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Phylogeny, biogeography and the stepwise evolutionary colonization of intertidal habitat in the Liparocephalini based on morphological and molecular characters (Coleoptera: Staphylinidae: Aleocharinae)

Kee-Jeong Ahn[a,*], Mi-Jeong Jeon[b] and Marc A. Branham[c]

[a]*Department of Biology, Chungnam National University, Daejeon 305-764, South Korea;* [b]*Biological Resources Research Department, National Institute of Biological Resources, Incheon 404-170, South Korea;* [c]*Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, USA*

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Abstract

A phylogenetic analysis of the tribe Liparocephalini Fenyes is presented based on morphological and molecular characters. The data set comprised 50 adult morphological characters, partial COI (907 bp), COII (366 bp) and 12S rDNA (325–355 bp), and nearly complete sequences of 18S rDNA (1768–1902 bp) for 21 species. Eighteen species of liparocephaline beetles from all eight genera and three outgroups, are included. The sequences were analysed separately and simultaneously with morphological characters by direct optimization in the program POY4 and by partitioned Bayesian analysis for the combined data. The direct optimization (DO) tree for the combined data under equal weighting, which also shows a minimum incongruence length difference value, resulted in a monophyletic Liparocephalini with the following patterns of phylogenetic relationships (outgroup ((*Baeostethus*, *Ianmoorea*) (*Paramblopusa* ((*Amblopusa*, *Halorhadinus*) (*Liparocephalus*, *Diaulota*))))). A sensitivity analysis using 16 different parameter sets for the combined data shows the monophyly of the liparocephalines and all its genera under all parameter sets. Bayesian analysis resulted in topological differences in comparison with the DO tree under equal weighting only in the position of the genus *Paramblopusa* and clade (*Amblopusa* + *Halorhadinus*), which were reversed. Historical biogeography and the stepwise evolutionary colonization of intertidal habitat in the Liparocephalini are discussed. Based on the biogeographical analyses, we hypothesize that the ancestor of the Liparocephalini occurred along the Panthallassan Ocean, the direct antecedent of the Pacific Ocean, followed by repeated dispersals to the Nearctic from the Palearctic. We also hypothesize that ancestors of the Liparocephalini appear to have arisen in the littoral zone of beaches and then colonized rocky reef areas in the low tidal zone later through high- to mid-tide zones.

A significant portion of the insect fauna of the marine littoral habitat is made up of beetles (Doyen, 1976), and the highest number of species belongs to the family Staphylinidae (Moore and Legner, 1976). The beetle family Staphylinidae is the largest in Coleoptera and in the whole Animal Kingdom. It currently contains over 55 440 species placed in about 3300 genera and 32 subfamilies (Grebennikov and Newton, 2009). Recent studies show that this is only a fraction of the true diversity. They represent one of the truly remarkable radiations in the history of life, characterized by dramatic habitat, micro-ecological and behavioural specialization within various lineages (Thayer, 2005). While many staphylinids are dominant generalist predators in leaf litter and soil communities, others have very specialized habits and habitats. For example, Staphylinidae is one of only a few lineages of insects to invade and diversify in marine littoral habitats. Over

*Corresponding author:
E-mail address: kjahn@cnu.ac.kr

390 species in 93 genera and seven subfamilies (Moore and Legner, 1976; Hammond, 2000; Frank and Ahn, unpublished data) are known to be exclusively confined to seashore habitats.

Multiple origins of marine littoral habitat colonization have occurred in the Staphylinidae (Ahn and Ashe, 2004; Hammond, 2000; Frank and Ahn, unpublished data). The subfamily Aleocharinae contains the largest number of seashore inhabiting species (185 species in 48 genera), almost half of all marine littoral staphylinids. Within this subfamily, the Liparocephalini is the second most speciose tribe (next to the Myllaenini), including 25 species in eight genera (Ahn and Ashe, 1996; Ahn, 2001, 2004), and is exclusively found in intertidal habitats such as within fine gravel, under seaweeds, beneath pebbles and stones, and inside rock crevices and empty barnacles on the rocky headland of the intertidal region (Table 1).

In earlier studies, the tribe Liparocephalini was classified within the polyphyletic tribe Phytosini or its equivalent (Seevers, 1978 and others). However, recent studies suggest that the Liparocephalini (*Amblopusa* Casey, *Baeostethus* Broun, *Diaulota* Casey, *Halorhadinus* Sawada, *Liparocephalus* Mäklin, *Ianmoorea* Ahn, *Paramblopusa* Ahn and Ashe, *Salinamexus* Moore and Legner) remains a clade distinct from the tribe Phytosini based on the synapomorphies: (i) galea with several setae present on mesal surface and the apex bearing several setae; and (ii) contiguous mesocoxal cavities (Ahn and Ashe, 1996; Ahn, 2001, 2004). The phylogenetic patterns of these more recent investigations have been reconstructed using morphological characters, wherein the monophyly of the Liparocephalini has been supported convincingly. However, the intergeneric relationships remain elusive, probably due to a paucity of morphological characters. For example, these analyses included between *ca.* 50 and 70 morphological characters for 24 species of liparocephalines and three outgroups. Although these studies provided insights into the relationships within the tribe and helped test both tribal and generic monophyly for many groups, the results did not firmly establish the generic and species relationships within the tribe. For example, the most recent study based on 50 adult characters (Ahn, 2004) showed the following pattern of generic relationships (*Salinamexus* ((*Halorhadinus*, *Moorea*) (*Amblopusa* (*Paramblopusa* (*Diaulota* (*Liparocephalus*, *Baeostethus*)))))), while Ahn and Ashe (1996) presented the intergeneric relationships of (*Salinamexus* (*Amblopusa* (*Paramblopusa* (*Diaulota* + *Liparocephalus*)))). Since the first cladistic analysis of the Liparocephalini (Ahn and Ashe, 1996), three more genera were added (Ahn, 2001, 2004; Leschen et al., 2002) to the Liparocephalini, but there is no consensus on the generic relationships within the tribe. In the present study, we have included molecular characters from four loci in order to elucidate the phylogenetic relationships of these beetles.

All liparocephaline taxa are found exclusively on the Pacific coasts from Korea and Japan, through Alaska to southern California and Baja California (Mexico), and New Zealand (Table 1). The six genera *Amblopusa*, *Diaulota*, *Halorhadinus*, *Liparocephalus*, *Paramblopusa* and *Salinamexus* are distributed along the Pacific seashores of the northern hemisphere. In contrast, two monotypic genera [*Baeostethus chiltoni* Broun and *Ianmoorea zealandica* (Ahn)] comprise subantarctic species that inhabit the New Zealand shores of the south Pacific Ocean. Leschen et al. (2002) argued that the trans-Pacific biogeographical pattern seen in Liparocephalini is due to contiguous distribution along the coastal margins of Pangea, accepting an early Mesozoic origin and the extinction of all other taxa confined to Gondwana.

The objectives of this paper are to test the monophyly of the tribe Liparocephalini and its included genera using morphological and molecular characters, to reconstruct the phylogenetic relationships of the genera and to present a hypothesis of the biogeography and evolution of intertidal habitat colonization among the liparocephalines. These results, taken together, provide a background for future studies of aleocharine phylogeny and the evolution of the marine littoral habitat preference and colonization among staphylinid beetles.

Materials and methods

Choice of taxa

One or several exemplar species of each genus in the Liparocephalini were chosen. The type species of each genus was included whenever possible. Eighteen out of 25 liparocephaline species from all eight genera were extracted for DNA sequence data. Exemplar taxa across three tribes (Phytosini, Homalotini and Athetini) and three genera [*Phytosus balticus* Kraatz, *Leptusa kitazawai* (Sawada), *Atheta tokiokai* Sawada] were selected to serve as outgroups. The genus *Atheta* Thomson was chosen as the root of the cladogram. Taxa studied, with their distributions, are listed in Table 2.

Morphological characters

Exemplar specimens for this study are deposited in the Chungnam National University Insect Collection (CNUIC, Daejeon) Korea and Snow Entomological Collection, Division of Entomology, KU Natural History Museum and Biodiversity Research Center, University of Kansas, Lawrence (KSEM).

Due to the small size of most aleocharines, dissections of specimens are essential for observing microscopic

Table 1
List of species, geographical distributions, seashore microhabitats and intertidal zones investigated in this study

Species	Distribution	Microhabitat	Intertidal zone
Amblopusa alaskana	Nearctic (AK)	Beneath pebbles and stones	High to mid-tide
A. magna	Palearctic (HK, RU)	Beneath pebbles and stones	High to mid-tide
Baeostethus chiltoni	Australasia (NZ)	Beneath pebbles (French and Smith, 1985; Danks, 1999)	Proximal to high tide
Diaulota alaskana	Nearctic (AK)	Inside empty barnacles and rock crevices	Mid- to low tide
D. aokii	Palearctic (KR, JP), Nearctic (AK)	Inside empty barnacles and rock crevices	Mid- to low tide
D. densissima	Nearctic (CAN, US-WA, OR, CA)	Inside empty barnacles and rock crevices	Very low tide
D. fulviventris	Nearctic (US-CA, MX)	Inside empty barnacles and rock crevices	Mid- to low tide
D. pacifica	Palearctic (KR, JP)	Inside empty barnacles and rock crevices	Mid- to low tide
D. uenoi	Palearctic (KR, JP)	Inside empty barnacles and rock crevices	Mid- to low tide
D. vandykei	Nearctic (US-CA)	Inside empty barnacles and rock crevices	Very low tide
Halorhadinus aequalis	Palearctic (KR, JP)	Beneath pebbles, stones and seaweeds	High to mid-tide
H. inaequalis	Palearctic (KR, JP)	Beneath pebbles, stones and seaweeds	High to mid-tide
Liparocephalus cordicollis	Nearctic (CAN, US-AK, WA, OR, CA)	Inside empty barnacles and rock crevices	Very low tide
L. littoralis	Palearctic (HK)	Inside empty barnacles and rock crevices	Mid- to low tide
Ianmoorea zealandica	Australasia (NZ)	Within fine gravels (Ahn, 2004)	Proximal to high tide
Paramblopusa borealis	Palearctic (HK, RU) Nearctic (CAN, US-AK, WA, OR)	Beneath pebbles and stones	High to mid-tide
P. eoa	Palearctic (HK)	Beneath pebbles and stones	High to mid-tide

CAN, Canada (British Columbia); JP, Japan (HK, Hokkaido); KR, Korea; MX, Mexico (Baja California); NZ, New Zealand; RU, Russia (Kuril Islands); US, USA (AK, Alaska; WA, Washington; OR, Oregon; CA, California).

Table 2
List of species, collection data and GenBank accession numbers investigated in this study (all sequences newly generated for this study)

Species	Collection locality	COI	COII	12S rDNA	18S rDNA
Amblopusa alaskana	Alaska, USA	FJ749928	FJ749949	FJ749887	FJ749907
A. magna	Hokkaido, Japan	FJ749929	FJ749950	FJ749888	FJ749908
Baeostethus chiltoni	Campbell Island, NZ	FJ749930	FJ749951	FJ749889	FJ749909
Diaulota alaskana	Homer, Alaska, USA	FJ749931	FJ749952	FJ749890	FJ749910
D. aokii	Chungnam, Korea	FJ749932	FJ749953	FJ749891	FJ749911
D. densissima	Oregon, USA	FJ749933	FJ749954	FJ749892	FJ749912
D. fulviventris	California, USA	FJ749934	FJ749955	FJ749893	FJ749913
D. pacifica	Gyeongnam, Korea	FJ749935	FJ749956	FJ749894	FJ749914
D. uenoi	Chungnam, Korea	FJ749936	FJ749957	FJ749895	FJ749915
D. vandykei	California, USA	FJ749937	FJ749958	FJ749896	FJ749916
Halorhadinus aequalis	Gyeongnam, Korea	FJ749938	FJ749959	FJ749897	FJ749917
H. inaequalis	Gyeongnam, Korea	FJ749939	FJ749960	FJ749898	FJ749918
Liparocephalus cordicollis	California, USA	FJ749940	FJ749961	FJ749899	FJ749919
L. littoralis	Hokkaido, Japan	FJ749941	FJ749962	FJ749900	FJ749920
Ianmoorea zealandica	Dunedin, New Zealand	FJ749942	FJ749963	FJ749901	FJ749924
Paramblopusa borealis	Hokkaido, Japan	FJ749943	FJ749964	FJ749902	FJ749921
P. eoa	Hokkaido, Japan	FJ749944	FJ749965	FJ749903	FJ749922
Salinamexus koreanus	Jeonnam, Korea	FJ749945	FJ749966	FJ749904	FJ749923
Phytosus balticus	Plymouth, UK	FJ749946	FJ749967	–	FJ749925
Leptusa kitazawai	Gangwon, Korea	FJ749947	FJ749968	FJ749905	FJ749926
Atheta tokiokai	Chungnam, Korea	FJ749927	FJ749948	FJ749886	FJ749906

structural features. The techniques for mounting and studying dissected specimens on microscope slides followed the methods of Ahn and Ashe (1996) and Hanley and Ashe (2003). The data matrix was originally scored from previous studies (Ahn and Ashe, 1996; Ahn, 2001, 2004) along with the inclusion of additional species (Appendices S1 and S2 in Supporting Information).

Selection of genes

Our molecular phylogenies are based on partial mitochondrial 12S rDNA, cytochrome *c* oxidase I and II sequences and nearly complete nuclear 18S rDNA sequences. Cytochrome *c* oxidase sequences, were selected to provide resolution at lower taxonomic levels (species and generic) (Maus et al., 2001), while 12S

rDNA and 18S rDNA sequences were used to provide resolution deeper within the tribe (Whiting et al., 1997; Caterino et al., 2005). Even though the use of 18S can be difficult for incorporation into phylogenetic analyses, due to length variation and extreme rate heterogeneity among regions as well as among taxa, it has been shown to be useful for resolving deep nodes between higher-level insect groups (see below). Those genes were selected to create a data matrix of rapid, medium and slowly evolving sequences.

DNA extraction, amplification and sequencing

We extracted DNA from the head and thorax of specimens preserved in alcohol (EtOH) or from dry specimens. Before DNA extraction, the abdomen was removed to prevent contamination by DNA of parasites or gut contents and to confirm species identification by the examination of the aedeagus and spermatheca. Total genomic DNA was extracted using Qiagen's DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Residual morphological materials were deposited in the CNUIC, Daejeon, Korea.

Amplification of the cytochrome *c* oxidase gene region was accomplished by amplifying three smaller fragments using the primers C1J1 2092/COJ 2680, C1JJ 2441/TL2N 3020 and C2J 3400/TKN 3782. These modified primers for COI were developed after obtaining preliminary sequences from primers listed by Simon et al. (1994). Primers for 12S rDNA were taken from Simon et al. (1994). 18S rDNA sequences are amplified in three fragments using 5′18s/519R, 515F/18sbi and 1055F/18L (Maddison et al., 1999). The primer 519R was modified from Maddison et al. (1999). All primers used in this study are listed in Table 3.

Typical polymerase chain reactions were prepared in 50-μL volumes containing *ca.* 1–3 μL template, 5 μL 10× buffer (Mg^{2+}-free), 4 μL $MgCl_2$ (25 mM), 4 μL dNTPs (2.5 mM each), 0.7 μL each primer (50 pmol), 0.3 μL TaKaRa Ex *Taq* polymerase (TaKaRa Shuzo Co., Tokyo, Japan) and 34.3 μL distilled water. The amplification involved 2 min denaturing at 94 °C, followed by 30 cycles of 30 s denaturing at 94 °C, 30 s primer annealing at 48 °C, *ca.* 52 °C and 1 min extension at 72 °C, after which a final 4 min extension at 72 °C was used.

PCR products were purified using a PCR Product Purification Kit (Roche, Indianapolis, IN, USA) and recovered in a volume of 30 μL H_2O. Amplified DNA was sequenced using a Perkin Elmer ABI377 Automated Sequencer (Applied Biosystems, Foster City, CA, USA) or outsourced to Genotech (Daejeon, South Korea). All sequences were generated in both directions and confirmed with sense and anti-sense strands.

DNA sequence editing

In total, 83 de novo sequences from 21 species (four genes for all 21 species, except for 12S from *P. balticus*) were generated for this study: 21 sequences of each COI, COII, 18S rDNA and 20 sequences of 12S rDNA. Chromatograms obtained from the automatic sequencer were read using the sequence-editing software SeqPup (Gilbert, 1999) and BioEdit (Hall, 1999).

18S sequence data were edited in MacGDE 2.2 (Linton, 2005), where they were divided according to primer-delimited regions and secondary structure in order to save computational time and to find more optimal trees during direct optimization (Giribet, 2001, 2005; Giribet and Wheeler, 2001). The sequences were divided into 16 fragments and all were used in the analysis.

The length of the 18S rDNA sequences included in the analysis was 1768–1902 bp; that of the 12S rDNA sequences, *ca.* 325–355 bp; of the COI sequences, *ca* 907 bp; and of the COII, *ca.* 366 bp. In total, we have included an average of 3510 bp for each taxon, from 3481 to 3530 bp, with the exception of 3041 bp for

Table 3
Primers used in this study

	Primer	Sequence	References
COI	C1J1 2092	5′-AGTTTTAGCAGGAGCAATTACTAT-3′	Brent et al. (1999)
	COJ 2680	5′-GAATCATTGAATAATTCCTGCT-3′	Modified
	C1JJ 2441	5′-CCAACAGGAGGAATTAAAATTTTTAGATGATTAGC-3′	Modified
	TL2N 3020	5′-GGAGCTTAAATCCAATACACTATTCTGCC-3′	Dobler and Müller (1999)
COII	C2J 3400	5′-ATTGGTCATCAATGATACTGA-3′	Simon et al. (1994)
	TKN 3782	5′-GAGACCATTACTTGCTTTCAGTCATCT-3′	Brent et al. (1999)
12S rDNA	12Sai	5′-AAACTAGGATTAGATACCCTACTAT-3′	Simon et al. (1994)
	12Sbi	5′-AAGAGCGACGGGCGATCTCT-3′	
18S rDNA	5′18s	5′-GACAACCTGGTTGATCCTGCCAGT-3′	Maddison et al. (1999)
	519R	5′-CACCGCGAGCGATGAACCRGCGGCGC-3′	Modified
	515F	5′-GTGCCAGCMGCCGCGG-3′	Maddison et al. (1999)
	18sbi	5′-GAGTCTCGTTCGTTATCGGA-3′	
	1055F	5′-GGTGGTGCATGGCCG-3′	
	18L	5′-CACCTACGGAAACCTTGTTACGACTT-3′	

P. balticus, which lacked a 12S sequence. All new sequences have been deposited in GenBank (Table 2).

Phylogenetic methods

Morphological characters only. The analysis was performed in TNT 1.1 (Goloboff et al., 2007) using the implicit enumeration option. Multistate characters were treated as unordered and all characters were equally weighted. The cladogram was rooted on *A. tokiokai*. Character distributions were examined using WinClada (Beta) 0.99 (Nixon, 1999a). The illustrated cladograms were prepared using WinClada and TreeView 1.6.6 (Page, 2001) and edited using Adobe illustrator CS.

Direct optimization and implied alignment. The molecules-only and simultaneous analyses (molecular + morphology) were conducted using direct optimization (DO; Wheeler, 1996) in the program POY4 (Varón et al., 2007). The two protein-coding genes (COI, COII) were analysed as "prealigned" because of no length variation. The sensitivity analysis (Wheeler, 1995) included equal weighting of all characters. In total, 16 different parameter sets were used both for the molecular characters only and for the combined characters (Table 4). In the simultaneous analyses, morphological characters were weighted equal to the highest of the molecular costs (= indel costs).

Tree search strategy followed Varón et al. (2007) and Giannini and Simmons (2003). First, random addition sequences (RAS), followed by alternating subtree pruning and regrafting (SPR) and tree bisection–reconnection (TBR) branch-swapping routines, were run to collect all shortest trees from each replication. Second, the parsimony ratchet (Nixon, 1999b) method was used to visit diverse parts of the tree space and to avoid tree searching becoming stuck on a local optimum. This approach contains multiple iterations of the same search strategy under different parameters.

The following tree-search options in POY4 were used: read ("mor.ss") transform (weight: 1) read (prealigned: ("cox1.fas", tcm:"111.txt")) read (prealigned: ("cox2.fas", tcm:"111.txt")) read ("12s.fas", "18s.fas") transform (tcm:"111.txt") build (250) swap (threshold: 5.0) select () perturb (transform (static_approx), iterations: 15, ratchet: (0.2,3)) select () report ("111.tre", trees: (total), "111con.tre", consensus, "111con.ps", graphconsensus). For simplicity, the above options showed commands for generating the data under only one parameter set, and "18s.fas" was actually divided into 16 segments.

We tested for data set congruence using the incongruence length difference (ILD) test (Mickevich and Farris, 1981; Farris et al., 1994). ILD values were calculated for each parameter set by subtracting the sum of the scores of all partitions from the score of the combined analysis of all partitions and normalizing it for the score of the combined analysis (Table 4).

In order to estimate branch support on a cladogram, we calculated both partitioned Bremer support (Bremer, 1988; Baker and DeSalle, 1997; Baker et al., 1998) and jackknife values. Partitioned Bremer support values were computed on the DO topologies with DO implied alignments (Wheeler, 2003; Giribet, 2005) via PAUP* 4.0b10 (Swofford, 1999) using a command file generated in TreeRot v.3 (Sorenson and Franzosa, 2007). Partitioned Bremer supports were computed several times to ensure consistency. We normalized these by dividing the total Bremer supports per partition by the total number of parsimony informative characters per partition to

Table 4
Tree length and ILD values for partitions including MOL + MOR, MOL, COI, COII, 12S rDNA and 18S rDNA at 16 different parameter set combinations

Ind:Tv:Ts	COI	COII	12S rDNA	18S rDNA	SUM	MOL	ILD (MOL)	MOR	MOL + MOR	ILD (MOL + MOR)
111	1299	518	437	480	2734	2842	0.0380014	121	3024	0.0558862
121	2008	768	743	677	4196	4367	0.0391573	242	4736	0.0629222
141	3405	1261	1347	1069	7082	7394	0.0421963	484	8141	0.0706301
181	6190	2244	2547	1841	12 822	13 430	0.0452717	968	14 922	0.0758611
211	1299	518	506	559	2882	2998	0.0386924	242	3361	0.0705147
221	2008	768	882	836	4494	4674	0.0385109	484	5406	0.0791712
241	3405	1261	1623	1382	7671	8010	0.0423220	968	9481	0.0888091
281	6190	2244	3103	2465	14 002	14 665	0.0452096	1936	17 606	0.0947404
411	1299	518	626	717	3160	3288	0.0389294	484	4017	0.0928553
421	2008	768	1111	1140	5027	5248	0.0421112	968	6688	0.1036184
441	3405	1261	2068	1970	8704	9149	0.0486391	1936	12 010	0.1140716
481	6190	2244	3976	3630	16 040	16 929	0.0525134	3872	22 623	0.1198337
811	1299	518	820	1009	3646	3840	0.0505208	968	5256	0.1221461
821	2008	768	1490	1712	5978	6337	0.0566514	1936	9135	0.1336617
841	3405	1261	2820	3106	10 592	11 321	0.0643936	3872	16 891	0.1436859
881	6190	2244	5480	5891	19 805	21 273	0.0690076	7744	32 383	0.1492758

MOL, molecular data combined; MOR, morphological data; COI, cytochrome *c* oxidase subunit I; COII, cytochrome *c* oxidase subunit II.

Table 5
Bremer support (Bs) values, jackknife values (Jk) and Bayesian posterior probability (Pp) for each clade on the cladogram with parameter set 1 : 1 : 1 (Fig. 3)

Clade	Total Bremer	Bremer Mor	Bremer COI	Bremer COII	Bremer 12S	Bremer 18S	Jk	Pp
A	–	–	–	–	–	–	100	–
B	17.00	−0.50	1.50	1.50	7.00	7.50	99	99
C	10.00	3.00	1.00	3	1.00	2.00	98	100
D	13.00	−2.50	2.00	2.50	4.00	7.00	99	100
E	3.00	−2.00	0	2.00	1.00	2.00	46	–
F	18.00	1.00	0	3.00	3.00	11.00	100	100
G	19.00	−4.00	4.00	5.00	0	14.00	99	100
H	12.00	3.00	3.00	1.00	0	5.00	96	92
I	99.00	4.00	28.67	13.00	10.33	43.00	100	100
J	9.00	−1.00	−8.00	2.00	5.00	11.00	93	100
K	15.00	2.00	−7.00	3.00	8.00	9.00	99	100
L	24.00	5.00	4.00	5.00	4.00	6.00	100	100
M	33.00	8.00	5.00	4.00	5.00	11.00	100	100
N	13.00	0	−3.00	4.00	4.00	8.00	99	100
O	3.00	0	0	0	2.00	1.00	7	99
P	4.00	0	0	−2.00	2.00	4.00	34	92
Q	4.00	0.60	1.00	−1.80	2.80	1.40	14	51
R	4.00	0.17	−2.50	0.33	3.17	2.83	–	–
S	41.00	0	21.00	11.00	9.00	0	100	100
Total	341.00	16.77	50.67	56.53	71.30	145.73		
Average	18.94	0.93	2.81	3.14	3.96	8.10		
Bs contribution (%)		4.92	14.86	16.58	20.91	42.74		
Total number PIC	697	47	273	136	95	146		
Total Bs/PIC	0.489	0.357	0.186	0.416	0.751	0.998		

PIC indicates the number of parsimony informative characters.

quantify the overall contribution of each partition (Table 5). Jackknife support values were also generated from the DO implied alignments via TNT 1.1 (Goloboff et al., 2007) with 1000 ($P = 0.36$) random replicates.

Bayesian analyses. Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). For the analysis, ClustalX (Thompson et al., 1997) was used to generate the alignment of 12S and 18S sequences using the default parameters. A substitution model for each partition was selected before the analysis and the composite model consisting of five submodels was used to run the Bayesian MCMC analysis. Selection of the appropriate model was performed using Modeltest (Posada and Crandall, 1998; Posada, 2003) to evaluate the performance of different models on the basis of the Akaike information criterion (AIC; Akaike, 1974). The AIC favoured the GTR + I + G model for COI, COII and 18S, and the TIM model for 12S. The TIM model is not implemented in MrBayes. Therefore we selected the GTR model for 12S because it was favoured by the hierarchical likelihood-ratio test (hLRT) and is the next more complex model.

All model parameters except tree topology and branch lengths were assumed to be independent across partitions. The rate prior was set to "variable" to allow all partitions to evolve under different rates. All other priors were set to default values (Ronquist and Huelsenbeck, 2003).

For morphological data, the Markov *k* (Mk) model (Lewis, 2001; Nylander et al., 2004) with character type "standard" and gamma-shaped rate variation was set. The number of free parameters in the evolutionary models examined ranged from 1 to 21.

Analyses comprised running four simultaneous chains for 3 000 000 generations, run repeatedly to ensure different starting points did not bias the resulting tree topologies and parameter estimates. Trees were sampled at intervals of 100 generations for a total of 30 000 trees. Convergence was judged by the stabilization of the standard deviation of the split frequencies below 0.01. The burn-in region, initially 25% of the samples (7500 total), were discarded and the remaining trees were summarized in 50% majority-rule consensus trees with MrBayes.

Biogeographical analysis and the evolution of intertidal habitat specialization

In order to elucidate the distributional history of the Liparocephalini, parsimony-based tree fitting and dispersal–vicariance analysis were employed. These analyses were implemented in the programs TreeFitter 1.0 (Ronquist, 2001) and DIVA 1.1 (Ronquist, 1997), respectively. For both analyses, the DO tree of combined data under equal weighting was used and three different distribution areas (Australasia, Nearctic and Palaearctic) were coded.

The Liparocephalini tree was fitted to the geological area cladogram using TreeFitter 1.0 (Ronquist, 2001; Sanmartín, 2007; Sanmartín and Ronquist, 2004) to investigate whether the biogeographical history reflects continental break-up. The costs for individual events were set to the default values in TreeFitter (0.01 for vicariance and duplication events, 1.0 for extinction and 2.0 for dispersal events). Widespread terminals were treated under the recent option (for detailed discussion see Sanmartín, 2007). A randomization test was used to assess the significance of the fit between the geological area cladogram and the Liparocephalini tree. TreeFitter generated 1000 random data sets for which the terminal distributions in the Liparocephalini tree and the areas in the geological area cladogram were randomly permuted. The significance (*P*) value was calculated as the percentage of random data sets that fit the area cladogram better than the nonpermuted Liparocephalini tree.

Additionally, we optimized the distribution data onto the Liparocephalini tree using DIVA 1.1 (Ronquist, 1997) to reconstruct the ancestral distribution of the Liparocephalini. DIVA infers ancestral distributions based on a three-dimensional cost matrix derived from a simple biogeographical model. This algorithm also identifies the optimal distributions of ancestral species by minimizing the number of dispersal and extinction events and does not require a general a priori hypothesis of area relationships.

In order to hypothesize the evolution of intertidal habitat specializations of these beetles (Table 1), four habitat specializations were coded: (0) proximal to high-tide zone, (1) high- to mid-tide zone, (2) mid- to low-tide zone and (3) very low-tide zone. These habitat data were scored for each species based on published literature (Hammond, 2000) as well as personal observations (Frank and Ahn, unpublished data) and were optimized across a cladogram (DO tree of combined data under equal weighting) using Mesquite 2.6 (Maddison and Maddison, 2009) under the parsimony option. A randomization test was used to investigate whether intertidal habitat specialization exhibits significant phylogenetic signal. Mesquite generated 1000 random data sets for which terminal taxa in the Liparocephalini tree were reshuffled. The significance (*P*) value was calculated as percentage of random data sets that have fewer steps in character than the nonmodified Liparocephalini tree.

Results

Morphological tree

The analysis resulted in two most parsimonious cladograms with a length of 121. The liparocephaline lineage is well supported as a whole, based on five synapomorphies. The reconstructed phylogeny of the liparocephaline genera is not substantially different from that of Ahn and Ashe (1996), Ahn (2001, 2004) and Leschen et al. (2002), with the following pattern of generic relationships (outgroup (*Salinamexus* (*Halorhadinus*, *Ianmoorea*, (*Amblopusa* (*Paramblopusa* (*Diaulota* (*Liparocephalus*, *Baeostethus*))))))). However, species of *Diaulota* did not form a monophyletic group.

DO tree of morphological and molecular characters combined

Simultaneous analysis of morphological and molecular characters under equal weighting resulted in a single most parsimonious cladogram with a length of 3024. We used this cladogram to depict the intergeneric relationships of the Liparocephalini because the parameter set (Ind:Tv:Ts = 1 : 1 : 1) scored a minimum ILD value in both the molecular only and combined data sets. The results from the sensitivity analyses are summarized in Table 4 and are illustrated as a Navajo rug appearing below each corresponding node on the tree (Fig. 1).

The genera *Baeostethus* + *Ianmoorea* form the sister group to the remaining liparocephaline genera (clade D = *Paramblopusa*, *Amblopusa*, *Halorhadinus*, *Liparocephalus*, *Diaulota*). However, *Salinamexus* is not recovered as a member of the Liparocephalini and its systematic position is discussed below. Within clade D, *Paramblopusa* shows a sister-group relationship to the remaining genera (clade E = *Amblopusa*, *Halorhadinus*, *Liparocephalus*, *Diaulota*). Within the clade E, *Amblopusa* and *Halorhadinus* form a monophyletic clade, which is sister to *Liparocephalus* and *Diaulota*. Within the most species-rich liparocephaline genus *Diaulota*, the following patterns of species relationships were discovered: (*D. pacifica* Sawada ((*D. uenoi* (Sawada) (*D. aokii* Sawada ((*D. fulviventris* Moore, *D. alaskana* Ahn) (*D. densissima* Casey, *D. vandykei* Moore))))).

Partitioned Bremer support and jackknife values are presented in Table 5 for the corresponding alphabetical nodes on the DO cladogram under equal weighting (Fig. 1). The contribution of each partition (total Bremer for the partition/total Bremer for all partitions) was as follows: morphology = 4.92%; COI = 14.86%; COII = 16.58%; 12S = 20.91%; 18S = 42.74% (Table 5).

Bayesian tree of morphological and molecular characters combined

Our Bayesian analysis resulted in an almost identical cladogram to the DO tree of combined data under equal weighting. It differed from the DO tree only in the position of the genus *Paramblopusa* and clade

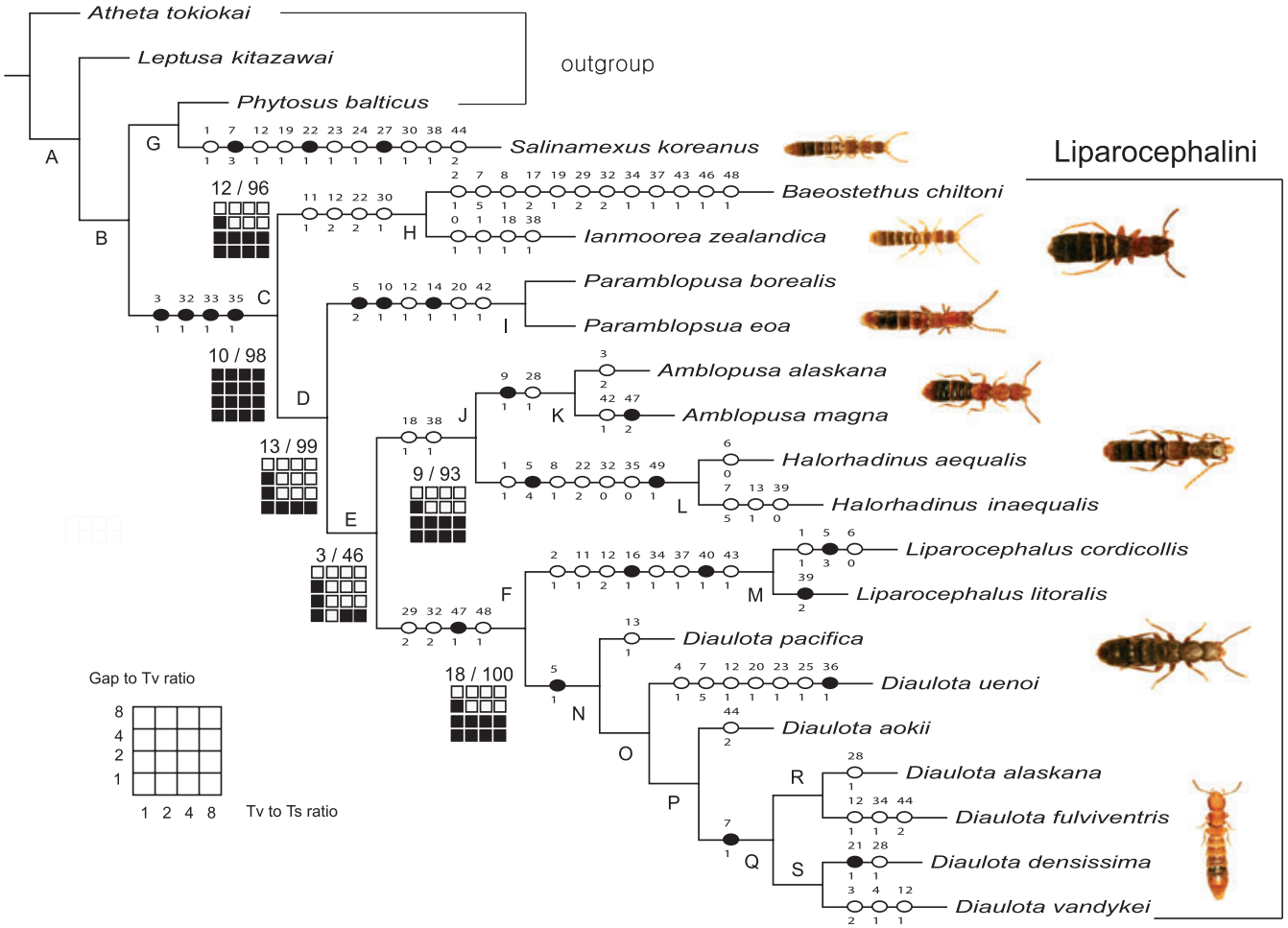

Fig. 1. A single most parsimonious cladogram analysed in POY (direct optimization) under equal weighting with Bremer support (left on Navajo rug) and jackknife values (right on Navajo rug). Navajo rugs representing the results of the sensitivity analysis (black squares indicate monophyly, white ones not). Unambiguously optimized morphological characters traced onto the cladogram. Unique characters are indicated by closed circles, homoplasies by open circles.

(*Amblopusa* + *Halorhadinus*), which were reversed (Fig. 2). Interestingly, using a traditional two-step parsimony analysis executed in ClustalW + PAUP* (tree not shown) showed intergeneric relationships identical to the Bayesian tree.

Biogeographical analysis and the evolution of intertidal habitat specialization

The best area cladogram retrieved by the exhaustive search in the TreeFitter was identical to the geological area cladogram (Australasia, (Palearctic, Nearctic)) with the cost of 2.16 (five vicariance, 11 duplication and two extinction events). Two terminal dispersals were not included in the cost because they are also present across all possible geological area cladograms (Sanmartín, 2007). The TreeFitter analyses indicate that the present-day distribution of the Liparocephalini is primarily a result of speciation within continents (11 sympatric speciation events) and vicariance (five events) between them. The randomization test gave a significant *P* value of 0 for the geological area cladogram, indicating that we can reject the null hypothesis of "no phylogenetic constraint" between the geological area cladogram and the Liparocephalini tree at the 0.05 significance level. The pattern of evolution in the Liparocephalini is consistent with a hypothesis of sequential continental break-up.

Dispersal–vicariance analysis showed that the optimal reconstruction required five dispersals (Fig. 3). The results suggest that the ancestor of the Liparocephalini was widely distributed in the Australasian and Palearctic regions, suggesting a contiguous distribution along the coastal margins of Pangaea. Results of our DIVA analysis suggest that repeated dispersal events occurred from the Palearctic to the Nearctic region (Fig. 3).

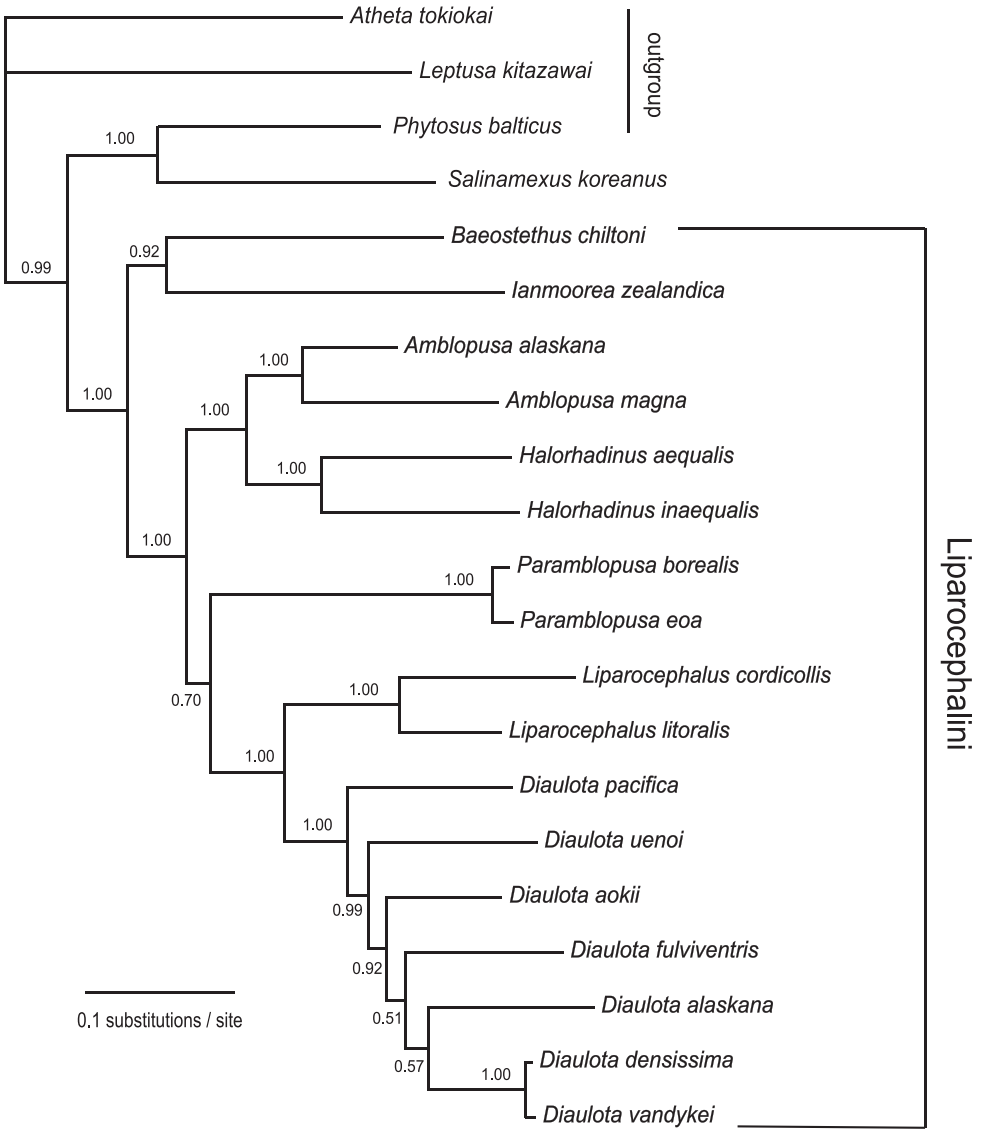

Fig. 2. Bayesian phylogenetic tree and posterior probabilities (Pp) for clades based on the combined data set under GTR + I + G model. Scale bar indicates number of expected substitutions per site.

Parsimony optimization of the intertidal habitat data indicated that the ancestral habitat specialization of the Liparocephalini, either proximal to high-tide zone or high- to mid-tide zone, is ambiguous. The evolution of intertidal habitat specialization in the Liparocephalini is represented by four changes: from the clade D, from the clade F, from the *Liparocephalus cordicollis* and from the clade (*Diaulota densissima* + *D. vandykei*) (Fig. 3). The randomization test gave a significant *P* value of 0, indicating that we can reject the null hypothesis of "no phylogenetic constraint" between the intertidal habitat specialization pattern and the Liparocephalini tree at the 0.05 significance level. The ecological pattern is consistent with a hypothesis of the stepwise evolutionary colonization of intertidal habitat in the Liparocephalini.

Discussion

Liparocephalini phylogeny

Monophyly of Liparocephalini. The monophyly of Liparocephalini has been demonstrated based on morphological characters (Ahn and Ashe, 1996; Ahn, 2001, 2004). We obtained consistent results from this study that a group of genera including *Baeostethus*, *Ianmoorea*, *Paramblopusa*, *Amblopusa*, *Halorhadinus*, *Liparocephalus* and *Diaulota* forms a monophyletic group. All our analyses (morphology only, DO trees of combined data under all 16 parameter sets and Bayesian analysis) support the monophyly of the Liparocephalini. However, *Salinamexus* is not recovered as a

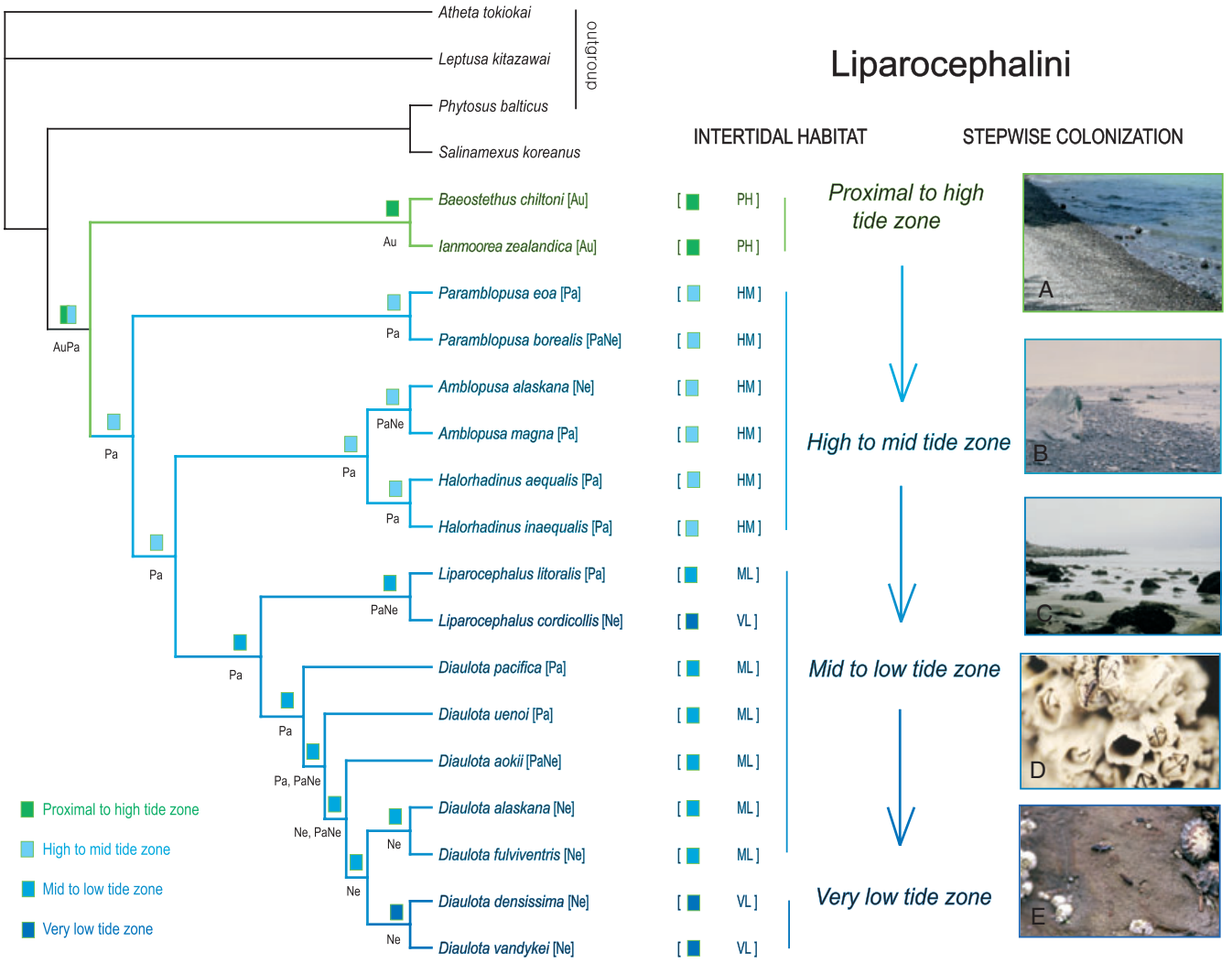

Fig. 3. The direct optimization tree (same as Fig. 1) with distributional data and seashore microhabitat. Ancestral distributions and ancestral intertidal habitat states were reconstructed using DIVA and Mesquite, respectively and plotted on the tree. Letters in parentheses indicate distribution and intertidal zone of each species: Au, Australasia; Pa, Palearctic; Ne, Neartic; PH, proximal to high tide zone; HM, high- to mid-tide zone; ML, mid- to low-tide zone; VL, very low-tide zone. Letters in picture indicate seashore microhabitats and intertidal zone of each species: A, under fine gravel proximal to high tide zone, New Zealand; B, under stones in high- to mid-tide zone, Alaska, USA; C, rocky reef area in mid- to low-tide zone, California, USA; D, *Diaulota aokii* on empty barnacles in low-tide zone, Anmyeondo Island, Korea; E, *Diaulota vandykei* on rocky reef area in very low-tide zone, California, USA.

member of Liparocephalini (see below for further discussion).

Monophyly of genera and intergeneric relationships. The monophyly of all of the liparocephaline genera was well supported by nearly all the analyses (molecular only, DO trees of combined data under all 16 parameter sets and Bayesian analysis). The single exception was the most speciose genus, *Diaulota*, which shows a non-monophyly with morphological data only. Most of the nodes showing intergeneric relationships are very well supported, with eight out of nine nodes possessing jackknife values of 90 or higher, total Bremer support of 9 or greater and posterior probabilities of 90 or higher in the Bayesian analysis.

Intergeneric relationships among Liparocephalini based on morphological characters were different from those based on combined characters, although there were some consistent relationships among the genera. Only the molecular character analysis with the following two parameter sets (1 : 1 : 1, 1 : 2 : 1) resulted in exactly the same topology as the simultaneous analysis under equal weighting.

The result of our DO trees of combined data with eight parameter sets of lower indel-to-transversion ratios (2 or less) and one parameter set (4 : 1 : 1) produced the

identical topology of intergeneric relationships ((*Baeostethus*, *Ianmoorea*) (*Paramblopusa* ((*Amblopusa* + *Halorhadinus*) (*Liparocephalus* + *Diaulota*)))) as under equal weighting. Four exceptions from the simultaneous analyses with four parameter sets (1 : 2 : 1, 2 : 2 : 1, 2 : 4 : 1, 2 : 8 : 1) were noted, showing the topological differences only in position between (*Amblopusa* + *Halorhadinus*) and *Paramblopusa*.

The intergeneric relationships among clade D (*Paramblopusa* ((*Amblopusa* + *Halorhadinus*) (*Liparocephalus* + *Diaulota*))) showed the identical topology in molecular data across all 16 parameter sets. On the contrary, DO trees of the combined data with three parameter sets of indel-to-transversion-to-transition ratios of (2 : 2 : 1, 2 : 4 : 1, 2 : 8 : 1) and Bayesian analysis supported the following relationships: ((*Amblopusa* + *Halorhadinus*) (*Paramblopusa* (*Liparocephalus* + *Diaulota*))). In addition, a very low jackknife value (46%) was generated on clade E.

Systematic position of the genus Baeostethus. The phylogenetic position of the genus *Baeostethus*, which occurs on subantarctic islands, has been problematic. Leschen et al. (2002) suggested that *Baeostethus* formed a sister-group relationship to the remaining whole liparocephaline genera. The authors based this hypothesis primarily on the biogeographical distribution of this genus (see below), and argued that the inadequate selection of outgroup taxa probably misled previous studies. However, the DO trees based on combined data from this study suggest that *Baeostethus* + *Ianmoorea* are actually the sister group of the remaining genera of the Liparocephalini, although we used essentially the same outgroups as in the previous studies (Ahn and Ashe, 1996; Ahn, 2004). In addition, our Bayesian analysis also supported the sister-group relationship of the genera *Baeostethus* and *Ianmoorea*.

The addition of increased weighting of morphological characters, for example, the DO trees of combined data with higher indel-to-change ratios (4 : 2 : 1, 4 : 4 : 1, 4 : 8 : 1, 8 : 1 : 1, 8 : 2 : 1, 8 : 4 : 1, 8 : 8 : 1), supports the sister-group relationship of *Baeostethus* and *Liparocephalus*, but places them in the most nested position on the cladogram. Possibly this is because they show many superficial morphological similarities externally, as hypothesized by Leschen et al. (2002).

Systematic position of the genus Salinamexus. *Salinamexus* species inhabit the Pacific coasts of Korea and Mexico. This genus is consistently recovered as a member of the Liparocephalini with morphological characters only. When this genus was not recovered as a member of the Liparocephalini, it was with the Phytosini in both our simultaneous analyses with four parameter sets (1 : 1 : 1, 1 : 2 : 1, 1 : 4 : 1, 2 : 1 : 1) and Bayesian analysis. All three synapomorphies (galea with several setae only on mesal surface and apex with setae; mesocoxal cavities contiguous; seta v absent on mentum) presented by Ahn (2004), based on 50 adult characters, were ambiguously optimized in this analysis on the DO tree with equal weighting. Instead, four other synapomorphies (3-1, 32-1, 33-1, 35-1; see Appendix S1 in Supporting Information) supported the monophyly of the Liparocephalini without *Salinamexus* in the DO tree under equal weighting. Therefore we tentatively exclude the genus from the tribe until the addition of more taxa and a more comprehensive analysis can provide a more robust picture of the phylogenetic position of this genus.

Data set contribution for DO trees. In order to determine how each type of data contributed to the support of the simultaneous analysis, we calculated partitioned Bremer support values for each node. Table 5 illustrates these support values for each data set summed across the simultaneous analysis tree under equal weighting. The relative contribution of our 18S data was very apparent in relation to the rest of our sequence data. While the other genes generated solid phylogenetic signal, 42.74% of the tree's total support is provided by the 18S data (COI = 14.86%; COII = 16.58%; 12S = 20.91%), whereas morphology influenced the topology very little (4.92%).

Our morphological data set contained only 47 parsimony informative characters, whereas COI provided 273. The relative contribution of each type of data across the tree (sum of partitioned Bremer support values divided by the total number of parsimony informative characters: see Baker and DeSalle, 1997; Ogden and Whiting, 2005) is summarized in Table 5 (morphology = 0.357; COI = 0.186, COII = 0.416; 12S = 0.751; 18S = 0.998). Morphological characters performed relatively well in supporting the monophyly of the Liparocephalini and most genera. The partitioned Bremer support (PBS) values on molecular data suggest substantial variation in the degree to which each gene partition contributes to the cladogram (Table 5). Nuclear genes contributed the most, particularly at higher taxonomic levels, while there was substantial variation in performance among mitochondrial genes. Of these, 12S and COII contributed relatively well at both higher and species levels.

Biogeography and the evolution of intertidal habitat specialization in the Liparocephalini

Phylogenies can address many issues in comparative evolution (Eldredge and Cracraft, 1980; Brooks and McLennan, 1991; Harvey and Pagel, 1991), and can provide a useful backdrop for interpreting the evolutionary histories of biogeography and other classes of organismal characteristics (Grandcolas et al., 2001;

Avise, 2004). To address evolutionary questions concerning biogeography and intertidal habitat specialization of the Liparocephalini, we reconstructed a phylogeny of the group. Interestingly, while the topologies generated across all methods of phylogeny reconstruction employed in this study (direct optimization parsimony analysis, partitioned Bayesian analysis and traditional two-step parsimony analysis) showed small topological differences, they all produced the same general topology. We redefine the tribe Liparocephalini to contain the genera *Baeostethus*, *Ianmoorea*, *Paramblopusa*, *Amblopusa*, *Halorhadinus*, *Liparocephalus* and *Diaulota* (see above). We used the phylogenetic results from the DO tree of combined data under equal weighting (Fig. 3) to examine distributional and intertidal habitat data for these beetles in order to develop a hypothesis of biogeography and habitat colonization within the Liparocephalini.

Historical biogeography. The results of our biogeographical analysis show that the distribution of Liparocephalini is congruent with the geological history. The biogeography of numerous organisms has been influenced by two geological events. These include the splitting of Pangea into Gondwana and Laurasia in the Mid-Jurassic 180–165 Ma (Sanmartín and Ronquist, 2004) and the isolation of the the Palearctic from the Nearctic by continental seaways in the Mid-Cretaceous 100–80 Ma (Sanmartín et al., 2001). The results from our TreeFitter analyses are congruent with these geological histories. There is strong concordance between the Liparocephalini tree and the Gondwana–Laurasia and the Palearctic–Nearctic sister area relationships displayed by the geological area cladogram. DIVA optimization places Australasia and the Palearctic at the ancestral node of the Liparocephalini tree, indicating that ancestors to the liparocephalines were restricted to both these areas. This result also supports the hypothesis of vicariance in the ancestral area of the Liparocephalini (Fig. 3).

Our analysis also suggests this beetle lineage underwent multiple dispersal events in the northern hemisphere. Five dispersal events were inferred through a DIVA analysis, while none (two terminal dispersal events) was recovered from a TreeFitter analysis in the northern hemisphere. These discrepancy between numbers of dispersal events hypothesized for this lineage may be due to the different ways in which TreeFitter and DIVA calculate the number of dispersal events (Sanmartín, 2007). Irrespective of the number of dispersal events generated with these programs, we can hypothesize that multiple dispersal events occurred from the Palearctic into the Nearctic.

Leschen et al. (2002) argued that the trans-Pacific biogeographical pattern seen in Liparocephalini is due to an early Mesozoic origin and the vicariance of Pangea. Our analyses support this hypothesis, implying that liparocephaline evolution can be traced back to ancestors widespread along the coasts of Pangea (Fig. 3). Leschen et al. (2002) provided distributional evidence supporting the possibility of a long history of *Baeostethus* on New Zealand. Moreover, they argued that the presence of a liparocephaline ancestor on the Gondwanan shoreline is consistent with the fossil record for staphylinids, which includes aleocharine-like taxa from the Jurassic (Carpenter, 1992).

A similar distribution pattern is seen in the intertidal staphylinine genus *Hadrotes* Mäklin (Staphylinidae), containing two species, one found on the Pacific shores of North America (California) and the other restricted to the shores of New Zealand. This distribution is also matched by the terrestrial aleocharine tribe Gymnusini, which is found in the Auckland Islands and is the sister taxon to the rest of the group, widespread throughout the Holarctic regions (Hammond, 1975; Klimaszewski, 1979; Ashe, 2000).

However, the alternative hypothesis—that the ancestor of the liparocephalines originated from Gondwana and later dispersed into Laurasia—cannot be rejected, because the sister-group relationship of the Liparocephalini is not unambiguously established and knowledge of the seashore inhabiting staphylinid beetle diversity throughout the trans-Pacific rim regions is limited, especially in the southern hemisphere. Therefore additional phylogenies for species distributed on the shores of the south Pacific, such as Australia and Chile, are necessary to confirm the hypothesis. We do not have enough data to support either of the two hypotheses at present, but it appears that the ancestor of the Liparocephalini occurred along the Panthallassan Ocean, the direct antecedent of the Pacific Ocean, followed by repeated dispersals into the Nearctic from the Palearctic.

The stepwise evolutionary transition of intertidal habitat colonization. Regular inhabitants of the marine littoral region can be divided into submarine and littoral species (Moore and Legner, 1976). Submarine species are those that tolerate being submerged in seawater and may continue their activities at a reduced rate when submerged (Meyerdirk, 1969; Topp and Ring, 1988). This contrasts with those of the littoral zone, which are killed by submergence in seawater (Topp and Ring, 1988). Most liparocephalines are submarine in that they live in the intertidal region and are subsequently submerged by seawater for at least part of each day.

By optimizing habitat specializations across the DO cladogram, the ancestral habitat of the Liparocephalini was recovered ambiguously, but either proximal to high-tide zone or high- to mid-tide zone. However, the common ancestor of these beetles could be hypothesized to inhabit the proximal to high-tide zone along

seashores, because the probable sister group shown in this study, *Phytosus* + *Salinamexus*, also occurs in the upper littoral zone. At present we do not have a confirmed sister group for the Liparocephalini and it is therefore difficult to determine the ancestral habitat clearly. Nevertheless, it appears unlikely that any sister-group candidate among all lineages of the subfamily Aleocharinae inhabits the mid- to high-intertidal zone or deeper tidal zones, even if this present sister-group relationship is determined to be incorrect.

Our optimization of habitat specialization across the tribe shows that the ancestral habitat of clade D (*Paramblopusa–Diaulota*) is the high- to mid-tide zone, indicating that the ancestor of the Liparocephalini colonized deeper intertidal habitat. Species of *Amblopusa*, *Paramblopusa* and *Halorhadinus* are restricted to high- to mid-tide zones. Later, these colonized into a little deeper intertidal habitat such as rocky reef areas in the mid- to low-tide zone, where members of *Diaulota* and *Liparocephalus* species are found. These species are submarine in that they live in the intertidal region and are subsequently submerged by seawater each day. Lastly, the ancestor of these colonized into the deepest intertidal habitat in which *L. cordicollis*, *Diaulota vandykei* and *D. densissima* occur. The results also reveal that these two lineages colonized independently in the very low intertidal zone, remaining submerged for a relatively long time. For that reason, they are called marine beetles and occupy the most nested position on the cladogram (Fig. 3). The general pattern of stepwise evolutionary transition of intertidal habitat can be hypothesized.

Therefore we hypothesize that the ancestors of the Liparocephalini appear to have arisen proximal to high-tide regions, such as in gravels and beneath stones on the beach, and colonized rocky reef habitat in the low-tide zone later through the high- and mid-tide zones. The genus *Diaulota*, with eight known species, is the most successful in terms of diversity and broad distribution [Pacific coasts of Korea, Japan, Kamchatka, Alaska, Canada, Washington, Oregon, California and Baja California (Mexico)]. However, the low species diversity of intertidal liparocephalines compared with the enormous aleocharine species diversity suggests that most have not diversified successfully in the harsh intertidal environment.

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References

Ahn, K.-J., 2001. Phylogenetic relationships of the intertidal genus *Halorhadinus* Sawada and key to the genera of the Liparocephalini (Coleoptera: Staphylinidae: Aleocharinae). Insect Syst. Evol. 32, 123–132.

Ahn, K.-J., 2004. *Moorea zealandica*, new genus and species from New Zealand with a discussion of its phylogenetic relationships (Coleoptera: Staphylinidae: Aleocharinae). N.Z. J. Zool. 31, 255–261.

Ahn, K.-J., Ashe, J.S., 1996. Phylogeny of the intertidal aleocharine tribe Liparocephalini (Coleoptera: Staphylinidae). Syst. Entomol. 21, 99–114.

Ahn, K.-J., Ashe, J.S., 2004. Phylogeny of the Myllaenini and related taxa (Coleoptera: Staphylinidae: Aleocharinae). Cladistics 20, 123–138.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–722.

Ashe, J.S., 2000. Mouthpart structure of *Stylogymnusa subantarctica* Hammond, 1975 (Coleoptera: Staphylinidae: Aleocharinae) with a reanalysis of the phylogenetic position of the genus. Zool. J. Linn. Soc. 130, 471–498.

Avise, J.C., 2004. Evolutionary Pathways in Nature: A Phylogenetic Approach. Cambridge University Press, Cambridge.

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilids. Syst. Biol. 46, 654–673.

Baker, R.H., Yu., X.B., DeSalle, R., 1998. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. Mol. Phylogenet. Evol. 9, 427–436.

Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42, 795–803.

Brent, C.E., Pedro, O., Hewitt, G.M., 1999. MtDNA phylogeography and recent intra-island diversification among Canary island *Calathus* beetles. Mol. Phylogenet. Evol. 13, 149–158.

Brooks, D.R., McLennan, D.A., 1991. Phylogeny, Ecology, and Behavior: A Research Program in Comparative Biology. University of Chicago Press, Chicago.

Carpenter, F.M., 1992. Treatise on invertebrate paleontology. Part R. Arthropoda 4. Vol. 4: Superclass Hexapoda. The University of Kansas, Lawrence, KS, USA and The Geological Society of America.

Caterino, M.S., Hunt, T., Vogler, A.P., 2005. On the constitution and phylogeny of Staphyliniformia (Insecta: Coleoptera). Mol. Phylogenet. Evol. 34, 655–672.

Danks, H.V., 1999. Life cycles in polar arthropods – flexible or programmed? Eur. J. Entomol. 96, 83–102.

Dobler, S., Muller, J.K., 1999. Resolving phylogeny at the family level by mitochondrial cytochrome oxidase: Phylogeny of carrion beetles (Coleoptera: Silphidae). Mol. Phylogenet. Evol. 15, 390–462.

Doyen, J.T., 1976. Marine beetles (Coleoptera excluding Staphylinidae). In: Cheng, L. (Ed.), Marine Insects. North Holland, Amsterdam, pp. 497–519.

Eldredge, N., Cracraft, J., 1980. Phylogenetic Patterns and the Evolutionary Process: Method and Theory in Comparative Biology. Columbia University Press, New York.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics, 1, 67–85.

French, D.D., Smith, V.R., 1985. A comparison between northern and southern tundras and related ecosystems. Polar Biol. 5, 5–21.

Giannini, N.P., Simmons, N.B., 2003. A phylogeny of megachiropteran bats (Mammalia: Chiroptera: Pteropodidae) based on direct optimization analysis of one nuclear and four mitochondrial genes. Cladistics 19, 496–511.

Gilbert, D.G., 1999. SeqPup, Version 0.9. Indiana University, Bloomington. http://iubio.bio.indiana.edu/soft/molbio/seqpup/java/seqpup-doc.html.

Giribet, G., 2001. Exploring the behavior of POY, a program for direct optimization of molecular data. Cladistics 17, S60–S70.

Giribet, G., 2005. Generating implied alignments under direct optimization using POY. Cladistics 21, 396–402.

Giribet, G., Wheeler, W.C., 2001. Some unusual small-subunit ribosomal RNA sequences of metazoans. Am. Mus. Novit. 3337, 1–14.

Goloboff, P., Farris, J., Nixon, K., 2007. T.N.T. Version 1.1: Tree Analysis Using New Technology. Program and documentation, available from the authors, and at http://www.zmuc.dk/public/phylogeny.

Grandcolas, P., Deleporte, P., Desutter-Grandcolas, L., Daugeron, C., 2001. Phylogenetics and ecology: as many characters as possible should be included in the cladistic analysis. Cladistics 17, 104–110.

Grebennikov, V.V., Newton, A.F., 2009. Good-bye Scydmaenidae, or why the ant-like stone beetles should become megadiverse Staphylinidae sensu latissimo (Coleoptera). Eur. J. Entomol. 106, 275–301.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NA. Nucleic Acids Symp. Ser. 41, 95–98.

Hammond, P.M., 1975. The phylogeny of a remarkable new genus and species of gymnusine staphylinid (Coleoptera) from the Auckland Islands. J. Entomol. 44, 153–173.

Hammond, P.M., 2000. Coastal Staphylinidae (rove beetles) in the British Isles, with special reference to saltmarshes. In: Sherwood, B.R., Gardiner, B.G., Harris, T. (Eds.), British Saltmarshes. Forrest Text, London, pp. 247–302.

Hanley, R.S., Ashe, J.S., 2003. Techniques for dissecting adult aleocharine beetles (Coleoptera: Staphylinidae). Bull. Entomol. Res. 93, 11–18.

Harvey, P.H., Pagel, M.D., 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.

Klimaszewski, J., 1979. A revision of the Gymnusini and Deinopsini of the world (Coleoptera: Staphylinidae, Aleocharinae). Agric. Can. Monogr. 25, 1–169.

Leschen, R.A.B., Bullians, M.S., Michaux, B., Ahn, K.-J., 2002. Systematics of *Baeostethus chiltoni*, a subantarctic liparocephaline (Coleoptera: Staphylinidae: Aleocharinae): a Pangean relic or a more recent immigrant? J. R. Soc. N. Z. 32, 189–201.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.

Linton, E.W., 2005. Macgde: Genetic Data Environment for Macos X Version 2.0. http://www.msu.edu/~lintone/macgde.

Maddison, W.P., Maddison, D.R., 2009. Mesquite: A Modular System for Evolutionary Analysis. Version 2.6. http://mesquiteproject.org.

Maddison, D.R., Baker, M.D., Ober, K.A., 1999. Phylogeny of carabid beetle as inferred from 18S ribosomal DNA (Coleoptera: Carabidae). Syst. Entomol. 24, 103–138.

Maus, C., Peschke, K., Dobler, S., 2001. Phylogeny of the genus *Aleochara* inferred from mitochondrial cytochrome oxidase sequences (Coleoptera: Staphylinidae). Mol. Phylogenet. Evol. 18, 202–216.

Meyerdirk, D.E., 1969. Adaptations of a Rove Beetle, *Diaulota Fulviventris* Moore (Coleoptera: Staphylinidae), to the Marine Environment. MSc Thesis, San Diego State College, San Diego, California.

Mickevich, M.L., Farris, J.S., 1981. The implications of congruence in *Menidia*. Syst. Zool. 30, 351–370.

Moore, I., Legner, E.F., 1976. Intertidal rove beetles (Coleoptera: Staphylinidae). In: Cheng, L. (Ed.), Marine Insects. North Holland, Amsterdam, pp. 521–551.

Nixon, K., 1999a. WinClada. Cornell University Herbarium, Ithaca.

Nixon, K., 1999b. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetics analysis of combined data. Syst. Biol. 53, 47–67.

Ogden, T.H., Whiting, M.F., 2005. Phylogeny of Ephemeroptera (mayflies) based on molecular evidence. Mol. Phylogenet. Evol. 37, 625–643.

Page, R.D.M., 2001. Tree View (Win32) 1.6.6. http://taxonomy.zoology.gla.ac.uk/rod/rod.htmlr.

Posada, D., 2003. Using Modeltest and PAUP* to select a model of nucleotide substitution. In: Baxevanis, A.D., Davison, D.B., Page, R.D.M., Petsko, G.A., Stein, L.D., Stormo, G.D. (Eds.), Current Protocols in Bioinformatics. John Wiley & Sons, Hoboken, NJ, pp. 6.5.1–6.5.14.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Ronquist, F., 1997. DIVA (Dispersal–Vicariance Analysis) Version 1.2. Computer program and manual available from Uppsala University, ftp.uu.se or ftp.systbot.uu.se.

Ronquist, F., 2001. TreeFitter v. 1.0. http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sanmartín, I., 2007. Event-based biogeography: integrating patterns, processes, and time. In: Ebach, M.C., Tangney, R.S. (Eds.), Biogeography in a Changing World. CRC Press, London, pp. 135–159.

Sanmartín, I., Ronquist, F., 2004. Southern hemisphere biogeography inferred by event-based models: plants versus animal patterns. Syst. Biol. 53, 216–243.

Sanmartín, I., Enghoff, H., Ronquiest, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc. 73, 345–390.

Seevers, C.H., 1978. A generic and tribal revision of the North American Aleocharinae (Coleoptera: Staphylinidae). Fieldiana, Zool. 71, 1–289.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primer. Ann. Entomol. Soc. Am. 87, 651–701.

Sorenson, M.D., Franzosa, E.A., 2007. TreeRot, Version 3. Boston University, Boston, MA.

Swofford, D.L., 1999. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0. Sinauer Associates, Sunderland, MA.

Thayer, M.K., 2005. Staphylinidae Latreille, 1802. In: Beutel, R.G., Leschen, R.A.B. (Eds.), Handbook of Zoology. Volume IV. Arthropoda: Insecta, Coleoptera, Beetles Volume 1: Morphology and Systematics. Walter de Gruyter, Berlin, pp. 296–344.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Topp, W., Ring, R.A., 1988. Adaptations of Coleoptera to the marine environment. II. Observations on rove beetles (Staphylinidae) from rocky shores. Can. J. Zool. 66, 2469–2474.

Varón, A., Vinh, L.S., Bomash, I., Wheeler, W.C., 2007. POY 4.0.2398 Beta. American Museum of Natural History, New York. http://research.amnh.org/scicomp/projects/poy.php.

Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44, 321–331.

Wheeler, W.C., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? Cladistics 12, 1–9.

Wheeler, W.C., 2003. Implied alignment: a synapomorphy-based multiple sequence alignment method and its use in cladogram search. Cladistics 19, 262–268.

Whiting, M.F., Carpenter, J.C., Wheeler, Q., Wheeler, W.C., 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Morphological character analysis

Appendix S2. Morphological character data matrix

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