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A COMPARISON OF THE RESPIRATORY SYSTEMS IN SOME CAVE AND SURFACE SPECIES OF SPIDERS (ARANEAE, DYSDERIDAE)

Matjaž Kuntner[1, 3], **Boris Sket**[1], and **Andrej Blejec**[2]**:** [1]Department of Biology, Biotechnical Faculty, University of Ljubljana, SI-1111 Ljubljana, Slovenia; [2]National Institute of Biology, POB 141, SI-1001 Ljubljana, Slovenia

ABSTRACT. We tested the hypothesis that the respiratory system of hypogean spiders is subject to regressive evolution by examining representatives of the family Dysderidae. This comparison included the epigean species *Dysdera ninnii* Canestrini 1868, and *Harpactea lepida* (C.L. Koch 1838), and the hypogean species *Stalita taenaria* Schiödte 1847, and *Parastalita stygia* (Joseph 1882). Both tube tracheae and book lungs of these species were measured and compared using 10 indices. Both the tracheal system and book lungs of the hypogean species were less developed than those of the epigean ones. We suggest that the cause is reduction of the respiratory system as a part of general structural reductions in the troglobites. This is consistent with the lower respiratory rates that characterize many troglobites.

True cave animals, called ‘‘troglobites,’’ are adapted to living in a very different environment from their surface relatives. Ecological conditions of the terrestrial underground environment are summarized here according to Vandel (1965) and Sket (1996). The most obvious and constant factor is the absence of light, consequently the absence of green plants and near absence of primary production, resulting in an energy-poor hypogean (subterranean) environment. The nearly constant temperature roughly equals the yearly average of the region. The chemical composition of the air in the caves that are well ventilated is similar to the surface atmosphere with a very slight increase of CO_2 concentration. However, in some caves CO_2 concentration may be significantly increased and O_2 concentration may be low (Vandel 1965; James *et al.* 1975; Whitten *et al.* 1987). Relative humidity in caves is normally 95–100%, so troglobites are hygrophilic and more sensitive to drying than epigean species (Vandel 1965).

Morphological adaptations of animals to the underground environment, or troglomorphisms, can be seen as gains or reductions. Typical hypogean arthropod gains are larger bodies and longer appendages, and increases in the number of ‘‘nonvisual’’ sensory organs. Typically, cuticle features such as wings, pigmentation and eyes are reduced (Vandel 1965; Sket 1985). In European cave spiders, all possible stages of depigmentation, eye reduction, and weakening of the integument can be observed (Deeleman-Reinhold 1975) along with gains such as elongation of appendages.

Regressive evolution or degenerative evolution in cave organisms, reviewed by Fong & Culver (1985), Kane & Richardson (1985), Poulson (1985), Romero (1985) and Sket (1985) is not restricted to morphological regression but can also be met with physiological and ethological changes. Typical changes in hypogean animals include decreased metabolic rate, slower ontogenetic development, and pedomorphosis. The respiratory metabolic rates of the studied cave species were as low as 3% of that of related surface species in isopod crustaceans and as low as 14% in troglophilic spiders (Vandel 1965; Hüppop 1985). The possible reasons of this reduction include relative ecological stability of the underground environment, lack of predators, low food availability (Hüppop 1985) and possibly higher CO_2 concentration (Whitten *et al.* 1987).

A number of authors present data about spider metabolism and respiratory physiology (e.g., Anderson 1970; Anderson & Prestwich 1980, 1982, 1985; Bromhall 1987; Dresco-Derouet 1969; Greenstone & Bennett 1980;

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[3] Current address: Department of Biological Sciences, George Washington University, 2023 G Street, N.W., Washington, DC 20052, USA

Opell 1987, 1990, 1992, 1998; Paul *et al.* 1987, 1989; Paul & Fincke 1989; Paul 1992; Prestwich 1983a, 1983b; Strazny & Perry 1984, 1987). Energetic adaptations allow spiders to have roughly half the value of the metabolic rate present in other poikilothermic animals of the same weight. During starvation periods, which in spiders can be prolonged, they have the ability to lower metabolic rates below the resting values. Spiders that have the lowest metabolic rates are adapted to living in the energy-poorest environments. The measured metabolic rates show positive correlation to the respiratory surface and volume. Although the cited studies did not include any troglobitic species, we can still assume that in the energy-poor cave environment the low metabolism in spiders can have impact on the structure of their respiratory system.

We studied the structure of the respiratory system in hypogean and epigean species of the spider family Dysderidae, which has many representatives in the Mediterranean, ranging from the most xerophylic epigean forms to the blind troglobites.

Dysderoidea includes families Dysderidae, Segestriidae, Oonopidae and Orsolobidae. Forster & Platnick (1985) claim that for Dysderoidea that their representatives have reduced book lungs, in the extreme case only four respiratory lamellae in some oonopids, and well developed paired tube tracheae, opening immediately behind the book lungs on the ventrolateral part of the abdomen. Levi (1967) and Winkler (1955) claim the above statement to be particularly adequate for Dysderidae.

In Dysderidae the tracheae extend into prosoma and enter the appendages (Winkler 1955; Novak 1967; Bromhall 1987; Foelix 1992). The dysderid heart is relatively small (Kaestner 1969). Spiders possessing prosomal tracheae have lower heart rates than spiders with tracheae limited to the abdomen (Bromhall 1987). The conclusion is that in Dysderidae the tracheae have a larger role in gas exchange than the book lungs, the latter being a less functional remnant of the evolution of this family.

We tested the hypothesis that reductions in the respiratory system occur during the course of evolution in phylogenetically old hypogean spider species. Thus, H_1 claims that the hypogean species have reduced respiratory systems relative to the epigean species. In contrast, H_0 states that there are no differences between hypogean and epigean species respiratory systems. The evidence supporting hypothesis H_1 is: (1) With few exceptions troglobites show lower metabolic rates than their epigean relatives; (2) High relative humidity in underground air coupled with thin integument of troglobitic species might allow additional gas exchange through their body surface, so their respiratory system need not to be strongly developed; (3) The majority of European karst caves are well aerated (Gams 1974: p. 123), and the differences between the cave and the surface atmospheres in such cases are small (James *et al.* 1975). A significant increase of CO_2 concentration and corresponding O_2 concentration decrease in karst caves is a rather unusual phenomenon.

METHODS

Species studied.—For the study of the respiratory system four different dysderid species belonging to three subfamilies (Deeleman-Reinhold & Deeleman 1988) were chosen (number and sex of the studied specimens in parentheses): the epigean species *Dysdera ninnii* Canestrini 1868 of the Dysderinae (6♀) and *Harpactea lepida* (C.L. Koch 1838) of the Harpacteinae (5♂, 1♀), and the hypogean species of Rhodinae *Stalita taenaria* Schiödte 1847 (3♂, 3♀), and *Parastalita stygia* (Joseph 1882; 3♂, 2♀, 1 immature). All these species were collected in Slovenia. The exact locality and habitat data of the examined material is given elsewhere (Kuntner 1998).

Preparation.—Spiders were dissected in 70% ethanol in a petri dish. Dorsal surfaces of the prosoma and of the abdomen were carefully removed. The animals were then gently heated in 10% KOH for 1 hour. They were then placed in a vial filled with distilled water and the vial was rigorously shaken. All soft tissues, eroded by KOH were thus removed. The chitinous cuticle was then stained overnight in chlorazol black mixed with glycerol, all integumental structures (including both components of the respiratory system) being colored black. Later the preparation was examined in water or further dissected and measured in glycerol.

Parameters measured and indices calculated.—Abbreviations of measured parame-

Table 1.—Parameters measured. Units in millimeters or mm^3 (*) except in NBL.

Abbreviation	Parameter	Description
PL	Prosoma length	dorsal view
OL	Opisthosoma length	dorsal view
F4	Femur IV length	prolateral view
BSW	Book lung stigma width	ventrolateral view
TSW	Tracheal stigma width	ventrolateral view
TL1	Cranial tracheal trunk length	dorsal view of the outer length of the curved main tracheal trunk
TC	Cranial tracheal curvature width	dorsal view
TL	Actual cranial tracheal trunk length	calculated from TL1 and TC
TW	Cranial tracheal trunk width	width in the middle of the trunk
TV	Cranial tracheal trunk volume*	calculated from TL and TW
CTL	Caudal tracheal tube length	dorsal view
TP	Cranial tracheal profile	circumference of the terminal part of the cranial tracheal trunk was calculated from the measurement using drawing and curvimeter, taking into account only the profile from which the tracheolae originate
CTP	Caudal tracheal profile	circumference of the terminal part of the caudal tracheal trunk using the same method as above
NBL	Number of book lung lamellae	examined under light microscope laterally

ters, their names and short descriptions are given in Table 1. They were measured for each specimen, using dissecting and compound light microscope with a micrometer. For parameters TP and CTP we used scale drawings and curvimeter to calculate circumferences of tracheal parts. Parameters TL and TV were calculated using formulae from Bronštejn & Semendjajev (1984). Ten indices were devised (Table 2) to compare the size and development of the respiratory systems between species and to reduce the influence of body size on studied parameters. Features tend to vary with size, surface and volume of the spider (*Harpactea lepida* was smaller in size than the rest of the species). This was taken into account in construction of indices as we tried to reduce variability of measured parameters within the pairs of surface and cave species.

Statistical analyses.—For analysis of differences among the surface and cave species, several statistical tests were applied: Wilcoxon rank sum (Mann-Whitney *U-test*), Kolmogorov-Smirnov, and Student *t-test*. Since different methods gave essentially the same results, only Wilcoxon rank sum is reported. There were generally no significant differences

Table 2.—Indices for comparison of the respiratory systems in species examined. Parameter abbreviations given in Table 1. Structure-index composition: L = length, S = surface, V = volume, N = number.

	Quotient	Description	Structure
I1	TL/PL^3	Relative cranial tracheal trunk length	L/V
I2	TW/PL	Relative cranial tracheal trunk width	L/L
I3	TV/PL^5	Relative cranial tracheal trunk volume	V/(S.V)
I4	CTL/PL^3	Relative caudal tracheal tube length	L/V
I5	TP/PL^2	Relative extent of tracheolae branching in the prosoma	L/S
I6	CTP/OL^2	Relative extent of tracheolae branching in the opisthosoma	L/S
I7	$TP/F4^2$	Relative extent of tracheolae branching in the appendages	L/S
I8	TSW/PL^2	Relative tracheal stigma width	L/S
I9	BSW/PL^2	Relative book lung stigma width	L/S
I10	NBL/PL	Relative number of book lung lamellae	N/L

Table 3.—Wilcoxon rank sum test of differences between species and species groups: — Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Comparison		
Index	*Stalita: Parastalita*	*Dysdera: Harpactea*	Cave: Surface
TL/PL^3	—	—	—
TW/PL	—	—	**
TV/PL^5	—	—	**
CTL/PL^3	*	—	***
TP/PL^2	—	—	***
CTP/OL^2	—	—	—
TP/F4^2	**	—	***
TSW/PL^2	—	—	***
BSW/PL^2	—	—	*
NBL/PL	—	—	***

among the cave or surface species (Table 3), so combined samples were eventually used to test the differences between cave and surface groups of species.

RESULTS

Both hypogean species lack eyes and have longer legs than both epigean species. In addition they are depigmented, have more setae on their legs, and appreciably more delicate cuticle. *Dysdera ninnii* has a well-developed tracheal system, but both cave species have less extensive tracheae. Although *Harpactea lepida* has relatively stout cranial tracheal trunks, these are relatively shorter than those of *D. ninnii*. However, no significant difference in all the indices between both surface species was observed (Table 3). Similarly, there was no significant difference in most of the indices between both cave species, the indices I4 and I7 being an exception (Table 3). Figure 1 shows the values of indices I1 to I10 for the studied species in the same order as listed in Table 2 (in the graphs the names of genera are given). In Table 3, Wilcoxon rank sum test of differences between both species pairs are presented.

Relative cranial tracheal trunk length (I1) was largest in *D. ninnii*, followed by *H. lepida* and both cave species. However, there was no significant difference between the epigean and hypogean pairs of species. Relative cranial tracheal trunk width (I2) showed similar values in both epigean species and was lower in both troglobites, both pairs of species showing significant difference. Relative cranial tracheal trunk volume (I3) showed a similar result to the previous index. Relative caudal tracheal tube length (I4) showed highly significant difference between both species pairs, and so did the next index—relative extent of tracheolae branching in the prosoma (I5). Relative extent of tracheolae branching in the opisthosoma (I6) was lower in the epigean pair of species, both species pairs showing no significant difference. Relative extent of tracheolae branching in the appendages (I7) was highest in *D. ninnii*, followed by *H. lepida, S. taenaria* and was lowest in the longlegged (more troglomorphic) *P. stygia*. There was a highly significant difference between the pairs of species. Relative tracheal stigma width (I8) was similar within the cave and surface species groups and showed a significant difference between them. The values for the relative book lung stigma width (I9) were again significantly higher in the epigean pair of species. Relative number of book lung lamellae (I10) showed again significantly higher values for the surface versus cave species pairs.

DISCUSSION

Dysdera ninnii has the most extensive tracheal system of the examined species. Both cranial and caudal tracheal trunks are strongly developed, and they branch into numerous tracheolae that supply with oxygen the prosomal organs, appendages and opisthosoma. Book lungs are also well developed, having up to 23 lamellae, but show considerable variability in their size (Kuntner 1998). The second epigean species, *Harpactea lepida*, shows similarly developed tracheal system and book lungs to *D. ninnii*, despite its smaller size. Although *H. lepida* and *D. ninnii* are both forest species, different ecological factors might influence their anatomy and physiology. However, as hypothesized, the examined surface species exhibit a well-developed respiratory system, even though they belong to different subfamilies.

The troglobites, *Stalita taenaria* and *Parastalita stygia*, both show reductions in the tracheal as well as book lung systems, compared to both surface species. Their cranial tracheal trunks are relatively shorter, narrower, and not as curved as in *D. ninnii*. They extend further into prosoma through the petiole be-

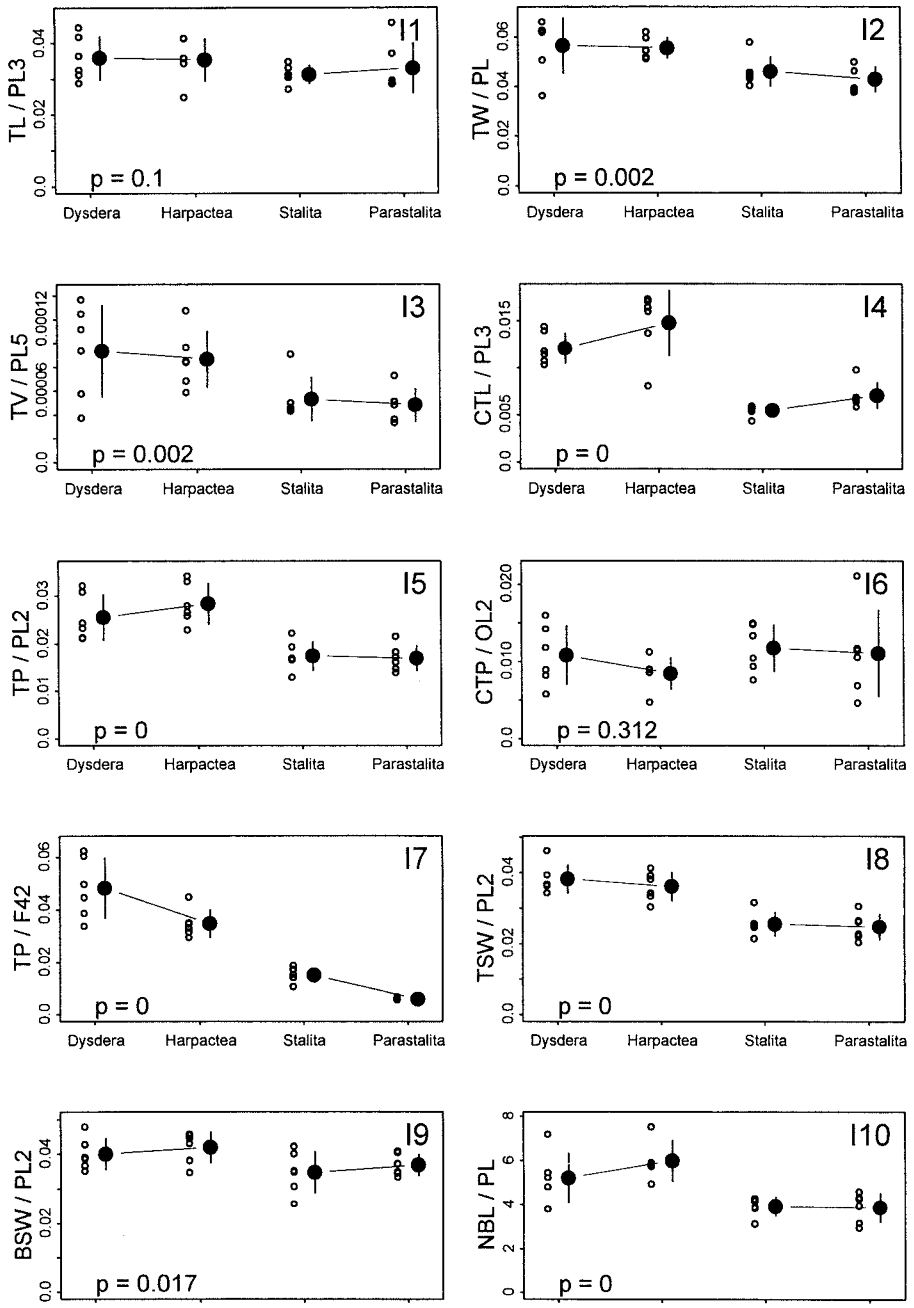

Figure 1.—Relative size and development of the respiratory system in epigean (*Dysdera ninnii, Harpactea lepida*) and hypogean (*Stalita taenaria, Parastalita stygia*) dysderid spiders from Slovenia, measured in six specimens each. For explanation of indices (I1–I10) see Table 2. (○ = Individual data, ● = Mean value ± SD, p = Wilcoxon rank sum significance level for differences between the surface and cave groups of species, groups indicated by the lines connecting mean values).

fore branching into tracheolae. The tracheolae bundle is much weaker in prosoma and fewer were observed to enter the legs. Their caudal tracheal tubes are greatly reduced compared to the ones in surface species, but the extent of the tracheolae branching in the opisthosoma shows no difference. The book lungs of both troglobites are also reduced and have a slightly different general appearance from those in the epigean species.

Both hypogean species showed very similar values of all the indices. Since they belong to a different subfamily than *Dysdera* and *Harpactea*, we cannot be sure that the supposedly epigean ancestors of hypogean Rhodinae had a stronger developed respiratory system, similar to that of *Dysdera* and *Harpactea*. However, we speculate that in both cave species it has been subject to regressive evolution. There are no comparable data on cave spider respiratory morphology in available araneological literature (e.g., Nentwig 1987). Yet, this seems to be another example of structural reduction in troglobites, similar to the reduction of other originally integumental structures in troglobitic spiders (Deeleman-Reinhold 1975) and other arthropods (Vandel 1965; Sket 1985). The cause for the reduction of the respiratory system in cave spiders still needs to be investigated further.

Opell (1990, 1998) states that tracheae and book lungs in the spider family Uloboridae are complementary respiratory structures; when one system is better developed the other is reduced. Opell concludes that the development of the two systems is governed by both spider's total respiratory demands and by the specificity of these demands. The more active species (with reduced webs) have relatively better developed tracheae, and the less active (orb-weaving) species have relatively better developed book lungs. If this is true for Dysderidae, future research could focus on possible compensating changes in both systems. Are both systems reduced in the troglobites or is there a shift in relative development of each system? As our study primarily treated the dysderid tracheae, future studies may reveal that the book lungs in the hypogean environment are more useful than in the epigean one.

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