Online Appendix A: Subterranean beetle morphological traits

Figure A1: Correlation between total body size and other morphological traits in blind subterranean diving beetles



Additional measurement data show that other morphological traits correlate very strongly with total body length in Australian subterranean diving beetles. This suggests that body size captures the interspecific variability associated with other traits and is therefore an appropriate single descriptor of species niche. Correlation values are indicated in each panel.

Figure A2: morphological differences among similarly-sized coexisting beetle species.



Three species of the genus *Paroster* (Dytscidae: Hydroporini) from the Napperby calcrete aquifer (aquifer indexed '49' in Table 1 and Table C1). This is the only aquifer in which we find that all pairs of coexisting species do not differ significantly in their mean body sizes. However, the species show a number of body shape modifications (note the very different head and pronotum widths) that could place them further apart in niche space, in agreement with the limiting similarity prediction. (Images made available by Chris Watts, South Australian Museum).

Online Appendix B: Phylogenetic structure of subterranean diving beetles

Figure B1. Molecular phylogeny and size ranks of subterranean species



Figure B1 shows a phylogenetic tree of diving beetles based on the molecular phylogeny published by Leijs et al. 2012 (PLoS ONE. 7: 1-8). The tree was obtained using bayesian methods (see Leijs et al. 2012 for details). The size ranks of the species are indicated with different branch colors. Species in the blue boxes indicate sympatric sister species. Colonization of the subterranean habitat occurred mainly in the terminal branches of the subterranean species (Leijs et al. 2003, 2012), and we assume that size differentiation would have occurred during or shortly after the environmental transition.

Figure B2. Testing phylogenetic independence of body sizes using Pagel's lambda.



Pagel's lambda (Pagel 1999. Nature. 401: 877-884; Freckleton et al. 2002. The American Naturalist. 160: 712-726) is a measure of phylogenetic correlation that varies between 0 (the trait is independent of phylogeny) and 1 (the trait covaries strictly with relatedness). The figure above represents a likelihood profile for Pagel's lambda calculated using a single phylogeny including both Bidessini and Hydroporini tribes (Leijs et al. 2012) and mean body size data of all species in the phylogeny. Maximum likelihood is reached for lambda=1e-6, indicating near-complete phylogenetic independence of beetle body size. The grey area represents the 95% confidence interval of the maximum likelihood estimate.

Figure B3. Testing phylogenetic independence of body size by comparing size ranks of sister species in the phylogeny.



Proportion of sister species pairs at same size rank

If species body size covaries with phylogeny one expects sister species to occupy the same size ranks across aquifers. A randomization test (Figure B3) shows that sister species are in fact not more often found at the same size rank than expected by chance. 31 sister species were detected in the set of communities, among which 13 belonged to the same size rank (see Figure B1, box b). To compute a null distribution of the proportion of sister species at the same size rank we randomly assigned 31 species to 1 of 3 ranks (small, medium and large). The proportion of species at the same size rank is recorded. The simulation is run 10000 times and a null distribution is plotted. The observed proportion of sister species pairs found at the same size rank (13/31) is represented by a vertical red line and is neither significantly lower nor greater than what can be expected under the null hypothesis.

Text B4

We use the size rank counts for the different tribes as a contingency table on which a Chi-Square test is applied (Figure B1, box a). The test indicates that the size rank occupied by a species is independent from the tribe it belongs to (Chi-square= 0.929, P= 0.628).

Online Appendix C: Body size structure of subterranean diving beetles	
Table C1: Body size differences among coexisting species across aquifers.	

Aquifer index as in	Size differences in	Size range overlap in
W&H 2009	main data set	compounded data set
1	S (2 species)	No overlap
2	S (2 species)	No overlap
3	S (2 species)	No overlap
6	S (2 species)	Overlap
10	S/NA (3 species)	No overlap
11	S (2 species)	No overlap
12	S/S (3 species)	No overlap
13	S/S (3 species)	No overlap
17	S/S (3 species)	No overlap
18	S (2 species)	No overlap
19	S (2 species)	No overlap
23	S/S (3 species)	No overlap
24	S/S (3 species)	No overlap
25	S/S (3 species)	No overlap
26	S/S (3 species)	No overlap
27	S/S (3species)	No overlap
28	S (2 species)	No overlap
29	NA	No overlap
30	NA	No overlap
36	S (2 species)	No overlap

37	NS/S (3 species)	No overlap
38	S (2 species)	No overlap
40	S (2 species)	No overlap
42	NA	No overlap
43	NA	No overlap
45	NA	No overlap
46	S/S (3 species)	No overlap
47	S (2 species)	No overlap
49	NS/NS (3 species)	Overlap
50	S (2 species)	No overlap
51	S/S/NS/NS (5	No overlap
	species)	
52	NA	No overlap

Results of ANOVA tests carried out on the species pairs recorded in all 26 multispecies aquifers listed in the main data set. 'S' indicates significant differences and 'NS' signals a non-significant result. Tests are performed on all pairs of coexisting species that are found consecutively on the body size axis. Aquifers 29, 30, 42, 43, 45, and 52 are not recorded in the main data set. In aquifer 10 the number of individuals was insufficient to perform an ANOVA test for one pair of species. The size range column shows whether the size ranges of coexisting species overlap in any of the 32 multi-species aquifers documented in a compounded data set (main data set and 6 additional aquifers documented in Watts and Humphreys (2009)). Note that in aquifer 3 a third, larger species is reported in Watts and Humphreys (2009) for which there is no size measurement in the main data set. Figure C1: distribution of beetle mean species body sizes



Body size data for all 1 (A), 2 (B) and 3-species (C) aquifers listed in the main data set. In each of the three panels the different colors correspond to species belonging to different size ranks.



Figure C2: Mean body size ratios of coexisting species in the compounded data set

The results of the analyses carried out on the compounded data set (main data set and Watts and Humphreys' data) do not differ from the ones using the main data set only and presented in the main text. Panel A shows that mean body size ratios among coexisting species (shown with their associated standard error bars) do not differ significantly (p value=0.18>0.05). Panel B represents the null distribution of standard deviations of simulated body size ratios under the hypothesis that species are distributed at random along the body size niche axis. The observed standard deviation is marked by a vertical black line and quantifies the variability of observed body size ratios. The observed variability falls within the smallest 5% of values occurring under the null hypothesis (grey area). From this we can conclude that the variability of observed species body size ratios is significantly less than expected by chance only.

Online appendix D:

Figure D1: Additional mortality rate in the limiting similarity model of body size evolution



The added mortality rate quantified by this function mitigates species' growth rates depending on their position on the niche axis. Edge species suffer maximum mortality whereas species closer to the centre of the axis experience lower losses. Edge species have fewer competitors than more central ones in the model and this additional term prevents them from taking over the community.



Figure D2: evolution of body size in a limiting similarity model with random fission speciation process

Results are identical to the ones obtained when speciation is implemented as a point mutation process (Fig. 3). Panels A to B represent an example of the evolution of a single community under the limiting similarity model from 20 species distributed at random (A) to 3 dominant, evenly-spaced species (B). Across 200 simulations, communities with different initial species number ranging from 1 to 20 all converge on average towards a similar low number of species (C). Distances between suitable size ranges converge towards a unique value through time and the standard deviation around dominant species' mean size differences decreases as a result (D).