

Additional file 1. Principal component analyses on raw data and phylogenetically independent contrasts of love-dart and reproductive morphology data.

	Raw		BI		BI with phylogenetic uncertainty		ML with or without phylogenetic uncertainty	
Love-dart variables	Dart PC1	Dart PC2	Dart PC1	Dart PC2	Dart PC1	Dart PC2	Dart PC1	Dart PC2
Number	0.298	0.882	0.359	0.884	0.351	0.876	0.333	0.912
Number of blades	0.586	-0.270	0.544	-0.345	0.550	-0.342	0.536	-0.357
Length of blade	0.545	-0.350	0.549	-0.306	0.551	-0.321	0.562	-0.203
Curve	0.521	0.165	0.523	0.074	0.520	0.110	0.535	0.003
Eigenvalue	2.320	0.933	2.416	0.828	2.379	0.851	2.404	0.853
%Variance	58.0	23.3	60.4	20.7	59.5	21.3	60.1	21.3
Gland variables	Gland PC1	Gland PC2	Gland PC1	Gland PC2	Gland PC1	Gland PC2	Gland PC1	Gland PC2
Number	0.626	-0.350	0.538	-0.534	0.548	-0.523	0.510	-0.574
Size	0.373	0.537	0.447	0.429	0.437	0.426	0.453	0.430
Shape	0.119	0.760	0.277	0.712	0.257	0.727	0.299	0.679
Placement	0.675	-0.106	0.659	-0.155	0.665	-0.130	0.667	-0.158
Eigenvalue	1.963	1.248	1.818	1.198	1.798	1.172	1.740	1.239
%Variance	49.1	31.2	45.4	29.9	45.0	29.3	43.5	31.0
Stylophore variables	Stylo. PC1	Stylo. PC2	Stylo. PC1	Stylo. PC2	Stylo. PC1	Stylo. PC2	Stylo. PC1	Stylo. PC2
Functional stylophore(s):								
Number	0.425	0.356	0.389	0.461	0.384	0.468	0.349	0.487
Size	0.005	0.668	-0.058	0.575	-0.065	0.568	-0.135	0.578
Placement	0.290	0.542	0.180	0.590	0.166	0.594	0.048	0.622
Vestigial stylophore(s):								
Number	0.525	-0.193	0.562	-0.102	0.565	-0.091	0.562	-0.006
Size	0.449	-0.219	0.437	-0.287	0.440	-0.289	0.479	-0.201
Placement	0.507	-0.221	0.553	-0.126	0.555	-0.115	0.559	-0.040
Eigenvalue	3.166	1.746	2.898	1.746	2.888	1.739	2.967	1.809
%Variance	52.8	29.1	48.3	29.1	48.1	29.0	49.4	30.1

Diverticulum variables	SRO PC1	SRO PC2	SRO PC1	SRO PC2	SRO PC1	SRO PC2	SRO PC1	SRO PC2
Length (relative to bursa tract)	0.602	-0.311	0.635	-0.288	0.636	-0.297	0.667	-0.238
Placement	0.591	-0.445	0.627	-0.350	0.631	-0.331	0.668	-0.230
Length (relative to organs)	0.536	0.840	0.451	0.892	0.444	0.896	0.331	0.944
Eigenvalue	2.174	0.528	1.681	0.827	1.664	0.837	1.490	0.940
%Variance	72.5	17.6	56.0	27.6	55.5	27.9	49.7	31.3

The independent contrasts are based on the raw data and the three alternative trees (see Methods), namely BI or ML with and without consideration of five cases of phylogenetic uncertainty. The ML tree topologies with and without phylogenetic uncertainty were identical. The table shows the eigenvectors for each variable in the different principal components (PC). These vectors are a measure for the weight of the variable in the PC. The eigenvalue and % variance are given for each PC. These values express how much of the total variance in the data is explained by that PC.

Additional file 2. Matrix of Pearson correlations (below diagonal) and their significance (above diagonal) for the comparison of the different principal components (PC) and the flagellum.

Raw data									
	Dart PC1	Dart PC2	Stylo. PC1	Stylo. PC2	Gland PC1	Gland PC2	SRO PC1	SRO PC2	Flag. length
Dart PC1		1.0000	0.2587	0.0002*	<0.0001*	0.0430	0.0011*	0.4666	0.0022*
Dart PC2	0.000		<0.0001*	0.1861	0.0009*	0.1333	0.1459	0.8554	0.6095
Stylo. PC1	0.161	0.688		1.0000	<0.0001*	0.0061	0.1833	0.2579	0.2492
Stylo. PC2	0.501	0.188	0.000		<0.0001*	0.0046*	0.1214	0.2866	0.0247
Gland PC1	0.618	0.451	0.669	0.570		1.0000	0.4722	0.8503	0.0048*
Gland PC2	0.285	-0.213	-0.379	0.391	0.000		0.0002*	0.8842	0.0152
SRO PC1	0.443	-0.207	-0.189	0.220	0.103	0.499		1.0000	<0.0001*
SRO PC2	0.104	-0.026	-0.161	0.152	-0.027	-0.021	0.000		0.4807
Flag. length	0.419	0.073	0.164	0.314	0.389	0.338	0.662	-0.101	

BI with phylogenetic uncertainty									
	Dart PC1	Dart PC2	Stylo. PC1	Stylo. PC2	Gland PC1	Gland PC2	SRO PC1	SRO PC2	Flag. length
Dart PC1		1.0000	0.0850	0.0205	<0.0001*	0.6273	0.0002*	0.8955	0.0173
Dart PC2	0.000		<0.0001*	0.0162	0.0039*	0.1548	0.2429	0.3931	0.3119
Stylo. PC1	0.246	0.623		1.0000	<0.0001*	0.0008*	0.6963	0.0961	0.0508
Stylo. PC2	0.327	0.338	0.000		<0.0001*	0.0667	0.0687	0.9909	0.0319
Gland PC1	0.658	0.401	0.532	0.592		1.0000	0.0026*	0.1883	0.0002*
Gland PC2	0.070	-0.204	-0.457	0.261	0.000		0.2697	0.7766	0.9798
SRO PC1	0.498	-0.168	0.057	0.260	0.417	0.159		1.0000	<0.0001*
SRO PC2	0.019	-0.123	-0.238	-0.002	-0.189	-0.041	0.000		0.2275
Flag. length	0.335	0.146	0.278	0.304	0.498	-0.004	0.595	-0.174	

ML with or without phylogenetic uncertainty									
	Dart PC1	Dart PC2	Stylo. PC1	Stylo. PC2	Gland PC1	Gland PC2	SRO PC1	SRO PC2	Flag. length
Dart PC1		1.0000	0.3007	0.0111	<0.0001*	0.7575	0.0026*	0.1328	0.0133
Dart PC2	0.000		<0.0001*	0.0167	0.0056	0.1341	0.7651	0.3278	0.1318
Stylo. PC1	0.149	0.659		1.0000	0.0025*	0.0010*	0.7190	0.1012	0.1276
Stylo. PC2	0.356	0.337	0.000		<0.0001*	0.5025	0.0756	0.5732	0.0255
Gland PC1	0.638	0.386	0.419	0.651		1.0000	0.0027*	0.6739	0.0002*
Gland PC2	0.045	-0.215	-0.451	0.097	0.000		0.3104	0.9574	0.8661
SRO PC1	0.417	-0.043	0.052	0.254	0.416	0.146		1.0000	<0.0001*
SRO PC2	0.216	-0.141	-0.234	0.082	-0.061	0.008	0.000		0.7943
Flag. length	0.348	0.216	0.218	0.316	0.505	-0.024	0.558	-0.038	

Results are shown for the raw data and the analyses based on PICs calculated from the BI with phylogenetic uncertainty and the ML trees either with or without phylogenetic uncertainty. Note that the ML trees with or without uncertainty are identical. *, Significance after Bonferroni correction.

Additional file 3. Information about the location and collectors of the species used in the analysis.

Species	Location	Collector(s)	Accession number
<i>Aegista vulgivaga</i> (Schumacher & Boettger, 1890)	Honshu Island, Kyoto (Japan)	A. Gulick (A16693)	AY014139
<i>Arianta arbustorum</i> (Linnaeus, 1758)	Mittenwald (Germany) Luzern (Switzerland)	N. Michiels & I. Vorndran S. Schumo (A79833)	AY014136
<i>Arion ater</i> (Linnaeus, 1758)	Münster (Germany)	J. Koene	AY014144
<i>Bradybaena similis</i> (Férussac, 1821)	Sendai (Japan)	S. Chiba	AY014138
<i>Cantareus aperta</i> (Born, 1778)	Roma (Italy)	C. Cavalieri	AY014129
<i>Cantareus aspersus</i> (Müller, 1774)	Amsterdam (The Netherlands)	F. Koene	AY014128
<i>Cepaea hortensis</i> (Müller, 1774)	Quebec City, QUE (Canada)	I. Picard	AY014131
<i>Cepaea nemoralis</i> (Linnaeus, 1758)	Münster (Germany)	J. Lange & J. Koene	AY014130
<i>Cernuella cisalpina</i> (Rossmässler, 1837)	Apulia (Italy)	D. Ferreri	AJ550955
<i>Cernuella hydruntina</i> (Kobelt, 1884)	Apulia (Italy)	D. Ferreri	AJ550957
<i>Cernuella virgata</i> (Da Costa, 1778)	Apulia (Italy)	D. Ferreri	AY014127
<i>Chilostoma cingulatum</i> (Studer, 1820)	Lucca (Italy)	M. Sosso	AJ550980
<i>Chilostoma glaciale</i> (Férussac, 1832)	Rocciamelone (Italy)	S. Schaparelli	AJ550979
<i>Chilostoma planospira</i> (Lamarck, 1822)	Lucca (Italy)	M. Sosso	AJ550981
<i>Cochlicella acuta</i> (Müller, 1774)	Roma (Italy)	C. Cavalieri	AY014126
<i>Deroceras reticulatum</i> (Müller, 1774)	Münster (Germany)	J. Koene	AY014119
<i>Discus rotundatus</i> (Müller, 1774)	Roma (Italy)	C. Cavalieri	AY014097
<i>Eobania vermiculata</i> (Müller, 1774)	Roma (Italy) Lecce (Italy) Nuoro, Sardegna (Italy) ‡	C. Cavalieri D. Ferreri M. Sosso	AJ550977
<i>Euhadra amaliae</i> (Kobelt, 1875)	(Japan)	M. Azuma §	AY014140
<i>Euhadra quaesita</i> (Dehayes, 1950)	Sendai (Japan)	A. Davison	AJ550970
<i>Euhadra sandai</i> (Kobelt, 1878)	(Japan)	M. Azuma §	AY014141
<i>Fruticicola fruticum</i> (Müller, 1774)	Münster (Germany)	J. Lange	AJ550971
<i>Helicella itala</i> (Linnaeus, 1758)	Bristol (United Kingdom)	J. Hutchinson	AJ550953
<i>Helicigona lapicida</i> (Linnaeus, 1758)	Bokova (Czech Republic)	H. Reise	AY014137
<i>Helix lucorum taurica</i> (Krynicky, 1833)	Crymea (Russia)	P. Balaban	AJ550975
<i>Helix pomatia</i> Linnaeus, 1758	Münster (Germany)	J. Koene	AJ550974
<i>Helminthoglypta nickliniana</i> (Lea, 1838)	San Mateo Co., CA (U.S.A.)	P. Liff-Grieff	AJ550973
<i>Helminthoglypta tudiculata</i> (Binney, 1843)	Wilmington, CA (U.S.A.)	P. Liff-Grieff	AJ550972
<i>Humboldtiana nuevoleonis</i> Pilsbry, 1927	Santiago, Nuevo León (México)	A. Correa	AJ550982
<i>Hygromia cinctella</i> (Draparnaud, 1801)	Bristol (United Kingdom)	J. Hutchinson	AJ550968
<i>Leptaxis erubescens</i> (Lowe, 1831)	Portela and Santa ‡ (Madeira)	P. Van Riel	AJ550969

Species	Location	Collector(s)	Accession number
<i>Marmorana scabriuscula</i> (Deshayes, 1890)	Sicily (Italy)	F. Giusti	AY014133
<i>Marmorana serpentina</i> (Férussac, 1821)	Nuoro, Sardegna (Italy)	M. Sosso	AJ550978
<i>Monacha cartusiana</i> (Müller, 1774)	Roma (Italy) ‡	C. Cavalieri	AJ550962
	Washington D.C. (U.S.A.)	D. Prasher & D. Robinson	
<i>Monacha parumcincta</i> (Pfeiffer, 1837)	Lecce (Italy)	D. Ferreri	AJ550961
<i>Monachoides vicinus</i> (Rossmässler, 1842)	Dolní Lomná (Czech Republic)	H. Reise	AJ550967
<i>Monadenia fidelis</i> (Gray, 1834)	Humboldt Co., CA (U.S.A.)	D. Yoshimoto	AY014142
<i>Otala lactea</i> (Müller, 1774)	Montréal, QUE (Canada)	R. Chase	AJ550976
<i>Perforatella bidentata</i> (Gmelin, 1791)	Görlitz (Germany)	H. Reise & J. Hutchinson	AJ550966
<i>Perforatella incarnata</i> (Müller, 1774)	Görlitz (Germany)	H. Reise & J. Hutchinson	AJ550965
<i>Polymita picta nigrolimbata</i> Torre, 1950	Santiago de Cuba (Cuba)	B. Reyes Tur	AJ550960
<i>Pseudotrachia rubiginosa</i> (Rossmässler, 1838)	Münster (Germany)	J. Koene	AJ550963
<i>Satsuma japonica</i> (Pfeiffer, 1847)	(Japan)	M. Azuma §	AY014122
<i>Theba pisana</i> (Müller, 1774)	Roma (Italy)	C. Cavalieri	AY014135
	Lecce (Italy)	D. Ferreri	
	Nuoro, Sardegna (Italy)	M. Sosso	
<i>Trichia hispida</i> (Linnaeus, 1758)	Münster (Germany)	J. Koene	AY014125
<i>Trichia striolata</i> (Pfeiffer, 1828)	Quebec City, QUE (Canada)	I. Picard	AJ550964
	Bristol (United Kingdom) ‡	J. Hutchinson	
<i>Vespericola columbiana</i> (Lea, 1838)	Washington State (U.S.A.)	Unknown (A2425D)	AY014120
<i>Xerarionta kellestii</i> (Forbes, 1850)	Santa Catalina Island, CA (U.S.A.)	P. Liff-Grieff	AJ550959
<i>Xeromunda durieui</i> (Pfeiffer, 1848)	Lecce (Italy)	D. Ferreri	AJ550956
<i>Xerosecta cespitum</i> (Draparnaud, 1805)	Lucca (Italy)	M. Sosso	AJ550958
<i>Xerotricha conspurcata</i> (Draparnaud, 1801)	Apulia (Italy)	D. Ferreri	AJ550954

Numbers behind collector names represent the catalog number in the malacological collection of the Academy of Natural Sciences of Philadelphia. The EMBL accession numbers for the new and published DNA sequences are also included. Published sequences all derive from Wade et al. (2001). ‡, The specimens from this location were used for sequencing; §, for these species the DNA sequence were available from EMBL but no specimens could be obtained for investigation. Therefore, the morphology data were based on Azuma (1995).

References

- Azuma M: *Colored Illustrations of the Land Snails of Japan*. Osaka: Hoikusha Publishing Co. Ltd.; 1995.
- Wade CM, Mordan PB, Clarke B: **The phylogeny of the land snails (Gastropoda: Pulmonata)**. *Proc. R. Soc. Lond. B* 2001, **268**:413- 422.

Additional file 4. Description of the data scored for the different reproductive organ characteristics.

Love-dart	
1. Number	0, 1, 2, 3, 4, 5, 6, 7, 8
2. Number of blades	0, 1, 2, 3, 4, 5, 6, 7, 8
3. Perpendicular blades on blades	1=Present
4. Length of blades (compared to total length)	0, 1/8, 1/4, 1/3, 1/2, 2/3, 3/4, 1
5. Curvature	1=Slightly curved 2=Curved 3=Curved and twisted
Stylophore(s)	
Functional stylophore(s)	
1. Number	0, 1, 2, 3, 4, 5, 6, 7, 8
2. Size (relative to reproductive organs)	1=Small 2=Medium 3=Big
3. Placement on vaginal duct	1=Near penis 2=Middle 3=Near bursa tract
Vestigial stylophore(s)	
4. Number	0, 1, 2, 3, 4, 5, 6, 7, 8
5. Size (relative to functional stylophore)	1=Small (less than half) 2=Medium (half) 3=Big (same)
6. Placement on vaginal duct	1=Above functional sac 2=Next to functional sac 3=Both
Glands	
1. Number	0, 1, 2, 3, 4, 5=many
2. Size (relative to reproductive organs)	1=Small 2=Medium 3=Big 4=Very big (as long as spermooviduct)
3. Type of branching	1=Tubular 2=Bifurcated 3=Branched 4=Branched and enlarged at end 5=Heavily branched 6=Flattened
4. Placement	1=On (top of) stylophore 2=In base of stylophore 3=On vaginal duct, at base of stylophore 4=On vaginal duct, halfway to bursa tract 5=On vaginal duct, near bursa tract

Diverticulum of spermatophore-receiving organ

- | | |
|---|---|
| 1. Presence | 1=Yes |
| 2. Length (relative to bursa tract) | 1=Shorter
2=Same
3=Less than twice as long
4=More than twice as long |
| 3. Placement (branching point from bursa tract) | 1=Near vaginal duct
2=Middle of bursa tract
3=Near bursa copulatrix |
| 4. Length (relative to reproductive organs) | 1=Shorter
2=Same
3=Less than twice as long
4=More than twice as long |

Flagellum

- | | |
|-------------------------------|---|
| 1. Presence | 1=Yes |
| 2. Length (relative to penis) | 1=Shorter
2=Same
3=Less than twice as long
4=More than twice as long |
-

When traits are absent these are scored as zero. All relative sizes were measured on the camera lucida drawings. When the size is indicated relative to the reproductive organs this refers to the posterior reproductive organs (excluding the albumen gland) thus making sure that they are independent of the size of the penis and bursa tract.

Additional file 5. The sequences for PCR and sequencing primers.

Primers

Forward (situated in 5.8S rRNA gene)

JOR58F 5' AACGCAAATGGCGGCCTCGG
JOR59F 5' CTCGGGTCCATCCCTCCGA

Reverse (located around position 850 of the 28S rRNA gene)

JOR28R850 5' GAAGACGGGTCTGGGTGGAATG

Internal

JOR28F50 5' TCCGACCTCAGATCGGACGAG
JOR28F400 5' CTTTGAAGAGAGAGTTCAAGAG
JOR28R401 5' GCGGTTTCACGTA CTCTTGAAC
JOR28F600 5' TACAAGCGGTTCCGGHGGCGG
JOR28R601 5' AAGAAGGGCTGGTAGGGACGC

Primers JOR58F, JOR59F, and JOR28R850 were used for PCR, whereby JOR59F was employed as the forward primer for *Cernuella cisalpina* and *Euhadra quaesita*, and JOR58F for all remaining species. Primers JOR28F50, JOR28F400, JOR28R401, JOR28F600, JOR28R601, and JOR28R850 were employed in sequencing reactions.