

Preference of antlion and wormlion larvae (Neuroptera: Myrmeleontidae; Diptera: Vermileonidae) for substrates according to substrate particle sizes

DUŠAN DEVETAK¹ and AMY E. ARNETT²

¹Department of Biology, Faculty of Natural Sciences and Mathematics, University of Maribor, Koroška cesta 160, SI-2000 Maribor, Slovenia; e-mail: dusan.devetak@guest.arnes.si

²Center for Biodiversity, Unity College, 90 Quaker Hill Road, Unity, ME 04915, U.S.A.; e-mail: aarnett@unity.edu

Key words. Neuroptera, Myrmeleontidae, Diptera, Vermileonidae, antlions, wormlions, substrate particle size, substrate selection, pit-builder, non-pit-builder, habitat selection

Abstract. Sand-dwelling wormlion and antlion larvae are predators with a highly specialized hunting strategy, which either construct efficient pitfall traps or bury themselves in the sand ambushing prey on the surface. We studied the role substrate particle size plays in these specialized predators. Working with thirteen species of antlions and one species of wormlion, we quantified the substrate particle size in which the species were naturally found. Based on these particle sizes, four substrate types were established: fine substrates, fine to medium substrates, medium substrates, and coarse substrates. Larvae preferring the fine substrates were the wormlion *Lampromyia* and the antlion *Myrmeleon hyalinus* originating from desert habitats. Larvae preferring fine to medium and medium substrates belonged to antlion genera *Cueta*, *Euroleon*, *Myrmeleon*, *Nophis* and *Synclisis* and antlion larvae preferring coarse substrates were in the genera *Distoleon* and *Neuroleon*. In addition to analyzing naturally-occurring substrate, we hypothesized that these insect larvae will prefer the substrate type that they are found in. Specifically, we tested substrate preference in a choice experiment for four species of antlions. This was then compared to the substrate that the larvae were naturally found in. Larvae of the four antlion species were allowed to choose among eight substrates differing according to their particle size. Our findings show that the majority of *M. hyalinus* larvae built pits in the finest sand fraction, and the majority of *N. microstenus* larvae were found in a coarser fraction. The other two species, *M. immaculatus* and *E. nostras*, constructed majority of their pits in a medium sand fraction. These results support the hypothesis that neuropteran larvae prefer specific substrates for pitbuilding or ambushing prey, and that this – in combination with other abiotic and biotic factors – may drive selection of appropriate habitats.

INTRODUCTION

Organisms respond to biotic and abiotic environmental factors optimally to maximize their fitness. Natural selection will favour individuals who choose the most suitable habitat and therefore the greatest number of successful offspring can be raised. Habitat selection may be affected by the spatial variation in the distribution of resources, presence of other organisms, physiological state of the individual or abiotic factors, such as physical properties of the environment (Janetos, 1986; Rosenzweig, 1995). In the past, habitat selection theory mostly focused on biotic factors while abiotic factors (such as temperature) were often neglected. As shown for red flour beetles, temperature is clearly an important factor in habitat selection of ectotherms and should be considered whenever thermal differences exist between habitats (Halliday & Blouin-Demers, 2014).

In certain terrestrial animals, habitat substrate can play an important role. In desert scorpions, sand-dwelling antlions, and gerbils which are restricted to sandy substrates, selective pressures on choosing the appropriate substrate should be strong since sandy substrate may affect feeding strategy. It influences the rate and ease with which food can be found (Brownell & Farley, 1979; Devetak, 2014) and plays a role in exposure to predators as well as the

likelihood of successfully evading an encountered predator (Kotler et al., 2001).

Larvae of antlions (Neuroptera: Myrmeleontidae) are predators living in a variety of habitats, ranging from arboreal and rocky habitats, living in dust and detritus, and up to true psammophily (Mansell, 1996, 1999). Sand-dwelling larvae, i.e. psammophily, culminate in the ability to modify the sandy environment into effective pitfall traps (Mansell, 1999).

While the majority of antlion species inhabit sandy substrate or loose soil, some build conical pitfall traps. These pit-building antlion larvae wait for their prey in the center of the pit. Using specialized traps is an advanced predatory strategy that evolved early in antlions (for review see Gepp & Hölzel, 1989; Scharf & Ovadia, 2006; Ruxton & Hansel, 2009; Gepp, 2010; Scharf et al., 2011). In contrast, the other sand-dwelling antlions are non-pit-builders, which bury themselves in the sand waiting for prey on the surface.

The other sand-dwelling insects, wormlions (Diptera: Vermileonidae) resemble the pit-building antlions in their hunting strategy, offering a classical example of convergent evolution, as they belong to different insect orders. Their behavioural traits have rarely been studied (Devetak, 2008; Dor et al., 2014).

Wormlion larvae lack long mandibles, but other parts of their elongated body are specialized for prey capture,

including the mouthparts and pseudopodium (Wheeler, 1930; Ludwig et al., 2001; Devetak, 2008; Dor et al., 2014). The pseudopodium is probably involved in the mechanical detection and seizure of prey. An abdominal comb with a row of long spines on the posterior part of the larval body is used to anchor it in the fine substrate (Ludwig et al., 2001; Dor et al., 2014). Studying foraging behavior of wormlions, Dor et al. (2014) found that wormlions' body mass and length were not tightly correlated with pit size, in contrast to antlions. Wormlions rarely co-occur with antlion larvae and it seems that antlions avoid areas inhabited by wormlions and vice versa. The larvae may simply prefer different substrates independent of the presence of heterospecifics (Devetak, 2008).

Foraging behaviour of pit-building antlions is not simply guided by a few factors as was once commonly accepted, but is much more complex and can vary greatly (e.g. Arnett & Gotelli, 2001; Scharf & Ovadia, 2006; Scharf et al., 2011; Dor et al., 2014). Habitat selection is an important component of fitness in sit-and-wait predators and particularly for pit-building antlions (Scharf & Ovadia, 2006). In pit-building antlions habitat suitability is based on sandy substrate depth (Loria et al., 2008; Scharf et al., 2009), particle size (Lucas, 1986; Farji-Brener, 2003; Ábrahám, 2006), substrate density (Devetak et al., 2012), prey availability and abundance (e.g. Griffiths, 1980), disturbance regime (Gotelli, 1993; Barkae et al., 2010), presence of conspecifics and heterospecifics (e.g. Matsura & Takano, 1989; Linton et al., 1991; Scharf et al., 2008b; Barkae et al., 2010) and microclimatic conditions. Microclimate can play an important role in pit-building decisions, e.g. shade (Topoff, 1977; Scharf et al., 2008c), photoperiod (Scharf et al., 2008c), soil temperature (Geiler, 1966; Marsh, 1987; Ábrahám, 2003), rain and soil moisture (Gotelli, 1993; Morrison, 2004).

Although substrate selection according to sand particle size in antlion larvae under laboratory conditions is well known (Youthed & Moran, 1969; Kitching, 1984; Allen & Croft, 1985; Loiterton & Magrath, 1996; Botz et al., 2003; Farji-Brenner, 2003; Devetak et al., 2005; Matsura et al., 2005), almost no research concerning substrate particle size composition has been carried out yet in natural habitats. Currently, the only comprehensive study in natural habitats, or antlion zones, has been conducted in Hungary (Ábrahám, 2003).

The aims of this study were to: (i) quantify the natural substrates of different psammophilous insect species according to their substrate particle sizes; (ii) test if antlion larvae are capable of discriminating between substrate fractions with different particle sizes; and (iii) examine if antlion species differ in their preference for a certain substrate type. We hypothesize that antlions will prefer substrates that are similar to that found in their natural habitat.

MATERIAL AND METHODS

Substrate particle sizes in natural habitats

Substrates of natural habitats of one wormlion and 13 antlion species from 17 sites were examined (Table 1). Substrate samples

were collected in natural antlion habitats using a 12 ml volume spoon. Sand or loose soil was collected with the spoon so that sand solely (in the case of non-pit-building antlions) or sand together with an antlion forming a pit was taken. Substrate from a pit and its surroundings was taken with the spoon three times so that the total volume of one sample was approximately 30–40 ml. Special care was devoted to sampling some species (e.g. *Myrmeleon formicarius* and *M. fasciatus*) living in very small patches of loose soil. In such patches we avoided sampling substrate in the surroundings of the pit where often soil quality drastically differed from that in the pit. Samples were transported in plastic bags separately and dried for two weeks at room temperature ($26 \pm 3^\circ\text{C}$) and relative humidity 30%.

Antlion larvae together with the substrate samples were collected during spring and summer months (from May to August), with an exception of *Myrmeleon yemenicus* larvae which were collected at the end of November (for details of its habitat – see Devetak et al., 2010). The majority of the larvae were reared to adults. For identification we used fundamental literature (for the larvae: Lucas & Stange, 1981; Badano & Pantaleoni, 2014; for adults: Aspöck et al., 1980).

Antlion natural substrate is a mixture of grains of different particle sizes. Substrate particle sizes were determined by sieving dry substrate samples with standard sieves used previously (see Devetak et al., 2005) and fractions composing a certain substrate were expressed in weight percent (w%). Mass of the fractions was determined by weighing dry substrate samples using a scale (Kern and Sohn GmbH, Balingen, Germany; accuracy of 0.001 g).

According to the particle-size composition, each sample is expressed as a mixture of the following fractions (listed in ascending series of sand particle sizes):

- (G1) fraction with particle sizes $< 60 \mu\text{m}$,
- (G2) fraction with particle sizes $60\text{--}110 \mu\text{m}$,
- (G3) fraction with particle sizes $110\text{--}230 \mu\text{m}$,
- (G4) fraction with particle sizes $230\text{--}540 \mu\text{m}$,
- (G5) fraction with particle sizes $540\text{--}1000 \mu\text{m}$,
- (G6) fraction with particle sizes $1000\text{--}1540 \mu\text{m}$,
- (G7) fraction with particle sizes $1540\text{--}1750 \mu\text{m}$,
- (G8) fraction with particle sizes $1750\text{--}2200 \mu\text{m}$,
- (G9) fraction with particle sizes $> 2200 \mu\text{m}$.

Substrate particle-size fractions representing > 20 w% in a certain sample are determined as dominant, and fractions representing $10\text{--}20$ w% as important (Table 1). Using the presence of dominant and important substrate particle-size fractions as a criterion, the following three basic categories were determined:

- (i) Fine substrates: ≥ 30 w% is composed of fractions G1–3;
- (ii) Medium substrates: ≥ 30 w% is composed of fractions G4–6;
- (iii) Coarse substrates: ≥ 30 w% is composed of fractions G7–9.

Preference for different sands of various particle sizes in choice experiments

In order to test the preference of larvae of different antlion species to different substrates, four antlion species were chosen: one naturally occurring in fine to medium sands (*Myrmeleon hyalinus*: Cyprus, $n = 27$), two occurring in medium sands (*Euroleon nostras*: Slovenia, $n = 25$; *Myrmeleon immaculatus*: USA, $n = 26$), and one typically found in coarse substrates (*Neuroleon microstenus*: Slovenia, $n = 26$). Prior to experimental manipulation the larvae were held in plastic cups filled with sand from their natural habitat, separated to avoid cannibalism. Room temperature was $26 \pm 3^\circ\text{C}$. Only third instar larvae were used in the experiments and larval stages were determined by measuring body length and head capsule width (Devetak et al., 2005).

TABLE 1. Substrates according to substrate particle sizes in natural habitats of antlions and wormlions. Abbreviations: PB = pit-builder; NPB – non-pit-builder; WL – wormlion; G1 – fraction with particle sizes <60 µm; G2 – 60–110 µm; G3 – 110–230 µm; G4 – 230–540 µm; G5 – 540–1000 µm; G6 – 1000–1540 µm; G7 – 1540–1750 µm; G8 – 1750–2200 µm; G9 – >2200 µm. N = number of samples.

Number / Locality / N	Habitat type/ Substrate type	Antlion species/ Status: PB, NPB	Dominant sub- strate particle size fraction (µm) (>20%)	Important sub- strate particle size fraction (µm) (10–20%)	Substrate type
1. Africa: Tunisia: Djerba: Midoun N = 12	Sand beach – semidesert/ fine sand	<i>Myrmeleon hyalinus</i> Olivier, 1811 – PB <i>Nophis teillardi</i> Navás, 1912 – NPB	G4, G3	G2	Fine to medium
2. Africa: Tunisia: Sahara: Douz N = 12	Sand desert/fine sand	<i>Myrmeleon hyalinus</i> Olivier, 1811 – PB	G2	G3	Fine
3. Africa: Tunisia: El-Jem N = 12	Sand desert/fine sand	<i>Myrmeleon fasciatus</i> (Navás, 1912) – PB	G4, G3	G2	Fine to medium
4. Asia: Cyprus: Salamis N = 12	Desert sand/fine sand	<i>Cueta lineosa</i> (Rambur, 1842) – PB <i>Myrmeleon hyalinus</i> Olivier, 1811 – PB	G4	G3	Medium
5. Asia: Yemen: Shibam- Kawkaban N = 12	Stony semidesert/ volcanic sand	<i>Myrmeleon yemenicus</i> Hölzel, 2002 – PB	G4	G3, G5	Medium
6. Asia: Yemen: Summarah N = 12	Stony semidesert: rock overhangs/volcanic soil	<i>Myrmeleon yemenicus</i> Hölzel, 2002 – PB	G4	G5, G6, G9	Medium
7. Europe: Albania: Berat N = 12	Sand dunes – river bank/ fine sand	<i>Cueta lineosa</i> (Rambur, 1842) – PB <i>Myrmeleon inconspicuus</i> (Rambur, 1842) – PB	G4, G3, G2		Fine to medium
8. Europe: Austria: Leibnitz, Seggauberg N = 12	Crevice in a wall/ sand	<i>Euroleon nostras</i> (Geoffroy in Fourcroy, 1785) – PB	G4, G2	G3, G6	Fine to medium
9. Europe: Croatia: Ilovik N = 12	Sand beach/sand	<i>Synclisis baetica</i> (Rambur, 1842) – NPB	G4	G3	Medium
10. Europe: Croatia: Rovinj: Villas Rubin N = 12	Grass-land with stones and sand/coarse sand	<i>Distoleon tetragrammicus</i> (Fabricius, 1798) – NPB <i>Neuroleon microstenus</i> (McLachlan, 1898) – NPB	G7, G9	G6, G8	Coarse
11. Europe: Greece: Kos Island: Tigaki N = 12	Sand beach/sand	<i>Myrmeleon hyalinus</i> Olivier, 1811 – PB	G4	G5	Medium
12. Europe: Hungary: Kiskunsági National Park, Bugacpuszta N = 12	Sand dunes/fine sand	<i>Myrmeleon bore</i> (Tjeder, 1941) – PB <i>Myrmeleon inconspicuus</i> Rambur, 1842 – PB	G3, G4	G2	Fine to medium
13. Europe: Slovenia: Boč N = 12	Under rock overhangs/ loose soil	<i>Euroleon nostras</i> (Geoffroy in Fourcroy, 1785) – PB	G4	G6, G9, G2, G3	Medium
14. Europe: Slovenia: Črni Kal N = 12	Grass-land with stones and sand/coarse sand	<i>Distoleon tetragrammicus</i> (Fabricius, 1798) – NPB <i>Neuroleon microstenus</i> (McLachlan, 1898) – NPB	G9	G6, G7, G4	Coarse
15. Europe: Slovenia: Pohorje: Slivniško Pohorje N = 12	Under rock overhangs/ loose soil	<i>Myrmeleon formicarius</i> Linnaeus, 1761 – PB	G9, G4	G6, G5	Medium
16. Europe: Spain: Barcelona: Parc Grúel N = 12	Rocky pockets/ fine powder	<i>Lampromyia iberica</i> Stuckenberg, 1971 – WL	G2, G1	G4, G3	Fine
17. North America: U.S.A.: Connecticut: Salmon River State Forest N = 12	Sandy soil with tall grass and medium-sized shrubs/ loose soil	<i>Myrmeleon immaculatus</i> De Geer, 1773 – PB	G4	G5, G6	Medium

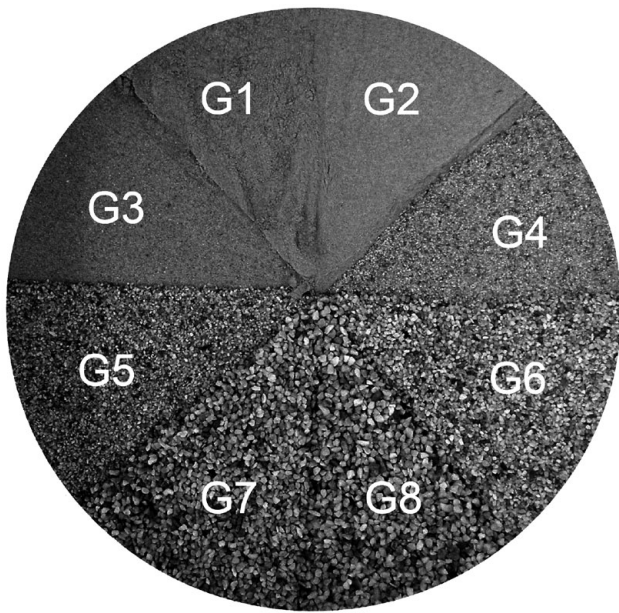


Fig. 1. Partitioning of substrates used in the choice experiment. G1 – fraction with particle sizes $<60 \mu\text{m}$; G2 – $60\text{--}110 \mu\text{m}$; G3 – $110\text{--}230 \mu\text{m}$; G4 – $230\text{--}540 \mu\text{m}$; G5 – $540\text{--}1000 \mu\text{m}$; G6 – $1000\text{--}1540 \mu\text{m}$; G7 – $1540\text{--}1750 \mu\text{m}$; G8 – $1750\text{--}2200 \mu\text{m}$.

Choice experiments started with a habituation period during which antlions were fed with ants. One *Lasius fuliginosus* ant was fed to each antlion per day for five days after which five days of food deprivation occurred. This handling was conducted to standardize the hunger level of the larvae and ensure that the physiological state of all larvae was approximately the same at the beginning of the experiment. After these ten days, a single antlion was placed in the center of a container with eight different sand fractions.

To obtain fractions with different particle sizes, comminuted sand (Gradis d.o.o., Maribor, Slovenia; grain sizes $0\text{--}4 \text{ mm}$) was sieved. The sand was dried for two weeks at room temperature ($26 \pm 3^\circ\text{C}$); the moisture content of the substrate was less than 1.5% by weight.

We anticipated that antlions should be capable of distinguishing among substrate types. We tested this hypothesis by giving antlions the chance to choose one of eight particle-size fractions. Each antlion was treated only once. Round plastic containers (diameter 20 cm; height 11 cm) were partitioned using a plexiglass partitioner divided into eight parts of equal size. Each compartment was carefully filled with 100 ml of sand of a certain particle-size fraction. The fractions G1–G8 were used in the experiment. Substrates were partitioned as shown in Fig. 1. The plexiglass partitioner was then carefully removed and an antlion was dropped into the center. After 24 h we recorded the fraction in which an antlion was present simply by watching the antlion's reaction to an ant released on the sand surface, or in the case of pit-builders, where a pit was built.

Statistical analysis

The programs GraphPad Prism and SPSS 17.0 for Windows were used for statistical analyses and graphical presentation. A chi-square test was used to determine differences in proportion of antlions present in each substrate type after 24 h.

RESULTS

Distribution of antlion species in different types of substrates in natural habitats

We determined substrate particle size composition of substrates in natural habitats for thirteen antlion and one wormlion species from eleven countries and four continents (Table 1; Fig. 2). Loose soil or sand from various habitat types was investigated – e.g. sand beaches, sand dunes, sand deserts, stony semideserts, river banks, grasslands, and under rock overhangs. According to the presence of certain substrate particle sizes the following four substrate groupings were recognized (Table 1):

(i) Fine substrates (fractions G1–3 $\geq 30 \text{ w\%}$ of the substrate).

The finest substrate is a mixture of powder and fine sand from Parc Grüel (Spain) with dominant fractions G2 and G1. The second finest sand is a Saharan sand from Douz (Tunisia) which is composed of G2 as a dominant fraction and G3 as important fraction (mean \pm SD: $73.5 \pm 4.5 \text{ w\%}$; $15.1 \pm 3.9 \text{ w\%}$).

(ii) Fine to medium substrates (fractions G1–6 $\geq 30 \text{ w\%}$).

Substrates with fine (G1–3) and medium fractions (G4–6) were collected in Djerba and El-Jem (both Tunisia), Berat (Albania), Leibnitz (Austria), and Bugacpuszta (Hungary).

(iii) Medium substrates (fractions G4–6 $\geq 30 \text{ w\%}$).

Medium substrates with important finer fractions (G1–3) were collected in Salamis (Cyprus), Shibam-Kawkaban (Yemen), and Ilovik (Croatia). Medium substrates with important medium fractions (G4–6) originated from Kos (Greece) and Salmon River State Forest (Connecticut, U.S.A.). Medium substrates with important coarser fractions (G7–9) were collected in Summarah (Yemen), Boč and Pohorje (both Slovenia).

(iv) Coarse substrates: (fractions G7–9 $\geq 30 \text{ w\%}$).

Coarse substrates originated from Rovinj (Croatia) and Črni Kal (Slovenia).

These results show that antlions occupy substrates of varying substrate size composition regardless of their prey capture strategy. Four of the investigated species (*Nophis teillardi*, *Neuroleon microstenus*, *Distoleon tetragrammicus*, and *Synclisis baetica*) were non-pit-builders, and the rest of the antlions were pit-builders.

The wormlion *Lampromyia iberica* was found in the finest sand with two dominant fractions G1 and G2 (mean \pm SD: $23.4 \pm 6.4 \text{ w\%}$; $27.7 \pm 3 \text{ w\%}$; Barcelona, Parc Grüel, Spain). The second finest sand is a Saharan sand from Douz (Tunisia) which is composed of G2 as a dominant fraction ($73.5 \pm 4.5 \text{ w\%}$). In the second finest substrate pit-building antlion *Myrmeleon hyalinus* was very common.

In contrast to fine substrates, coarse substrates were convenient for non-pit-building antlions. Two non-pit-builders, *Neuroleon microstenus* and *Distoleon tetragrammicus*, were found in the two coarsest substrates (Črni Kal, Slovenia: dominant fraction G9: mean \pm SD: $33.8 \pm 4.6 \text{ w\%}$; Rovinj, Croatia: dominant fractions G7 and G9: mean \pm SD: $27.7 \pm 3 \text{ w\%}$; $21.4 \pm 6.3 \text{ w\%}$).

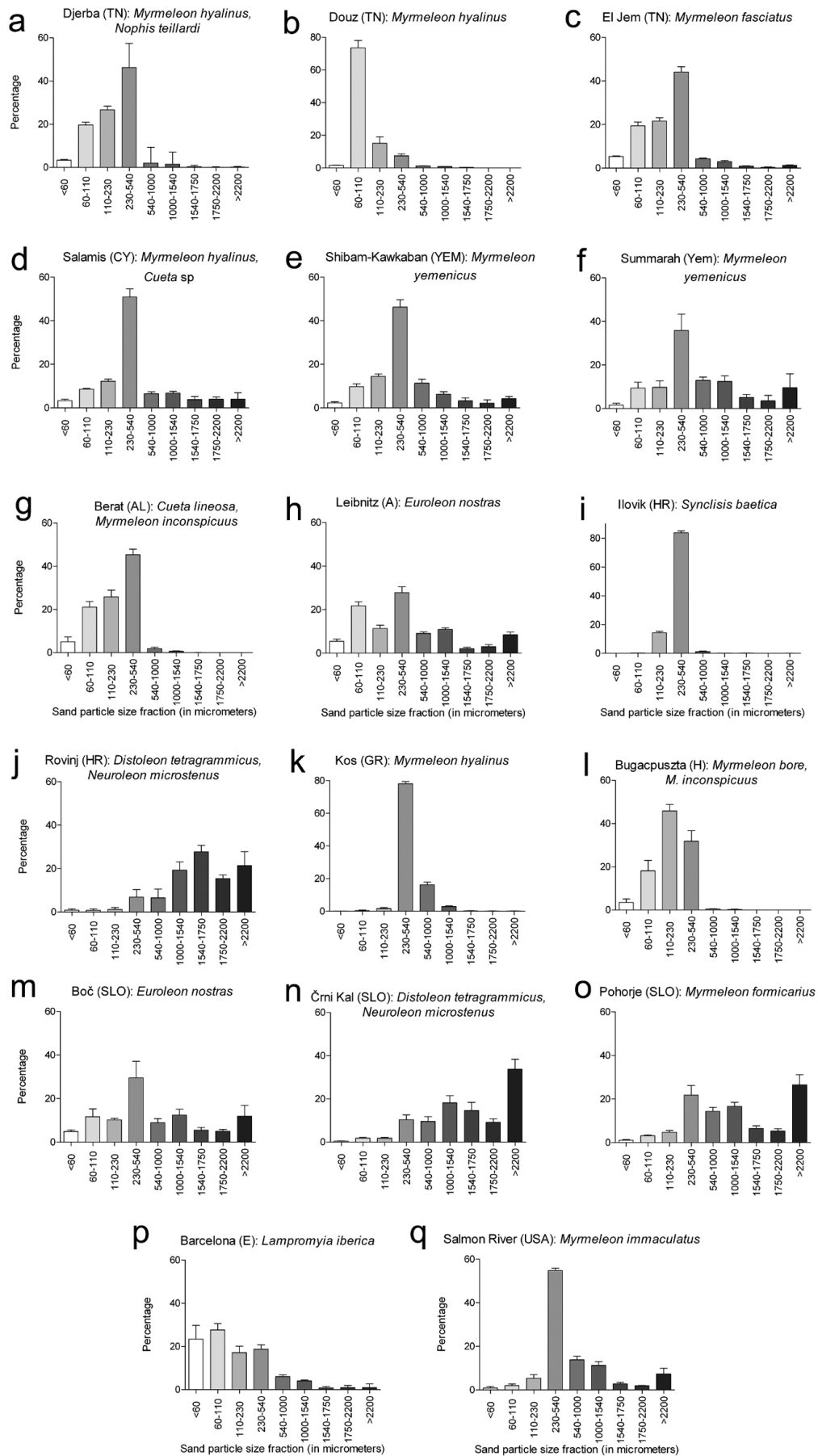


Fig. 2. Average percentage of different substrate particle size composition of substrates in natural antlion habitats. In abscissa is shown substrate particle size composition in micrometers. Vertical bars represent SD.

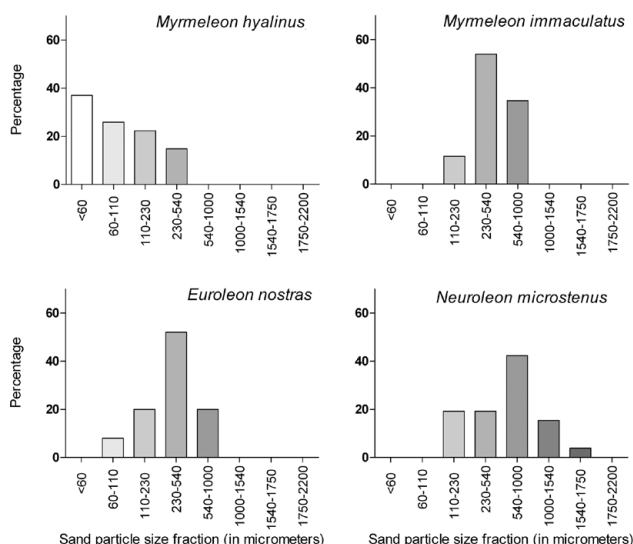


Fig. 3. Choice of different substrate fractions according to their particle sizes in four antlion species.

One non-pit-builder *Nophis teillardii* and the pit-building antlions *M. hyalinus*, *M. fasciatus*, *M. inconspicuus*, *M. bore*, *Euroleon nostras* and *Cueta lineosa* were all found naturally occurring in fine to medium substrates. In medium substrates with important finer fractions pit-builders (*M. hyalinus*, *M. yemenicus*, *C. lineosa*) and a non-pit-builder (*Synclisis baetica*) were found. In medium substrates with important medium fractions two pit-builders were collected (*M. hyalinus*, *M. immaculatus*). Pit-building species were also found in medium substrates with important coarser fractions, namely *M. yemenicus*, *M. formicarius* and *E. nostras*.

According to the substrate particle sizes in which larvae were naturally found, the investigated larvae can be classified into four categories (Table 2). A few larvae were recorded in only one type of substrate – in fine substrate (wormlion *Lampromyia*) or in coarse substrate (*Neuroleon* and *Distoleon*). The antlion *M. hyalinus* tolerates a wide

range of finer substrates, from fine sands to medium substrates with finer (or medium) fractions. *Euroleon nostras* is an antlion species tolerating a wide range of medium substrates with important fine or coarser fractions.

Choice experiment examining substrate particle-size preference

Four antlion species were tested for substrate particle-size preference by giving antlion larvae the choice to choose one of eight substrate fractions.

Each larva dropped into the centre of the fractions moved in an apparently haphazard direction on, or just underneath, the substrate surface. This locomotion, called doodling (Topoff, 1977) in pit-builders, may assist in finding a suitable location for submerging or constructing the pit. During pit construction, antlions moved in large circles or loops, and some larvae, in the eight substrate-choice tests, encountered more than one particle-size fraction before constructing their pits in the most convenient one (Fig. 3). The larvae never built the pits between two neighbouring fractions but always chose one fraction.

A significant number of *M. hyalinus* larvae constructed pits in the finest sand fraction (G1: 37%; $\chi^2 = 120.49$, $P < 0.0001$, $n = 27$ larvae). On the contrary, the majority of *N. microstenus* larvae were found in a coarser fraction (G5; 42.3%; $\chi^2 = 122.31$, $P < 0.0001$, $n = 26$ larvae) and substantial numbers in G6 and G7 (15.4% and 3.9%). The other two species, *M. immaculatus* and *E. nostras*, constructed the majority of their pitfall traps in a medium sand fraction, G4 (53.9% and 52%; *M. immaculatus*: $\chi^2 = 238.77$, $P < 0.0001$, $n = 26$ larvae; *E. nostras*: $\chi^2 = 185.44$, $P < 0.0001$, $n = 25$ larvae).

Our results show that antlion species have a clear preference for a certain substrate type: *M. hyalinus* occurs naturally in fine to medium substrates and predominantly chose that particle size in the choice test, *N. microstenus* in coarse substrates, and *M. immaculatus* and *E. nostras* in medium substrates. As the chi-square test revealed, preferences for

TABLE 2. Classification of psammophilous insect larvae according to their occurrence in the substrates with different particle sizes.

Category	Species
I. Larvae preferring fine substrates	<i>Lampromyia iberica</i>
	<i>Myrmeleon hyalinus</i> (Tunisia: Sahara)
	<i>Cueta lineosa</i> (Albania)
	<i>Euroleon nostras</i> (Austria)
II. Larvae preferring fine to medium substrates	<i>Myrmeleon bore</i>
	<i>Myrmeleon hyalinus</i> (Tunisia: Djerba)
	<i>Myrmeleon fasciatus</i>
	<i>Myrmeleon inconspicuus</i>
	<i>Nophis teillardii</i>
III. Larvae preferring medium substrates	<i>Cueta lineosa</i> (Cyprus)
	<i>Euroleon nostras</i> (Slovenia)
	<i>Myrmeleon formicarius</i>
	<i>Myrmeleon hyalinus</i> (Cyprus, Greece)
	<i>Myrmeleon immaculatus</i>
IV. Larvae preferring coarse substrates	<i>Myrmeleon yemenicus</i>
	<i>Synclisis baetica</i>
	<i>Distoleon tetragrammicus</i>
	<i>Neuroleon microstenus</i>

TABLE 3. Chi-square test of substrate particle-size preference in four antlion species. The left lower side shows Chi-square results and the right upper side provides the respective P values. d.f. = 7. ns = not significant.

	<i>M. hyalinus</i>	<i>M. immaculatus</i>	<i>E. nostras</i>	<i>N. microstenus</i>
<i>M. hyalinus</i>	–	P = 0.0077	P = 0.0012	P = 0.0217
<i>M. immaculatus</i>	X ² = 19.14	–	P = 0.5308 (ns)	P = 0.0022
<i>E. nostras</i>	X ² = 23.93	X ² = 6.08	–	P = 0.0037
<i>N. microstenus</i>	X ² = 16.40	X ² = 22.36	X ² = 21.07	–

the type of the substrate differed between the substrate categories, and did not differ within the category (i.e. within the medium sand) (Table 3).

DISCUSSION

Antlion and wormlion larvae are sand-dwelling insects, predominantly found in arid and semiarid regions, occupying fine sand or loose soil. Wormlions and many antlion species are highly specialized trap-building predators, whose foraging behaviour is not simply guided by a few factors but is much more complex, influenced by a number of variables (e.g. Arnett & Gotelli, 2001; Scharf & Ovidia, 2006; Scharf et al., 2011; Dor et al., 2014). Antlions occur in “antlion zones” (Gotelli, 1993), characterized by a specific combination of habitat variables such as sand particle size, sand density, density of plants, shade, and shelter (Scharf et al., 2008b; Devetak et al., 2012). Topoff (1977) believed that substrate particle size is less important because antlions can build pits in a wide range of substrate particle sizes. He therefore proposed shade as a major factor because *M. immaculatus*, the subject of his research, inhabits shaded habitats. Yet our findings suggest that sand particle size in determining habitat is also important for antlions and wormlions.

In natural habitats, larvae will experience sand as a mixture of material with different substrate particle sizes. Substrates in different geographical areas differ according to their substrate particle sizes. In Israel, for example, sands along the Mediterranean coast are coarser than those in the desert (Scharf et al., 2008a). *M. hyalinus* occurs in both habitats there, i.e. in coastal and desert sands (Scharf et al., 2008a), and it fits to our finding that it is a relatively generalist antlion in its habitat preference. The generalistic nature of *M. hyalinus* was previously demonstrated by Barkae et al. (2012). In experimental conditions, when two antlion species were exposed to two different substrates, the prey capture success of the habitat generalist *M. hyalinus* was higher than that of the habitat specialist *C. lineosa* (Barkae et al., 2012).

For antlions and wormlions sand particle size appears to be of key importance for efficient sit-and-wait and sit-and-pursue predatory strategies. In natural habitats, pit-constructing antlion larvae are capable of discriminating between areas of substrate differing in particle size, choosing fine to medium sands and avoiding coarser sands (Lucas, 1986; Loiterton & Magrath, 1996; Botz et al., 2003; Devetak et al., 2005). The physical characters of substrate influence predation strategies in antlions, including selection of convenient pit-building sites, pit construction, prey

recognition, etc. (Kitching, 1984; Allen & Croft, 1985; Klokočovnik et al., 2012).

In accordance with previous studies (Youthed & Moran, 1969; Kitching, 1984; Botz et al., 2003; Devetak et al., 2005; Ábrahám, 2006), we have shown that sand particle size plays an important role in pit-building decisions in two of our investigated antlion species (*E. nostras* and *M. immaculatus*). *E. nostras* larvae readily build pits in a fraction with a particle size of 0.23–0.54 mm, but in a fraction with a particle size of 1–1.54 mm only occasionally (Devetak et al., 2005). No pits are built in a coarser sand fraction (1.54–2.2 mm). This finding is in accordance with the present study where eight sand fractions were offered to *E. nostras* larvae. In African and Australian *Myrmeleon* species, the majority of pits were constructed in medium sand fractions (0.2–1 mm) (Youthed & Moran, 1969; Kitching, 1984).

The hypothesis suggested by Devetak et al. (2005) that larger sand particles are probably more difficult to handle for larvae, and thus they require more time to construct pits, was later rejected (Klokočovnik et al., 2012). In a previous study, the duration of three stages of pit construction – i.e. excavation of the initial furrow, deepening of a pit, and finishing the pit – was the longest in the most convenient particle size, i.e. medium sand fraction (0.23–0.54 mm), but not in coarser sands (>1 mm) (Klokočovnik et al., 2012). Furthermore, substrate particle size has an effect on the frequency of jerks produced during sand tossing; the frequency was lower in coarser sand and increased with decreasing sand particle size (Klokočovnik et al., 2012). Probably the morphology of antlion species also influences the preference for a certain substrate particle-size fraction. As it is shown here in the choice experiment, *M. hyalinus* prefers the finest sand fraction and the possible explanation is that this preference is a consequence of its slender head. The ratio head capsule width/head capsule length (HW/HL) in *M. hyalinus* is 0.75, in contrast to HW/HL ranging from 0.81 to 0.89 in the other Mediterranean Myrmeleontini species (Badano & Pantaleoni, 2014).

Analysing some of the physical properties of substrate vibrations and measuring behavioural reactions, Devetak et al. (2007) showed that larvae of the antlion *E. nostras* detect their arthropod prey by sensing the vibrations that arthropods generate during locomotory activity. Frequency of prey signals and sand particle size both influence the propagation properties of vibratory signals. Vibrations in finer sand are attenuated more strongly than in coarser sand and, consequently, an antlion detects its prey at a shorter distance (Devetak, 2014). The most convenient sands for

prey detection for all species are considered to be medium-sized particles.

Little is known about the role substrate particle size may play in the behaviour of animals, although it is known that substrate particle size influences pit construction behaviour of *E. nostras* larvae (Klokočovnik et al., 2012). They found that different sand particle-size fractions changed behaviour of antlions in terms of the occurrence and duration of particular behavioural stages. In sand composed of larger particles the larvae occasionally constructed irregular traps with a figure eight shape, and they crossed the centre of the cone during deepening. In the coarsest substrate the antlions did not build pits (Klokočovnik et al., 2012).

In this study, in samples from natural antlion habitats, we show that thirteen antlion and one wormlion species occupy a variety of substrates, differing according to their densities (Table 1). Each antlion species clearly preferred a substrate of certain particle size regardless of its predatory strategy. The question arises whether each antlion species prefers a substrate similar to its own habitat of origin. From this and previous studies (Botz et al., 2003; Devetak et al., 2005) it is evident that, in laboratory conditions, particle size affects pit-building decision and pit size in pit-building antlion larvae. Samples of antlion habitat showed that antlions are found naturally in coarser substrates than they chose in choice experiments, although *Neuroleon microstenus* and *Distoleon* chose substrate of similar course quality in laboratory conditions. *Neuroleon* differs significantly in prey capturing strategy from pit-builders and shows a plasticity in behaviour (Klokočovnik & Devetak, 2014). This could be a reason that *Neuroleon* prefers to forage for prey in coarser substrates.

Among psammophilous insects, wormlions are highly specialized for the finest substrates, consequently their larvae often occur in powders (Wheeler, 1930; Devetak, 2008). But why do antlion larvae in natural habitats build pits in substrates with an extreme range of particle sizes? Besides the fact that the density of psammophilous larvae plays important role, a probable explanation is that females lay eggs in the only substrate available that is appropriate for the larvae. Antlion larvae are sedentary predators that rarely move great distances. Matura et al. (2005) investigated substrate selection based on particle-size composition in *M. bore* in Japan, and found that the selection of oviposition substrates by adult antlion females was the primary factor governing spatial distribution of individuals in the field, and that larval movement would not be very important.

Particle-size composition of substrate of the pit and its surroundings is very complex. Ábrahám (2006) studied particle size composition of substrates in Hungary in antlion zones of four pit-building antlion species (*E. nostras*, *M. bore*, *M. formicarius* and *M. inconspicuus*) and found that the structure of the substrate depends on the position of a sample regarding the pit. The finest substrate was found inside the pit, and the sizes of the particles collected from the edges of the pit were bigger than the ones taken from the pit slope but smaller than the ones from a point

3 cm away from the pit edge (Ábrahám, 2006). The larvae move the finer particles to line the pit and they throw out the coarser particles (Lucas, 1982; Gepp, 2010; Ábrahám, 2006).

It is also known that sand particle size affects pit morphology. In the substrate with fine particles, antlions build pits with the highest pit angle (Botz et al., 2003). Pits constructed in fine sands are more effective because escape of potential prey is hindered due to the steeper slope of the pit wall (Lucas, 1982; Botz et al., 2003; Fertin & Casas, 2006; Devetak et al., 2012). Pitfall traps are effective when slopes are steep enough to guide prey to predator's jaws without any attack or with small effort of the antlion, and shallow enough to avoid the likelihood of avalanches typical of crater angles (Fertin & Casas, 2006). On the other hand, for small sand-dwelling insects it is also inconvenient to dig and move in substrate with extremely large particles.

In summary, we have found that for sand-dwelling antlion and wormlion larvae the composition of substrates in natural habitats and the effect of substrate particle size on site selection are important. In natural habitats, different antlion and wormlion species occupy places with different substrate particle sizes, and fall into four categories: (i) Larvae preferring fine substrates are the wormlion *Lampromyia* and antlion *Myrmeleon hyalinus* originating from desert habitats. (ii) Larvae preferring fine to medium or (iii) medium substrates belong to antlion genera *Cueta*, *Euroleon*, *Myrmeleon*, *Nophis* and *Synclisis*. (iv) Larvae preferring coarse substrates are *Distoleon* and *Neuroleon*. In the choice experiment, four species of antlion primarily chose medium grained substrates, except for *M. hyalinus*, which preferred fine grains, similar to the grains found in its natural habitat.

ACKNOWLEDGEMENTS. We are grateful to I. Scharf and an anonymous referee for the thorough and constructive review, which improved the manuscript. We thank F. Janžekovič for advice on the statistical analysis. We are grateful to C. Kehlmaier for identification of *Lampromyia iberica*. This research was supported partly by the Slovene Research Agency and partly by the research project Biodiversity of the Neuropterida in the Balkan (RP BioDiv Neuropterida Balkan – ALBH 2013).

REFERENCES

- ÁBRAHÁM L. 2003: Temperature tolerance and predatory strategy of pit-building ant-lion larvae (Neuroptera: Myrmeleontidae). — *Acta Phytopathol. Entomol. Hung.* **38**: 167–179.
- ÁBRAHÁM L. 2006: Pit building ant-lion larvae effect to the distribution of the substrate particles in their microhabitats. — *Natura Somogyiensis* **9**: 167–185.
- ALLEN G.R. & CROFT D.B. 1985: Soil particle size and the pit morphology of the Australian ant-lions *Myrmeleon diminutus* and *M. pictifrons* (Neuroptera: Myrmeleontidae). — *Aust. J. Zool.* **33**: 863–874.
- ARNETT A.E. & GOTELLI N.J. 2001: Pit-building decisions of larval ant lions: effects of larval age, temperature, food, and population source. — *J. Insect Behav.* **14**: 89–97.
- ASPÖCK H., ASPÖCK U. & HÖLZEL H. (UNTER MITARBEIT VON RAUSCH H.) 1980: *Die Neuropteren Europas. Eine zusammenfassende Darstellung der Systematik, Ökologie und Chorologie der*

- Neuropteroidea (Megaloptera, Raphidioptera, Planipennia) Europas. 2 Vols.* Goecke & Evers, Krefeld, 495 + 355 pp.
- BADANO D. & PANTALEONI R.A. 2014: The larvae of European Myrmeleontidae (Neuroptera). — *Zootaxa* **3762**: 1–71.
- BARKAE E.D., SCHARF I., SUBACH A. & OVADIA O. 2010: The involvement of sand disturbance, cannibalism and intra-guild predation in competitive interactions among pit-building antlion larvae. — *Zoology* **113**: 308–315.
- BARKAE E.D., SCHARF I., ABRAMSKY Z. & OVADIA O. 2012: Jack of all trades, master of all: a positive association between habitat niche breadth and foraging performance in pit-building antlion larvae. — *PLoS ONE* **7**: e33506.
- BOTZ J.T., LOUDON C., BARGER J.B., OLAFSEN J.S. & STEEPLES D.W. 2003: Effects of slope and particle size on ant locomotion: implications for choice of substrate by antlions. — *J. Kans. Entomol. Soc.* **76**: 426–435.
- BROWNELL P. & FARLEY R.D. 1979: Prey-localizing behaviour of the nocturnal desert scorpion, *Paruroctonus mesaensis*: orientation to substrate vibrations. — *Anim. Behav.* **27**: 185–193.
- DEVETAK D. 2005: Effects of larval antlions *Euroleon nostras* (Neuroptera, Myrmeleontidae) and their pits on the escape-time of ants. — *Physiol. Entomol.* **30**: 82–86.
- DEVETAK D. 2008: Substrate particle size-preference of wormlion *Vermileo vermileo* (Diptera: Vermileonidae) larvae and their interaction with antlions. — *Eur. J. Entomol.* **105**: 631–635.
- DEVETAK D. 2014: Sand-borne vibrations in prey detection and orientation of antlions. In Cocroft R.B., Gogala M., Hill P.S.M. & Wessel A. (eds): *Studying Vibrational Communication, Animal Signals and Communication 3*. Springer, Heidelberg, pp. 319–330.
- DEVETAK D., ŠPERNJAK A. & JANŽEKOVIČ F. 2005: Substrate particle size affects pit building decision and pit size in the antlion larvae *Euroleon nostras* (Neuroptera: Myrmeleontidae). — *Physiol. Entomol.* **30**: 158–163.
- DEVETAK D., MENCINGER-VRAČKO B., DEVETAK M., MARHL M. & ŠPERNJAK A. 2007: Sand as a medium for transmission of vibratory signals of prey in antlions *Euroleon nostras* (Neuroptera: Myrmeleontidae). — *Physiol. Entomol.* **32**: 268–274.
- DEVETAK D., LIPOVŠEK S. & PABST M.-A. 2010: Morphology and biology of the antlion *Myrmeleon yemenicus* Hölzel, 2002 (Neuroptera, Myrmeleontidae). — *Zootaxa* **2531**: 48–56.
- DEVETAK D., NOVAK T. & JANŽEKOVIČ F. 2012: Effect of substrate density on behaviour of antlion larvae (Neuroptera: Myrmeleontidae). — *Acta Oecol.* **43**: 1–7.
- DOR R., ROSENSTEIN S. & SCHARF I. 2014: Foraging behaviour of a neglected pit-building predator: the wormlion. — *Anim. Behav.* **93**: 69–76.
- FARJI-BRENER A.G. 2003: Microhabitat selection by antlion larvae, *Myrmeleon crudelis*: effect of soil particle size on pit-trap design and prey capture. — *J. Insect Behav.* **16**: 783–796.
- FERTIN A. & CASAS J. 2006: Efficiency of antlion trap construction. — *J. Exp. Biol.* **209**: 3510–3515.
- GEILER H. 1966: Über die Wirkung der Sonneneinstrahlung auf Aktivität und Position der Larven von *Euroleon nostras* Fourcr. (= *Myrmeleon europaeus* McLachl.) in den Trichterbodenfallen. — *Z. Morph. Ökol. Tiere* **56**: 260–274.
- GEPP J. 2010: *Ameisenlöwen und Ameisenjungfern. Myrmeleontidae*. Westarp Wissenschaften, Hohenwarsleben, 168 pp.
- GEPP J. & HÖLZEL H. 1989: *Ameisenlöwen und Ameisenjungfern – Myrmeleontidae*. Ziemsen, Wittenberg Lutherstadt, 108 pp.
- GOTELLI N.J. 1993: Ant lion zones: causes of high-density predator aggregations. — *Ecology* **74**: 226–237.
- GREENVILLE A.C. & DICKMAN C.R. 2009: Factors affecting habitat selection in a specialist fossorial skink. — *Biol. J. Linn. Soc.* **97**: 531–544.
- GRIFFITHS D. 1980: The feeding biology of ant-lion larvae: prey capture, handling and utilization. — *J. Anim. Ecol.* **49**: 99–125.
- HALLIDAY W.D. & BLOUIN-DEMERS G. 2014: Red flour beetles balance thermoregulation and food acquisition via density-dependent habitat selection. — *J. Zool.* **294**: 198–205.
- JANETOS A.C. 1986: Web site selection: Are we asking the right questions? In Shear W.A. (ed.): *Spiders: Webs, Behavior and Evolution*. Stanford University Press, Stanford, CA, pp. 9–22.
- KITCHING R.L. 1984: Some biological and physical determinants of pit size in larvae of *Myrmeleon pictifrons* Gerstaecker (Neuroptera: Myrmeleontidae). — *J. Aust. Entomol. Soc.* **23**: 179–184.
- KLOKOČOVNIK V. & DEVETAK D. 2014: Pit-builder vs non-pit-builder: advantage of trap building strategy in antlion larvae does not mean greater behaviour diversity. — *Behaviour* **151**: 653–668.
- KLOKOČOVNIK V., DEVETAK D. & ORLAČNIK M. 2012: Behavioral plasticity and variation in pit construction of antlion larvae in substrates with different particle sizes. — *Ethology* **118**: 1–9.
- KOTLER B.P., BROWN J.S., OLDFIELD A., THORSON J. & COHEN D. 2001: Foraging substrate and escape substrate: patch use by three species of gerbils. — *Ecology* **82**: 1781–1790.
- LINTON M.C., CROWLEY P.H., WILLIAMS J.T., DILLON P.M., ARAL H., STROHMEIER K.L. & WOOD C. 1991: Pit relocation by antlion larvae: a simple model and laboratory test. — *Evol. Ecol.* **5**: 93–104.
- LOITERTON S.J. & MAGRATH R.D. 1996: Substrate type affects partial prey consumption by larvae of the antlion *Myrmeleon acer* (Neuroptera: Myrmeleontidae). — *Aust. J. Zool.* **44**: 589–597.
- LORIA R., SCHARF I., SUBACH A. & OVADIA O. 2008: The interplay between foraging mode, habitat structure, and predator presence in antlions. — *Behav. Ecol. Sociobiol.* **62**: 1185–1192.
- LUCAS J.R. 1982: The biophysics of pit construction by antlion larvae (*Myrmeleon*, Neuroptera). — *Anim. Behav.* **30**: 651–664.
- LUCAS J.R. 1986: Antlion pit construction and kleptoparasitic prey. — *Fla Entomol.* **69**: 702–710.
- LUCAS J.R. & STANGE L.A. 1981: Key and descriptions to the *Myrmeleon* larvae of Florida (Neuroptera: Myrmeleontidae). — *Fla Entomol.* **64**: 207–216.
- LUDWIG P., MELZER R.R. & EHRHARDT V. 2001: Larval morphology and classification of wormlions (Diptera, Vermileonidae). — *Mitt. Dtsch. Ges. Allg. Entomol.* **13**: 89–94.
- MANSSELL M.W. 1996: Predation strategies and evolution in antlions (Insecta: Neuroptera: Myrmeleontidae). In Canard M., Aspöck H. & Mansell M.W. (eds): *Pure and Applied Research in Neuropterology*. Sacco, Toulouse, pp. 161–169.
- MANSSELL M.W. 1999: Evolution and success of antlions (Neuroptera: Neuroptera: Myrmeleontidae). — *Stapfia 60 (Neue Folge)* **138**: 49–58.
- MARSH A.C. 1987: Thermal responses and temperature tolerance of a desert ant-lion larva. — *J. Thermal Biol.* **12**: 295–300.
- MATSURA T. & TAKANO H. 1989: Pit-relocation of antlion larvae in relation to their density. — *Res. Popul. Ecol.* **31**: 225–234.
- MATSURA T., YAMAGA Y. & ITOH M. 2005: Substrate selection for pit making and oviposition in an antlion, *Myrmeleon bore* Tjeder, in terms of sand particle size. — *Entomol. Sci.* **8**: 347–353.
- MORRISON L.W. 2004: Spatiotemporal variation in antlion (Neuroptera, Myrmeleontidae) density and impacts on ant (Hymenoptera: Formicidae) and generalized arthropod foraging. — *Ann. Entomol. Soc. Am.* **97**: 913–922.
- ROSENZWEIG M.L. 1995: *Species Diversity in Space and Time*. Cambridge University Press, Cambridge, UK, 436 pp.
- RUXTON G.D. & HANSELL M.H. 2009: Why are pitfall traps so rare in the natural world? — *Evol. Ecol.* **23**: 181–186.

- SCHARF I. & OVADIA O. 2006: Factors influencing site abandonment and site selection in a sit-and-wait predator: A review of pit-building antlion larvae. — *J. Insect Behav.* **19**: 197–218.
- SCHARF I., FILIN I., GOLAN M., BUCHSHTAV M., SUBACH A. & OVADIA O. 2008a: A comparison between desert and Mediterranean antlion populations: differences in life history and morphology. — *J. Evol. Biol.* **21**: 162–172.
- SCHARF I., HOLLENDER Y., SUBACH A. & OVADIA O. 2008b: Effect of spatial pattern and microhabitat on pit construction and relocation in *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) larvae. — *Ecol. Entomol.* **33**: 337–345.
- SCHARF I., SUBACH A. & OVADIA O. 2008c: Foraging behaviour and habitat selection in pit-building antlion larvae in constant light or dark conditions. — *Anim. Behav.* **76**: 2049–2057.
- SCHARF I., GOLAN B. & OVADIA O. 2009: The effect of sand depth, feeding regime, density, and body mass on the foraging behaviour of a pit-building antlion. — *Ecol. Entomol.* **34**: 26–33.
- SCHARF I., LUBIN Y. & OVADIA O. 2011: Foraging decisions and behavioural flexibility in trap-building predators: a review. — *Biol. Rev.* **86**: 626–639.
- TOPOFF H. 1977: The pit and the antlion. — *Nat. Hist.* **86**: 64–71.
- WHEELER W.M. 1930: *Demons of the Dust*. Kegan Paul, Trench, Trubner & Co., London, UK, 378 pp.
- YOUTHED G.J. & MORAN V.C. 1969: Pit construction by myrmeleontid larvae. — *J. Insect Physiol.* **15**: 867–875.

Received January 15, 2015; revised and accepted February 24, 2015
Prepublished online March 20, 2015