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Diversity and Nesting Preferences of *Camponotus lateralis* Group Species on Western Balkan Peninsula (Hymenoptera: Formicidae)

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ABSTRACT

On western Balkan Peninsula *Camponotus lateralis* group Emery, 1925 is represented by three species: *Camponotus lateralis* (Olivier, 1791), *C. piceus* (Leach, 1825) and *C. dalmaticus* (Nylander, 1849). *Camponotus lateralis* group is founded by ecologically subordinated ants that are dominantly adapted to habitats in Mediterranean region. In contrast to *Camponotus lateralis* and *C. dalmaticus*, *C. piceus* is also found in the Dinaric region on open hot meadows. In microhabitat level, *Camponotus piceus* is building colonies in ground and open thermophilic Mediterranean and Dinaric grassland habitats. *Camponotus lateralis* and *C. dalmaticus* are associated with woodland and shrub land habitats in the Mediterranean region. *Camponotus lateralis* construct colonies in dry wood, however 11% of colony findings were under stone. Similar to *Camponotus lateralis*, *Camponotus dalmaticus* build nests in rotten wood parts, roots and tree trunks that were underground. Collected field data indicate that overlap in colony construction niche between analysed species is low due to different habitat and nesting site preferences.

Key words: *Camponotus*, *Myrmentoma*, ants, nesting preferences, Western Balkan Peninsula.

INTRODUCTION

Ants of *Camponotus lateralis* group Emery, 1925 belong to the subgenus *Myrmentoma* Forel, 1912. Morphologically *Camponotus lateralis* group species differ from other groups within the subgenus *Myrmentoma* by flattened dorsal surface of the propodeum. The posterior propodeum can be curved in or flat and the dorsal surface of the thorax is either with or without mesopropodeal depression (Radchenko, 1997).

Biosystematic data indicate that the diversity of the *Camponotus lateralis* group on western Balkan Peninsulais is larger than expected: two undescribed species provisionally named by Seifert (2007) as *Camponotus lateralis* sp. 2 and *C. piceus* sp. 2, Seifert (2007) were reported for the investigated area. According to a recent unpublished investigation of Seifert, *C. lateralis* sp. 2 is still a discrete intraspecific morph of *C. lateralis* meanwhile the heterospecificity of *C. piceus* sp. 2 from *Camponotus piceus* could be clearly confirmed (Seifert pers. comm.).

Camponotus honaziensis Karaman and Aktaç, 2013 has similarities with *Camponotus lateralis* sp. 2. It is possible that *Camponotus honaziensis* from Montenegro; reported by Bračko *et al.* (2014); actually represents *Camponotus lateralis* sp. 2. According to Borowiec and Salata (2013) *Camponotus honaziensis* is equal to *Camponotus lateralis* sp. 2 and it is widely distributed in Mediterranean areas.

Camponotus lateralis group species are a common element in the ant fauna of the Mediterranean region, but it is still a less known component of woodland and grassland habitats in the western Balkan Peninsula due to their subordinated position in ant communities and cryptic nests.

Aside from faunistical investigations, data regarding species autecology and general biology of *Camponotus lateralis* group are scarce (Müller, 1923; Zimmermann, 1934; Kaudewitz, 1955; Seifert, 2007). With this in mind, the primary goal of the current study was to investigate diversity of *Camponotus lateralis* group in the western Balkan Peninsula and to gather general zoological field data which would improve the understanding of autecology of *Camponotus lateralis* group. To determine habitat structure parameters that are important for nesting aggregation and habitat preference of *Camponotus lateralis* group on western Balkan peninsula we investigated number of colonies in regard to their geographical region, habitat type, habitat structure and nesting site microhabitat preferences.

MATERIAL AND METHODS

The researched area of western Balkan Peninsula included: Montenegro, Bosnia-Herzegovina and Croatia. In the areas above we investigated presence of *Camponotus lateralis* group species within three regions: Mediterranean, Dinaric Alps and Continental region. The field investigations were conducted from May to September between 2013 and 2015.

Investigated Sites

In Montenegro the investigated areas were: 1-Herceg Novi, Savinjska wood (lat. 42.453°; lon. 18.552°). In Croatia: 2-Konavle, Lovorno: (lat. 42.547°; E18.361°), 3-Dubrovnik, Velika and Mala Petka: (lat. 42.650°; lon. 18.071°), 4-Peljesac Peninsula, the municipality Janjina (lat. 42.923°; lon. 17.427°), 5-Split (lat. 43.511°; lon. 16.413°) and 6-Pula (lat. 44.881°; lon. 13.881°). In Bosnia-Herzegovina field investigations were conducted in the Mediterranean part, 7-Neum (lat. 42.924; lon. 17.616), 8-Blagaj (lat. 43.257°; lon. 17.898°) and in the Dinaric areas: 9-Velez (lat. 43.348°; lon 17.895°), 10-Diva Grabovica (lat. 43.596°; lon. 17.712°), 11-Sarajevo (lat. 43.858°; lon. 18.447°), 12-Visegrad (lat. 43.773°; lon.19.274°), 13-Zavidovici (lat. 44.424°; lon. 18.177°), 14-Sanski Most (lat. 44.767°; lon. 16.668°), 15-Bihac (lat. 44.812°; lon. 15.870°), 16-Martin Brod (lat. 44.502°; lon. 16.156°), 17-Drvar (lat. 44.379°; lon. 16.384°). In Continental region of Bosnia and Herzegovina we examined three localities: 18-Orasje (lat. 45.028°; lon. 18.717°), 19-Brcko (lat. 44.863°; lon. 18.831°) and 20-Janja (lat. 44.663°; lon. 19.258°) (Fig. 1).

Studied Habitats

Mediterranean forest habitats were represented with *Quercetalia ilicis* and *Pinion heldreichi* forest. Mediterranean scrub lands habitats were represented with: *Ostryo-Quercetum illicis* and *Erico-Cistetalia* habitats. Mediterranean grassland habitats were represented with: *Thero-Brachypodietalia*, *Scorzoneratalia villosae* meadows.

Dinaric forest and scrub lands habitats were represented by: *Pinetum sylvestris dinaricum*, *Ostryo-Fagetum*, *Carpinion betuli*. Dinaric grassland habitats: *Arrenateretea*, *Brometalia erecti, Calluno-Festucion capillatae*, *Festuco-Brometalia*, *Festucion vallesiacae*.

Continental forest and scrub lands habitats were represented by: *Quercetum patraeae-cerris pannonicum*, *Quercetalia pubescentis.* Continental grassland habitats: *Molinion caeruleae, Deschampsietum cespitosae*.

Sampling Methods

On 20 locations and 61 sub locations we have applied the active sampling method. On each sub locality we investigated on three sites that were recognized as a forest, a scrub land and a grassland habitats type. Each of the habitat types was actively sampled on10 plots of 7X7 meters and no more than 5 meters between plots. In total 1830 plots were analyzed.

Sampled ants were stored in 96% ethanol and identified using identification keys (Agosti and Collingwood, 1987; Seifert, 2007) then deposited in the Laboratory for Animal ecology at the Faculty of Science in Sarajevo. All samples were labelled and cross referenced with field protocol numbers.

Habitat Structure Analysis

The field protocol included information on sample code, municipality, locality, date, GPS coordinate, altitude, slope, exposition, region (Mediterranean, Dinaric and Continental) and the level of habitat degradation (forest, scrub land and grassland/ ruderal). Ant colony characteristics are: nest type, placement of the nest and height of the nest above ground. Analysis of forests sublevels included: percentage of the stone coverage and stone diameter, moss height and relative coverage, perennial plant level height and relative coverage, bush level height and relative coverage. Upper storey, tree level height, relative coverage and average diameter breast height were included in the protocol.

The field protocol was applied to record information on newly found colonies and on the scouting workers. Transect line intersect method: a 100 meters in length was used for the assessment of the relative abundance of habitat structure parameters. The transect line was either placed in the center of the colony or where workers were found. Along a transect the newly found workers were considered as individuals from the same colony if find on 7x7 plots. In case of finding a new colony in the transect new data were collected on a separate protocol. Analysed structural parameters

were measured five times within the transect (starting point, 25, 50, 75, and 100 m of the transect).

Specimens Identification

Camponotus lateralis group species were identified using taxonomical keys Agosti and Collingwood (1987), Seifert (2007) and for *Camponotus honazienzis* we used original description according to Karaman and Aktaç (2013). *Camponotus piceus* sp. 2 and *Camponotus lateralis* sp. 2 morphs were identified by morphometric characters according to Seifert (2007).

Continuous descriptive data of habitat structures were analysed by descriptive statistics, ANOVA and post hoc Newman-Keuls test. Connection between habitat structural parameters and number of ant colony findings was tested by linear regression.

RESULTS

Field investigations confirmed that three ant species from *Camponotus lateralis* group are present in the investigated area: *Camponotus lateralis* (Olivier, 1791), *C. dalmaticus* (Nylander, 1849) and *C. piceus* (Leach, 1825). The distinct hairy morph of *Camponotus lateralis* ("*Camponotus lateralis* sp. 2" of Seifert, 2007) was collected from one colony in Montenegro, Herceg Novi (Savinjska wood), second finding were workers sampled in May 2013 from Neum. Morph of *Camponotus piceus* ("*Camponotus piceus* sp. 2" of Seifert, 2007) was collected from Bosnia-Herzegovina: Mostar (Diva Grabovica), Sarajevo (Dariva) and Croatia: Konavle (road from Lovorno to Kuna Konavoska) (Fig. 1). On the investigated area we found and collected samples from 10 colonies of *Camponotus piceus* sp. 2.

In the investigated areas we have collected 128 colonies: *Camponotus dalmaticus* (17.2% sampled colonies), *Camponotus lateralis* (38.3%) and *Camponotus piceus* (44.5%).

Most of the *Camponotus lateralis* group samples were collected in the Mediterranean region, 78.3%. In the Mediterranean region we collected 93.4% of *Camponotus dalmaticus*, 94.4% of *Camponotus lateralis* and 59.1% of *Camponotus piceus* colonies. Due to unequal intensity of sampling between different regions and habitat types, relative abundance of *Camponotus lateralis* group findings was separately computed for each analysed category (Table 1).

In the Mediterranean region of 1.050 plots the total number of sampled colonies was 104 or 9.9%. In the Dinaric region we investigated 660 plots and the total number of positive plots was 24 or (3.6%) (Table 1).

Relative number of colonies shows that *Camponotus lateralis* and *C. dalmaticus* are more common in the Mediterranean regions, while *C. piceus* has similar abundancy in the Mediterranean and the Dinaric regions (Table 1). On 120 plots in the Continental region we did not detect *Camponotus lateralis* group species.

Fig. 1. Camponotus lateralis group distribution map on western Balkan peninsula: The circle with a number indicates locations: 1-Herceg Novi and Savinjska wood, 2-Konavle, Lovorno, 3-Dubrovnik, Velika and Mala Petka, 4-Peljesac, Janjina, 5-Split, 6-Pula, 7-Neum, 8-Blagaj, 9-Velez, 10-Diva Grabovica, 11-Sarajevo, 12-Visegrad, 13-Zavidovici, 14-Sanski Most, 15-Bihac, 16-Martin Brod, 17-Drvar, 18-Orasje, 19-Brcko, 20-Janja; letters: L-*Camponotus lateralis*, L2-*C. lateralis* sp. 2, D-*C. dalmaticus*, P.-*C. piceus*, P2-*Camponotus piceus* sp. 2.

Table 1. Absolute number and percentages of tested plots with *Camponotus lateralis* group colonies in the western Balkan Peninsula.

Region	Mediterranean	Dinaric
Total number of testing sites = 1830	**1050**	**660**
Camponotus lateralis	(47) 0.044	(2) 0.30
Camponotus dalmaticus	(21) 0.020	(1) 0.10
Camponotus piceus	(36) 0.034	(21) 3.20

Relative distribution of *Camponotus lateralis* group species in the Mediterranean and the Dinaric region was analysed in order to determine nesting preferences toward habitat type. In the Mediterranean forest habitats colonies of *Camponotus lateralis* and *C. dalmaticus* had the highest abundance. Colonies of *Camponotus piceus* were dominantly built in the Mediterranean grassland habitats (Table 2).

Table 2. Absolute number and percentages of positive testing surfaces with *Camponotus lateralis* group colonies: M-mediterranean, D-dinaric, C-continental, f-forest habitat, s-scrub land habitat, g-grassland habitat.

Region	M f	M s	M g	D f	D s	D g
Total number of testing sites = 1830	**350**	**350**	**350**	**220**	**220**	**220**
Camponotus lateralis	9.4	3.4	0.5	0.0	0.0	0.9
Camponotus dalmaticus	3.7	2.3	0.0	0.0	0.0	0.5
Camponotus piceus	0.6	3.7	6.0	1.8	2.3	5.5
	Absolute frequency					
Camponotus lateralis	33	12	2	0	0	2
Camponotus dalmaticus	13	8	0	0	0	1
Camponotus piceus	2	13	21	4	5	12

The findings of *Camponotus lateralis* group were concentrated in the range of 0-200 meters above sea level. Within the range of 0-200 meter altitude we collected 91.8% (45/49) samples of *Camponotus lateralis,* 95.5% (21/22) samples of *Camponotus dalmaticus* and 26.3% (15/57) samples of *Camponotus piceus* (Table 3).

Table 3. Absolute number and percentages of findings of *Camponotus lateralis* group species in relation to altitude.

Altitude	0-100	100-200	200-300	300-400	400-500	500-600	over 600
Number of plots	**690**	**360**	**360**	**60**	**150**	**150**	**60**
Camponotus lateralis	3.9	5.0	0.6	1.7	0.0	0.7	3.9
Camponotus dalmaticus	1.0	1.4	1.9	5.0	0.0	0.0	1.0
Camponotus piceus	2.6	4.4	1.7	15.0	0.0	4.7	2.6
	Absolute frequency						
Camponotus lateralis	27	18	2	1	0	1	0
Camponotus dalmaticus	7	5	7	3	0	0	0
Camponotus piceus	18	16	6	9	0	7	1

Due to positively skewed data beside arithmetic, the geometric mean and median were calculated (Table 4). Colony construction site preferences in the investigated group show that *Camponotus piceus* build colonies dominantly in ground which represents 84.2% of findings and 15.8% in ground under rocks. *Camponotus lateralis* build colonies dominantly in dry dead tree trunks (65.3% of colonies) (Table 5). All of the *Camponotus lateralis* colonies were found either in dry hard dead wood trunks or in dry trees branches. The number of *Camponotus lateralis* colonies built under stone was 6.1% (Table 5).

The colonies of *Camponotus lateralis* that were under rocks were a combination of wood and soil in a sort of galleries extending in the ground. However, one colony of *Camponotus lateralis* was built in a concrete wall.

Table 4. *Camponotus lateralis* group species average elevation above sea level preferences in Western Balkan Peninsula.

Structural parameter in habitat	Species (n=number of cases)	Average ± standard deviation (minimal-maximal value)	Geometric mean – Median
Altitude (m)	*C. dalmaticus* (n=22)	176.6±112.0 (15.0, 376.0)	128.8 – 191.5
	C. lateralis (n=49)	111.9±96.8 (16.0, 568.0)	85.7 – 81.0
	C. piceus (n=57)	233.2±195,5 (14.0, 725.0)	168.2 – 198.0

Height of colonies built in tree trunks and branches in *Camponotus lateralis* group was analysed. Colonies of *Camponotus lateralis* were 0-264 cm higher over ground, mode = 20. In *Camponotus dalmaticus* colonies built in tree logs and branches were at height 0-20 cm, mode = 5.

Table 5. Absolute and relative numbers of findings of colonies regarding place of colony construction; absolute number (A) and percentages (P) of findings.

		Dry log	Rotten log	Dry branch on tree	Under bark	In ground	Under rock
Camponotus lateralis	A	32	4	8	2	0	3
	P	65.3%	8.2%	16.3%	4.1%	0.0%	6.1%
Camponotus dalmaticus	A	2	13	2	0	0	5
	P	9.1%	59.1%	9.1%	0.0%	0.0%	22.7%
Camponotus piceus	A	0	0	0	0	48	9
	P	0.0%	0.0%	0.0%	0.0%	84.2%	15.8%

All three species are building colonies dominantly in habitats in south and south-east exposition; *Camponotus piceus* 53.5%, *C. lateralis* 51.8% and *C. dalmaticus* 22.7% of detected colonies. The average ground slope in habitats of *Camponotus lateralis* was in range 0°- 60°, mode = 5°, in *Camponotus dalmaticus* 0°- 40°, mode = 10° and in *Camponotus piceus* 0°- 45°, mode = 15°.

Analysis of structural parameters in habitats where colonies of *Camponotus lateralis* group were constructed indicated that all three species are building colonies on moderately rocky substrates. Most of the colonies were found in habitats with 10%-40% of surface covered with stones; in *Camponotus lateralis* 73.1% of colony findings, *C. piceus* 73.7% and *C. dalmaticus* 81.9%.

Trees level shows most consistency and clearly indicates a differentiation between analysed *Camponotus lateralis* species. Linear regression analysis indicated that *Camponotus piceus* has a negative linear correlation with trees level coverage $y = -0.3014x + 24.571$, $R^2 = 0.5258$, $p < 0,00$, while *Camponotus lateralis* and *C. dalmaticus* show positive regression.

Statistically significant difference by ANOVA and post hoc Newman-Keuls test at ($p<5\%$)in tree diameter was detected between *Camponotus lateralis* and *C. piceus* habitats.

CONCLUSIONS AND DISCUSSION

In western Balkan Peninsula, we have confirmed the presence of *Camponotus lateralis* sp. 2, and *C. piceus* sp. 2 morphs. Confirmed morphs had sympatric distribution with *Camponotus lateralis* and *C. piceus*. *Camponotus lateralis* sp. 2 was represented by colony from Herceg Novi (Savina wood) and samples of *Camponotus lateralis* sp. 2 workers from Neum are representing the northern most findings.

Nesting preferences of *Camponotus lateralis* group species can be explained by ants' temperature preferences. The ant nest temperature can significantly affect the development time of immature stages (Porter, 1988, Heinrich, 1993). Cold climate ants are building ant hills over the ground and construct colonies on sites with higher insolation (Brian and Brian, 1951; Pontin, 1960; Lach *et al.*, 2010). Temperate forests ants as *Camponotus ligniperdus* and *C. herculeaneus* build colonies usually in wood. Wood is a good insulator and prevents rapid temperature changes in colonies. North temperate ant species also occupy dead logs and stumps that are heating more rapidly below bark or in surface galleries (Hölldobler and Wilson, 1990). According to our findings *Camponotus lateralis* and *Camponotus dalmaticus* are thermophiles species with narrower niche in compare to *Camponotus piceus*. Colonies of *Camponotus lateralis* and *C. dalmaticus* are aggregated in the Mediterranean habitats on lower elevations Colwell *et al*. (2008) which indicate the thermophilic nature.

In the Mediterranean regions, the main differences between *Camponotus lateralis* group species were in their habitats preferences. In the Mediterranean regions *Camponotus piceus* was building colonies in open grassland habitats while *Camponotus lateralis* and *C. dalmaticus* were building in forest and scrub land habitats. Colonies of *Camponotus lateralis* were found in habitats with largest tree diameters which indicates a preference toward old forests. Compared to other *Camponotus lateralis* group species, *Camponotus lateralis* shows a variety of micro habitats for colony construction and had the broadest ecological niche on western Balkan Peninsula. In contrast to our investigations Zimmermann (1934) has discovered nests *of Camponotus lateralis* in south Dalmatia but only in ground. Our data do not indicate regional preferences towards soil as nesting place for *Camponotus lateralis* or local intraspecific differences in the investigated area.

The main detected difference in colony construction between *Camponotus lateralis* and *C. dalmaticus* was in their tree choices of different decay level. Colonies of *Camponotus dalmaticus* were detected in rotten wet tree logs and in dead rotten tree roots, these were dispersing in surrounding ground. In total 60% of the *Camponotus dalmaticus* colonies existed in rotten logs or in underground dead tree roots.

In Dinaric regions with sub Mediterranean climate *Camponotus lateralis* group species show similar distribution pattern to the Mediterranean populations. And in Dinaric habitats with continental climate influence, only *Camponotus piceus* was present.

Camponotus lateralis and *C. dalmaticus* inhabit woodland and scrub land habitats with very little direct sun radiation. In hot Mediterranean climate *Camponotus lateralis*

and *C. dalmaticus* are building colonies more often in wood, then in soil. By using wood for colonies constructions investigated species were probably compensating lower temperatures in shaded woodland habitats. Species using rocks, stumps, or logs as nest sites are vulnerable to low humidity and high temperature than those nesting in the soil (Gösswald, 1938). Chen *et al.*, (2002) found that shaded, woodland, habitats of *Camponotus vicinus* have lower temperatures and greater humidity in comparison to open habitats. In our opinion *Camponotus lateralis* in woodland habitats build colonies more often in wood and over ground to compensate higher humidity in shaded habitats.

Open grassland Mediterranean habitats are characterized by high ground temperatures due higher insulations and also by higher temperature variation (Cros *et al.*, 1997).

In the Mediterranean and Dinaric regions, we found *Camponotus piceus* colonies in scrub land and grassland habitats. Colonies of *Camponotus piceus* were built uniformly without ant hill and with one inconspicuous opening in ground. In the Mediterranean habitats only one colony of *Camponotus piceus* was built under stone and in the Dinaric region six colonies were found under stones. The colonies in ground provide protection from high day temperatures in exothermic grasslands that are inhabited by *Camponotus piceus.* In soil, below ground, humidity and temperature have a low degree of variability (Hölldobler and Wilson, 1990). In the Dinaric habitats colonies of *Camponotus piceus* were built on grounds with higher inclination, southern expositions and often under stone. It is possible that *Camponotus piceus* also use stones for faster colony heating, like some other cold adapted ant species (Hölldobler and Wilson, 1990).

Differences in colony construction sites between *Camponotus piceus* and *C. piceus* sp. 2 were analysed. Data indicate that *Camponotus piceus* sp. 2 build colonies in ground without anthill. Colonies of *Camponotus piceus* sp. 2 were on habitats with larger inclination and with 40% to 80% stone coverage. *Camponotus piceus* sp. 2 colonies and workers were more often collected in the Mediterranean regions on higher elevations over 300 meters and in the Dinaric regions over 400 meters. Findings of *Camponotus piceus* sp. 2 colony on Mt. Velez was finding of *Camponotus piceus* with highest elevation over 800 meters. In the context of altitudinal and thermal preferences within *Camponotus lateralis* group we find that *C. piceus* have the widest ecological valence. Literature data indicate that *Camponotus piceus* also has greater northern distribution compared to *Camponotus lateralis* and *C. dalmaticus* (Radchenko, 1997).

The results of our studies indicate ecological niche partitioning in the dimension of nest construction between three closely related *Camponotus lateralis* group species. Nest constructing differences between analysed species show spatial allocation. In the Mediterranean regions the competition between queens of *Camponotus lateralis* group probably maintain detected differences in the place of colony construction. In the Dinaric regions the main factor of absence of *Camponotus lateralis* and *C. dalmaticus* was temperature.

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