica pratensis in the studied area was discovered during field work in August and is located in the vicinity of the village of Bliznak (42°10'3.00"N 27°18'32.04"E, 308 m above sea level). Initially, trophobiotic workers of ants were found on the spot on a wild pear tree, located on a hay meadow and near a small river. The locality is actively visited by people and regularly mowed. Near the nest is a pumping station of the village. Cattle grazing in the area is also noticeable. There is an asphalt road about 100 of the open nest. The nest is young, small in size, probably in the area due to active haymaking and other human activities is often destroyed. A fresh blackberry twig, which is covered with nest material, also speaks of the recent construction of the nest.

Fig. 37. Locality 4.


Fig. 38. General view from the locality 4.


Fig. 39. View from the nest from locality 4.

Fig. 40. View from the nest from locality 4.;

Fig. 41. View from the nest from locality 4 and participant from the Turkish team – prof. Yılmaz Çamlitepe.

Date: 26.09.2020	Nest № 8	Locality № 4	Locality name: near Bliznak vill.			GPS: 42.16743 27.3072			
Nest features: small nest under *Rubus* sp.									
Nest small diameter (cm):	Nest large diameter (cm):	Nest height (cm):	Nest volume (dm^3):	Flattened/ steeper dommed	Nest material	Polydomy (P) /Monodom (M)	Distance between satellite nests (cm)	Altitude (m)	Nest exposure
38	47	20	15,90	flatted	soil, plant dry stems	M	N/A	308	N/A
Habitat features/Threats: Habitat is near a road, haymaking									

Fig. 42. ID-card for the nest of locality 4.

An obligatory part of the methodology during the field work requires from each established nest to be collected 30 ant individuals and fixed in ethanol in separate tubes for the subsequent laboratory work. This is necessary to verify the identification, which requires a microscopic technique and to establish the intraspecific variation in the morphological characteristics of the body.

Determination of the threats of the European red wood ants

The Strandzha Mountain cross-border region does not differ from other regions in terms of threats to the species. Populations of red wood ants, including the European red wood ant, are under

increasing anthropogenic pressure by **direct and indirect human damage**. This is mainly determined by the destruction of their habitats, as well as the fragmentation of the forest communities with which they are directly connected. Less common is the deliberate destruction of their nests by owners of meadows and private yards, as evidenced by local people in the area. Hay meadows, where the species is often found, are subject to active human activity and the possibility of their nests being destroyed is taken into account.

Intensive logging in forests is often reported in the Strandzha Mountains. The long-term effects of **habitat loss, habitat fragmentation** will have to be monitored on red wood ant populations. Although the species prefers light and warm places, their nests are always close to a group of trees or forest, which provide the necessary microclimate and rich nutrient base. Trees and shrubs are also needed for the almost obligatory symbiosis with aphids, from which they receive sugar-rich sweet excrements, called honeydew.

Due to the significantly weak development of crop production in the Strandzha Mountains, the **negative impact of insecticides** is a relatively weak threat to the nests of the European red wood ant in the area. On the other hand, the risk of **forest fires** is not to be underestimated. Given that the target species occurs near forests, the attention of the population should be increased to prevent them.

During the current study, the greatest threat to the survival of the largest group of nests established in the Bulgarian part of Strandzha, namely the vicinity of the town of Malko Tarnovo, where a **photovoltaic park** is being built. The related infrastructure - **road construction**, **machinery damage**, active human presence, digging a 2-meter-wide trench for laying cables to photovoltaics, which passes literally up to 4 of the ant nest, will most likely negatively affect the state of the species. Additionally, in August 2020, a mass removal of trees near the nests from the wetland was carried out, which will affect the hygroregime of the site and its drying. This will deprive the inhabitants of the largest nest (№ 1) of the prey needed to feed them - mainly other insects. Necessary actions in such cases are **wiring protection and marking the nests** to avoid their accidental destruction.

Fig. 43. Active construction work in the locality 1.

IV. Laboratory studies

The laboratory work is performed in order to confirm the species using a microscopic technique (fig. 44) and to perform morphometric and metric studies to determine the affiliation to one of the two known ecomorphs of the species. A Zeiss stereomicroscope, model Stemi, was used for the study.

Fig. 44. Laboratory equipment and processing of collected materials.

The collected specimens - 240 (30 from each of the eight nests) are prepared dry according to the standard method for ants using entomological pins, triangular entomological cards with dimensions 12/4 mm and entomological glue.

Verification of identification was performed using modern scientific literature and diagnostic features used by Seifert (2018): “Scape slender: SL/SMAX 9.94–11.74. Frons matt. This overall impression is produced by a stronger more reticulate microsculpture. More xerothermic, woodland and woodland–steppe habitats. Discriminant for entire western Palaearctic [with CS in mm] –0.355 CL/CW–6.909 SL/CS+3.06 SL/SMAX–10.774 PEW/CS+0.418 CS–19.5>0.”

Measurement data (head width (HW), ½ by the number of erect hairs on the occipital border on one side of the head (NBH), the length of the longest hair from the occipital margin (LBH) and the number of erect hairs along the outer edge of the posterior tibia (NHTI) (fig. 45) they enter in tabular form.

Fig. 45. Morphometric and metric traits used in the identification of *F. pratensis* (photos: AntWeb)

Seifert (1992) found that there were statistically significant differences in some morphometric measurements and haetotaxy between two ecomorphs of the species *Formica pratensis*. The N-morph is characterized by a larger number of hairs than the P-morph, which has fewer hairs. Various preferences for environmental conditions have also been noted, with N-morph being more thermophilic (preferring warmer habitats) and typically more widespread in Europe. The mean values for the N- and P-morph are as follows:

	N-екоморфа	**P-екоморфа**
HW	1771.8 +/- 218.5	1815.9 +/- 217.5
NBH	28.17 +/- 6.42	17.87 +/- 5.74
LBH	140.6 +/- 20.1	109.7 +/- 26.2
NHTI	25.12 +/- 5.11	17.82 +/- 5.21

Table 1. Mean values of the characters used by Seifert (1992).

The performed measurements (Table 2-9 of the appendix) and the counting of the number of hairs on the head and posterior tibia of the ant specimens collected from the Bulgarian part of Strandzha show values closer to those of *Formica pratensis* ecomorph N (*nigricans*). This is in line with the findings so far in other parts of the country that in the southern parts this morph is more common, while in the more northern parts the P-morph is present. The location of Strandzha in southeastern Bulgaria, the climate with a transitional-Mediterranean character and the relatively low altitude fully correspond to the preferred environmental conditions for the established ecological morph of the species.

Training and joint activities

At the end of August, the Bulgarian team visited the study area in Turkey and conducted joint fieldwork with the team of specialists from the University of Edirne (Fig. 46). The activities included searching for nests, methodologically filling in the field forms and taking into account the parameters of the nests, surveying the terrain and the nests themselves, placing information boards and enclosures. We also participated in the working meeting in Demirkoy with representatives of the leading partner of the project - Dayko, where they discussed the state of research in both countries, organizational and methodological issues, conservation policies.

Fig. 46. Photos from the visits of the Bulgarian team to Turkey and the joint work

Fig. 47. Photo of the leader of the scientific team from the training conducted in Burgas.

Activities to promote the results of the project

During the field work, footage was taken by a professional team for the activities performed by the team in search of ant nests. To support the information on the site under construction, macrophotographs of the target species were made in natural conditions to be used to enrich the photo gallery.

V. Analysis of the situation, conclusions, recommendations and proposals for development of conservation programmes

1. Analysis of the situation

The work on this project was aimed at establishing the current state of the population of red wood ants in the cross-border region of Strandzha Mountain in a joint work of the Bulgarian and Turkish scientific team. As a result, it was found:

- Lack of modern, targeted research on red forest ants in Bulgaria. This calls into question the data reported mainly in the middle and end of the last century on the composition and distribution of the five species of red wood ants in the country. Many of the findings noted in the literature were not made by narrow specialists in the taxonomy of ants, which calls into question their reliability, given that red wood ants often vary in morphology and even hybridize with each other. Data from some areas are not based on published localities, which further requires their confirmation. Such is the case with the publication of *Formica rufa* from the Bulgarian part of Strandzha Mountain, which requires a revision of this announcement, as so far there is no established collected material from the area. In addition, the environmental requirements of this species does not imply to be found. This species has not been also found in the Turkish part of Strandzha Mountain. The only confirmed species from the group of red wood ants in the relatively low altitude and with a transitional Mediterranean climate Strandzha Mountain is the European red wood ant - *Formica pratensis*, which is expected due to the more thermophilic nature of the species. Prior to the start of this project, only 2 localities of the species were published relatively recently (2010) in the scientific literature - from the vicinity of the town of Malko Tarnovo and the village of Rezovo. The availability of suitable conditions for the species in the area implies its wider distribution in conducting a more targeted study. In addition, it is known that in the Turkish part of the mountain the species *Formica pratensis* was found in the last study with a much larger number of nests. It is possible that this difference is due both to the targeted long-term research by specialists there and to the higher altitude of the mountain in the Turkish part of the cross-border area.

- During the study period of this project were visited by the scientific team 30 assessed as suitable places in search of the species, but it was found in 4, all of them located on the periphery of urban areas such as - the vicinity of Malko Tarnovo (2 of them), the surroundings of the village of Brashlyan and the village of Bliznak. The total number of localized nests from the team visits is 8. The results show that in targeted research, despite the short period of research and the limited number of experts, the found nests are 4 times more than known so far, given the studies on ant fauna in Strandzha mountains date back to 1890.
- The data collected by the team on the ecological preferences of the species *Formica pratensis* in the study area confirm its affinity to relatively open and sunny places where they build their nests, but always near a forest and wetland. In all identified cases, workers of the species visited aphid colonies located in nearby trees, from which they collected sweet excrement to supplement their basic protein diet. Six of the established nests are monodomic and only two had an active exchange of workers (polydomic). The size of the mound of the nests varies from small to medium-sized, with a predominance of the flat dome. In most cases, the dome is made of dry plant parts, glued with soil, and sparsely live plants were observed on them.
- Laboratory studies have shown that in the Bulgarian part of Strandzha there is an ecological morphotype - N (*nigricans*), which fully supports what is known so far about its distribution in the southern parts of Bulgaria.
- The location of the established nests in the urban areas emphasizes the need for efforts in their protection by informing the owners of the terrains about their existence, their important ecological role and special attention in order to reduce the risk of flood destruction.
- The joint work of the Bulgarian and Turkish teams proved to be extremely important for synchronizing the work on the study of the target species in the cross-border area, the opportunities for exchange of experience and data, as well as for planning future activities for conservation of natural resources. The Turkish scientific team has a solid base of collected information on the European red wood ant, works together with forestries and conservation organizations that support it in the search for new nests and the protection of existing ones. The nest identification cards developed by the Turkish specialists allowed the two teams to work according to the same methodology, to exchange and compare the obtained data.
- The training of stakeholders and institutions, conducted in Burgas, showed the need to synchronize the efforts of scientific experts, NGOs, management of Strandzha Nature Park, local authorities and control authorities in order to protect nature and seek sustainability.

2. Conclusions

The work of the scientific team on the project managed to conduct the first targeted study on the European red wood ant in the cross-border area. The physico-geographical features of Strandzha, the presence of suitable habitats and the data from the Turkish part of the mountain for the species suggest its wider distribution.

The intensive search for the species in a large number of places and its discovery in only 4 out of 30 shows that the species is relatively rare. The available data at this stage show that the efforts of the experts, supported by the interested institutions, organizations and citizens, to collect new information on the localities of the species in the mountains must continue. However, in order to carry out its protection activities, it is necessary to conduct long-term monitoring and assessment of threats in specific places. For this purpose, the already developed monitoring methodologies and field forms from the National System for Monitoring of Biological Diversity can be used. For the best effect on the conservation of the species, the measures should be joint on both sides of the border area.

3. Recommendations and proposals for development of conservation programmes

Nest inventory and habitat characterization

One of the main conclusions from the scientific analysis of the previous studies of the state of the population of *F. pratensis* is that the accumulated georefered data for the Bulgarian part of Strandzha are extremely insufficient to assess the state of the population of the species. This requires continued collection of new data on the distribution and condition of the species *Formica pratensis* throughout the Strandzha Park. This can be done in the following ways:

• through targeted research of the experts - for this purpose project financing is needed, providing for a large volume of field work and attracting more entomologists;

• by purposefully collecting information about the species from various organizations in the government sector, such as the Executive Forest Agency and its regional divisions, the Strandja Park Administration, the Southeast State Forest Enterprise;

• by collecting basic and supporting information from various projects and programs of non-governmental organizations;

• by collecting data from citizen science, which can be done through specialized Internet platforms, such as iNaturalist and SmartBirds, as well as through non-specialized platforms, such as some groups on Facebook (for example "Insects and Entomologists") or by personal correspondence. The iNaturalist platform (www.inaturalist.org), a joint initiative of the California Academy of Sciences and the National Geographic Society, has over 1,385,000 users and over 53,000,000 observations of worldwide biodiversity, enabling the accumulation of georefered data with the potential for control of their authenticity. It can work with mobile devices, which allows its easy and

effective use for documenting animals, plants and other organisms. The platform currently has over 27,300 records of more than 4,000 species of organisms for Bulgaria. These recordings have been published by over 900 users of the platform - an almost unused capacity. The platform supports the creation of projects that support the organization and analysis of information and its sharing. SmartBirds (www.smartbirds.org) is a Bulgarian platform developed by the Bulgarian Society for the Protection of Birds, whose scope was subsequently extended to larger areas and other organisms. Mobile applications are also available to work with this platform, which greatly facilitates the entry of georeferenced field data;

• by extracting information from various databases, such as GBIF (Global Biodiversity Information Facility) - an international platform with scientific information on biodiversity.

In the case of collecting information from governmental and non-governmental organizations and citizens, the data must be verified by experts through a field visit, complete documentation of the nests by filling in the appropriate form and identification of the species in laboratory conditions. Particularly important in this case is the reliable identification of the species, which can only be done by experts.

Data management

The institutions concerned should collect the new data in databases compliant with the Darwin Core International Biodiversity Database Standard (DwC). DwC is a standard adopted by the TDWG (Taxonomic Databases Working Group), now called Biodiversity Information Standards, and allows the exchange of data between modern applications for biodiversity analysis. Compliance of all collected data with this standard is mandatory for all modern platforms for which they have to exchange information.

Assessment of the conservation status of the species

At both national and regional level, after the initial collection of information, the conservation status of the species should be assessed according to the IUCN criteria (IUCN 2012, 2015, 2017), as the main indicator should be the location and number of nests. This includes calculating the Extent of occurrence (EOO) and Area of Ocupancy (AOO) in Bulgaria and in Strandzha using the IUCN-recommended GeoCAT tool. This will be able to prepare a distribution map. With the help of the recommended Heatmap tool, the regions in Bulgaria and in Strandzha with increased concentration of the species can be identified and the areas important for the species can be determined.

Monitoring

So far known nests, as well as those that will be found in the future, should be included in a system for periodic monitoring of the species, according to the methodology provided in the National Biodiversity Monitoring System (http://eea.government.bg/bg/bio/nsmbr/prakticheskо-rakovodstvo-metodiki-za-monitoring-i-otsenka/Formica_Method.pdf). The monitoring data must be filled in the field form provided by the methodology (http://eea.government.bg/bg/bio/opos/activities-results/Formica_Formulqr.pdf). Monitoring should be carried out regularly and with such frequency as to allow the documentation of phenological changes in nests - for example every 6 months. Specimens of morphometric measurements should be extracted from each newly found nest. These measurements must be made by appropriate optical techniques by experts.

The monitoring will provide evaluating the success of conservation and training studies and of stakeholders, determination of changes in nest numbers and habitats by regular controlling of present nests.

Conservation of *Formica pratensis* colonies

As a consequence of the assessment of the conservation status of the species and the determination of the areas important for it, measures must be taken for the protection of both its habitats and the specific nests.

Given the ecological characteristics of *F. pratensis*, it should be borne in mind that the maintenance of the use of pastures, meadows and surrounding forests, as well as the water regime near the nest, are of particular importance for the protection of nests.

Specific habitat conservation measures should be proposed by experts, separately for each of the species important for the conservation of the species, taking into account the threats to the species in the specific area.

The protection of the specific nests should be in accordance with the anthropogenic pressure of the area, the environmental conditions and the distance from the urban area. When ant nests are located in or in the immediate vicinity of settlements, they should be fenced and marked with informative plates, except in cases where this could lead to conflicts with the local community or cause vandalism directed at the nests. The fencing and marking of the nests must be done with materials that would not be of interest for household use or would not have value when sold on the secondary market. In remote areas, nests should not be fenced off, as this may attract unwanted attention to malicious activity.

Raising awareness

All the activities of the interested scientific experts and institutions would not be effective in the long run if there was a lack or insufficient information about the importance of red wood ants and

the activities for their protection. That is why a constant and targeted campaign is needed, aimed at children, but also at local forestry, municipalities, park management and protected areas by meetings, seminars, practices. These activities should be supported also be Forestry Operation Directorates.

The responsible attitude and increased motivation to keep these species in their natural environment in good functioning condition can also improve the collection of data on their distribution and possible threats in specific places, if they know who to inform and how to gather the necessary information. Therefore, it is important to develop and establish a working platform to fill in the signals, motivate and engage local structures in the protection of red wood ants as part of the local natural wealth.

APPLICATION: Morphometric and meristic data from the laboratory study of the ant specimens from 8 nests of *Formica pratensis* in the Bulgarian part of the border Strandzha mountain

specimen number	HW (мкм)	NBH	LBH	NHTI
Nest 1				
Specimen 1	1621.62	21	135.14	12
Specimen 2	1756.76	20	135.14	19
Specimen 3	1756.76	16	162.16	17
Specimen 4	1513.51	17	108.11	21
Specimen 5	1810.81	18	135.14	18
Specimen 6	1864.86	19	108.11	23
Specimen 7	1783.78	18	189.19	13
Specimen 8	1864.86	24	135.14	19
Specimen 9	1540.54	20	135.14	20
Specimen 10	1513.51	17	135.14	18
Specimen 11	2000.00	23	135.14	23
Specimen 12	1675.68	20	162.16	15
Specimen 13	1675.68	25	108.11	14
Specimen 14	1675.68	23	135.14	19
Specimen 15	1621.62	18	108.11	20
Specimen 16	1594.59	24	162.16	18
Specimen 17	1837.84	21	108.11	17
Specimen 18	1648.65	25	135.14	18
Specimen 19	1783.78	23	162.16	19
Specimen 20	1540.54	19	135.14	21
Specimen 21	1621.62	20	108.11	25
Specimen 22	1810.81	23	135.14	23
Specimen 23	1675.68	24	108.11	20
Specimen 24	1756.76	25	135.14	21
Specimen 25	1594.59	19	108.11	26
Specimen 26	1567.57	24	108.11	14
Specimen 27	1756.76	26	135.14	21
Specimen 28	1648.65	25	108.11	24
Specimen 29	1810.81	22	135.14	20
Specimen 30	1675.68	21	135.14	24
average	1700	21.33	131.53	19.4

Nest 2	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1837.84	16	175.44	21
Specimen 2	1756.76	15	175.44	19
Specimen 3	1891.89	18	105.26	24
Specimen 4	1351.35	10	87.72	17
Specimen 5	1837.84	15	157.89	27
Specimen 6	1459.46	15	105.26	20
Specimen 7	1621.62	13	122.81	18
Specimen 8	1324.32	12	87.72	15
Specimen 9	1405.41	15	70.18	16
Specimen 10	1297.3	16	87.72	17
Specimen 11	1972.97	23	87.72	17
Specimen 12	1297.3	13	70.18	12
Specimen 13	1837.84	20	87.72	26
Specimen 14	1702.7	21	87.72	20
Specimen 15	1864.86	23	87.72	21
Specimen 16	1621.62	22	105.26	16
Specimen 17	1891.89	16	70.18	12
Specimen 18	1432.43	16	87.72	16
Specimen 19	1756.76	20	105.26	14
Specimen 20	1378.38	15	70.18	13
Specimen 21	1891.89	21	122.81	29
Specimen 22	1756.76	15	105.26	26
Specimen 23	1810.81	22	105.26	27
Specimen 24	1891.89	21	87.72	23
Specimen 25	1459.46	18	105.26	17
Specimen 26	1702.7	16	87.72	17
Specimen 27	1486.49	23	87.72	20
Specimen 28	1459.46	12	87.72	18
Specimen 29	1783.78	19	105.26	23
Specimen 30	1162.16	15	87.72	15
average	1631.53	17.2	100.58	19.2

Nest 3	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1675.68	19	122.81	16
Specimen 2	1837.84	20	122.81	18
Specimen 3	1756.76	19	140.35	14
Specimen 4	1756.76	23	192.98	21
Specimen 5	1891.89	17	175.44	19
Specimen 6	1675.68	19	175.44	16
Specimen 7	1891.89	25	192.98	17
Specimen 8	1621.62	23	157.89	14
Specimen 9	1405.41	22	105.26	15
Specimen 10	1486.49	24	87.72	14
Specimen 11	1756.76	24	157.89	15
Specimen 12	1486.49	24	105.26	16
Specimen 13	1891.89	23	105.26	23
Specimen 14	1540.54	20	105.26	9
Specimen 15	1837.84	17	105.26	15
Specimen 16	1756.76	20	140.35	20
Specimen 17	1567.57	22	87.72	17
Specimen 18	1810.81	23	122.81	19
Specimen 19	1540.54	20	87.72	16
Specimen 20	1540.54	18	122.81	17
Specimen 21	1837.84	18	140.35	18
Specimen 22	1783.78	28	140.35	17
Specimen 23	1810.81	22	122.81	20
Specimen 24	1675.68	19	105.26	18
Specimen 25	1513.51	14	140.35	19
Specimen 26	1297.3	17	87.72	13
Specimen 27	1648.65	22	87.72	10
Specimen 28	1675.68	22	87.72	10
Specimen 29	1567.57	20	105.26	9
Specimen 30	1891.89	30	87.72	16
average	1681.08	21.13	123.98	16.03

Nest 4	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1432.43	13	70.18	6
Specimen 2	1810.81	26	87.72	18
Specimen 3	1864.86	25	105.26	19
Specimen 4	1540.54	22	87.72	18
Specimen 5	1351.35	16	87.72	13
Specimen 6	1567.57	12	105.26	15
Specimen 7	1621.62	19	87.72	18
Specimen 8	1081.08	16	87.72	13
Specimen 9	1432.43	16	87.72	14
Specimen 10	1216.22	4	87.72	5
Specimen 11	1891.89	6	70.18	3
Specimen 12	1135.14	13	87.72	13
Specimen 13	1891.89	17	70.18	17
Specimen 14	1486.49	13	70.18	10
Specimen 15	1621.62	13	87.72	15
Specimen 16	1216.22	16	87.72	13
Specimen 17	1891.89	17	87.72	17
Specimen 18	1756.76	14	87.72	8
Specimen 19	1891.89	18	105.26	19
Specimen 20	1891.89	19	87.72	14
Specimen 21	1567.57	21	87.72	16
Specimen 22	1432.43	8	87.72	0
Specimen 23	1810.81	24	105.26	26
Specimen 24	1729.73	18	87.72	18
Specimen 25	1270.27	16	70.18	7
Specimen 26	1783.78	23	105.26	19
Specimen 27	1189.19	8	70.18	6
Specimen 28	1108.11	3	70.18	0
Specimen 29	1486.49	25	87.72	14
Specimen 30	1486.49	11	87.72	21
average	1548.65	15.73	86.55	13.17

Nest 5	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1675.68	13	87.72	17
Specimen 2	1891.89	20	87.72	18
Specimen 3	1756.76	18	87.72	16
Specimen 4	1621.62	17	70.18	16
Specimen 5	1891.89	14	87.72	20
Specimen 6	1756.76	18	105.26	17
Specimen 7	1621.62	11	70.18	24
Specimen 8	1945.95	24	105.26	23
Specimen 9	1891.89	20	70.18	20
Specimen 10	1756.76	15	87.72	22
Specimen 11	1891.89	10	52.63	12
Specimen 12	1675.68	18	70.18	17
Specimen 13	1864.86	11	87.72	22
Specimen 14	1891.89	20	70.18	23
Specimen 15	1702.7	16	70.18	20
Specimen 16	1837.84	15	87.72	16
Specimen 17	1756.76	16	70.18	17
Specimen 18	1891.89	23	122.81	18
Specimen 19	1783.78	10	70.18	17
Specimen 20	1729.73	21	70.18	20
Specimen 21	1810.81	18	87.72	23
Specimen 22	1675.68	16	70.18	24
Specimen 23	1648.65	14	105.26	17
Specimen 24	1783.78	15	70.18	16
Specimen 25	1702.7	16	70.18	14
Specimen 26	1891.89	19	105.26	20
Specimen 27	1864.86	16	70.18	16
Specimen 28	1891.89	16	70.18	17
Specimen 29	1756.76	14	105.26	17
Specimen 30	1729.73	17	87.72	19
average	1786.49	16.37	82.46	18.6

Nest 6	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1756.76	19	122.81	17
Specimen 2	1891.89	16	105.26	18
Specimen 3	1783.78	19	87.72	20
Specimen 4	1891.89	17	122.81	13
Specimen 5	1945.95	10	70.18	12
Specimen 6	1918.92	10	122.81	25
Specimen 7	1945.95	20	87.72	20
Specimen 8	1891.89	16	87.72	14
Specimen 9	1891.89	16	105.26	13
Specimen 10	1756.76	17	105.26	16
Specimen 11	1621.62	16	87.72	16
Specimen 12	1945.95	17	87.72	13
Specimen 13	1675.68	11	105.26	12
Specimen 14	1837.84	15	70.18	19
Specimen 15	1864.86	14	122.81	17
Specimen 16	1891.89	13	87.72	13
Specimen 17	1945.95	16	122.81	18
Specimen 18	1918.92	18	122.81	20
Specimen 19	1783.78	15	87.72	18
Specimen 20	1891.89	16	70.18	19
Specimen 21	1810.81	11	105.26	16
Specimen 22	1756.76	11	105.26	16
Specimen 23	1891.89	20	105.26	15
Specimen 24	1837.84	14	105.26	18
Specimen 25	1783.78	14	105.26	14
Specimen 26	1810.81	18	122.81	19
Specimen 27	1891.89	20	122.81	18
Specimen 28	1837.84	16	105.26	20
Specimen 29	1918.92	22	70.18	21
Specimen 30	1945.95	23	122.81	14
average	1851.35	16	101.75	16.8

Nest 7	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1675.68	19	87.72	16
Specimen 2	1675.68	7	70.18	17
Specimen 3	1540.54	10	87.72	9
Specimen 4	1405.41	13	87.72	3
Specimen 5	1810.81	12	87.72	12
Specimen 6	1567.57	16	105.26	10
Specimen 7	1621.62	15	105.26	11
Specimen 8	2351.35	16	87.72	10
Specimen 9	1810.81	15	70.18	10
Specimen 10	1837.84	12	70.18	4
Specimen 11	1486.49	17	70.18	8
Specimen 12	1702.7	12	105.26	10
Specimen 13	1594.59	13	70.18	12
Specimen 14	1594.59	11	105.26	11
Specimen 15	1621.62	11	70.18	9
Specimen 16	1837.84	12	70.18	12
Specimen 17	1675.68	14	87.72	10
Specimen 18	1837.84	15	87.72	10
Specimen 19	1675.68	19	87.72	9
Specimen 20	1837.84	13	105.26	8
Specimen 21	1891.89	16	87.72	7
Specimen 22	1891.89	10	87.72	15
Specimen 23	1756.76	17	70.18	9
Specimen 24	1783.78	10	70.18	11
Specimen 25	1945.95	17	87.72	17
Specimen 26	1567.57	14	105.26	11
Specimen 27	1729.73	16	105.26	9
Specimen 28	1702.7	14	87.72	9
Specimen 29	1702.7	10	87.72	10
Specimen 30	1756.76	9	70.18	11
average	1729.73	13.5	85.96	10.33

Nest 8	HW (мкм)	NBH	LBH	NHTI
Specimen 1	1729.73	13	70.18	11
Specimen 2	1756.76	20	122.81	8
Specimen 3	1351.35	9	87.72	18
Specimen 4	1972.97	21	122.81	16
Specimen 5	891.89	10	35.09	9
Specimen 6	1459.46	15	70.18	11
Specimen 7	1837.84	15	70.18	12
Specimen 8	1945.95	25	122.81	19
Specimen 9	1837.84	14	70.18	13
Specimen 10	1378.38	13	70.18	17
Specimen 11	1621.62	11	87.72	11
Specimen 12	1675.68	19	87.72	18
Specimen 13	1432.43	21	70.18	14
Specimen 14	1378.38	16	70.18	10
Specimen 15	1729.73	18	122.81	9
Specimen 16	1837.84	19	87.72	15
Specimen 17	1513.51	13	70.18	12
Specimen 18	1891.89	17	122.81	10
Specimen 19	1486.49	14	70.18	16
Specimen 20	1729.73	17	70.18	9
Specimen 21	1594.59	15	87.72	11
Specimen 22	1486.49	14	70.18	17
Specimen 23	1432.43	14	87.72	12
Specimen 24	1837.84	11	70.18	13
Specimen 25	1864.86	16	105.26	13
Specimen 26	1459.46	17	70.18	18
Specimen 27	1567.57	15	70.18	18
Specimen 28	1783.78	11	70.18	12
Specimen 29	1405.41	12	70.18	14
Specimen 30	1648.65	21	122.81	16
average	1618.02	15.53	84.21	13.4

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