

Supplementary figures and tables for :

The *achaete-scute* complex contains a single gene that controls bristle development in the semi-aquatic bugs

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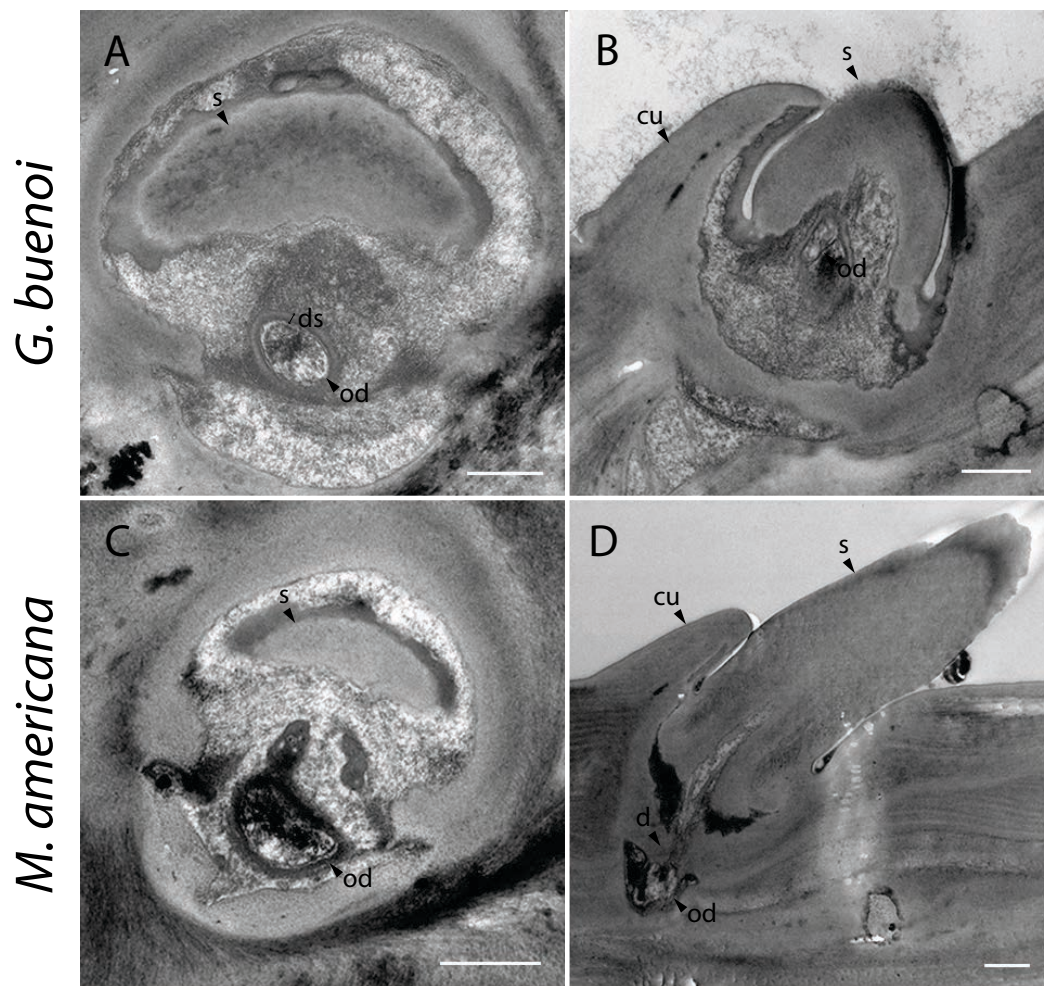


Figure S1. Ultrastructure of leg bristles in *G. buenoi* and *M. americana*. The TEM clichés show the presence of an outer dendritic segment underneath leg bristle in both *G. buenoi* (A, B) and *M. americana* (C, D). cu: cuticle, ds: dendritic sheath, oe: outer dendritic segment, s: shaft.

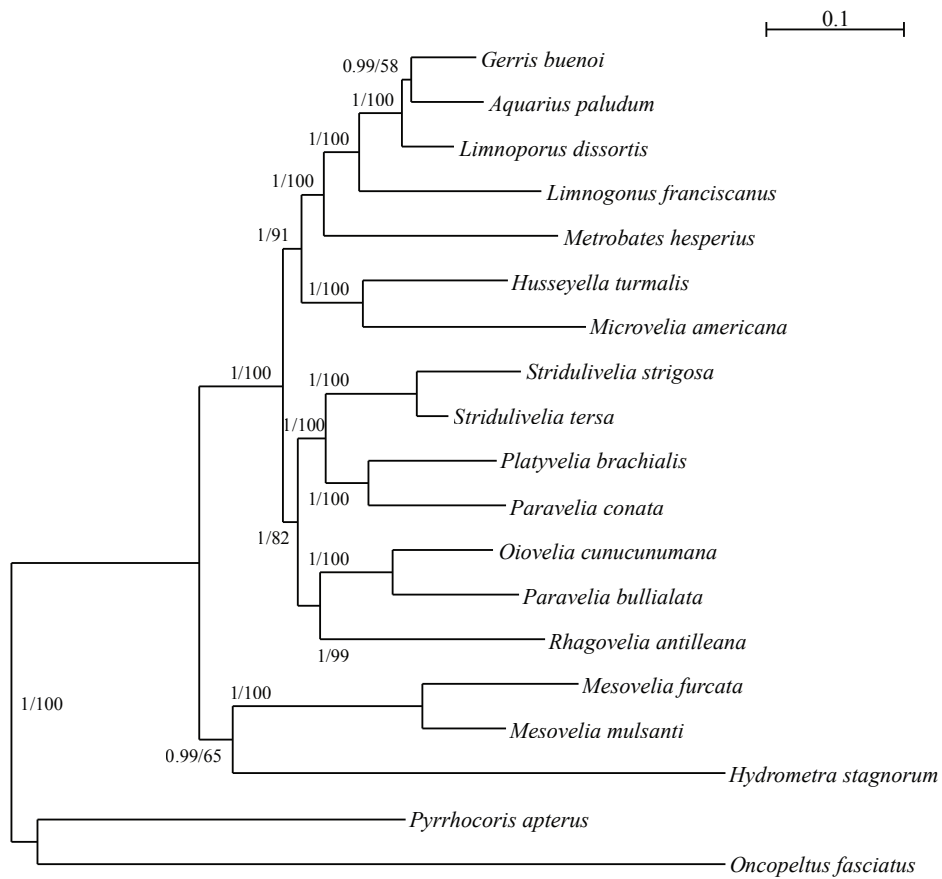


Figure S2. Phylograms of the Gerromorpha phylogeny built upon 14 markers. Support values obtained after 100 bootstrap replicates and Bayesian posterior probabilities are shown for all branches.

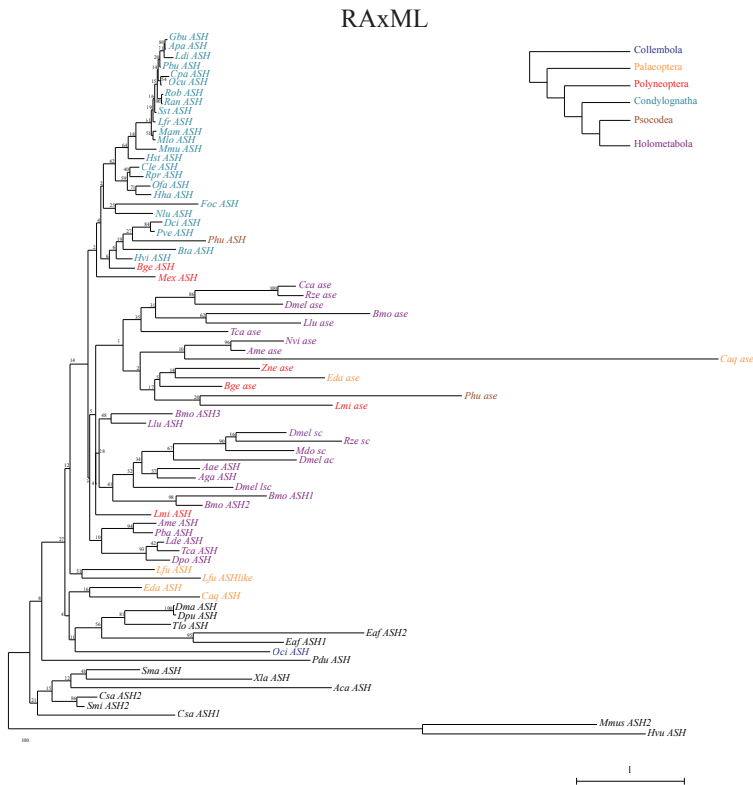


Figure S3. Phylograms of the 77-taxon analyses. RAxML Maximum-likelihood analyses and MrBayes Bayesian analyses were conducted under the LG+ Γ model and the GTR+ Γ model, respectively. Support values obtained after 100 bootstrap replicates and Bayesian posterior probabilities are shown for all branches. Scale bar indicates number of changes per site.



Figure S4. Variability in penetrance of *ASH* RNAi in *G. buenoi*. Top panel: wild-type leg L1; bottom panel: phenotypic variation in *dsASH* among five treated individuals.

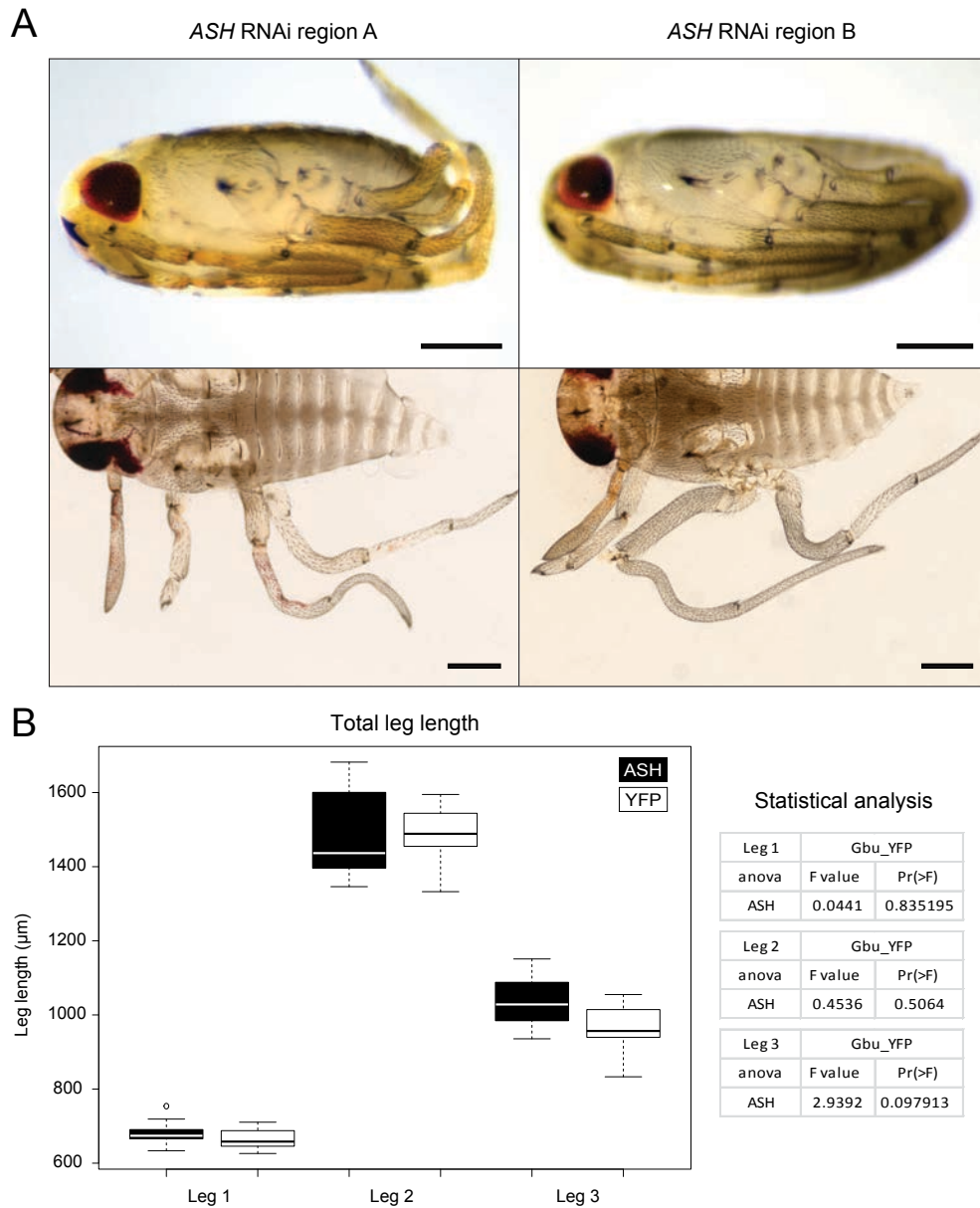


Figure S5. Phenotypic characterization of *ASH* RNAi individuals in *G. buenoi*. (A) The two non-overlapping subregions A and B targeted by RNAi show phenotypes similar to those obtained when the full *ASH* coding region is targeted. (B) The total leg length is not affected in *ASH* RNAi individuals (full construct).

Gene	Species	Accession	Gene	Species	Accession		
Antp	<i>Limnogonus franciscanus</i>	LT986725	cyt b	<i>Limnogonus franciscanus</i>	LT986731		
	<i>Metrobates hesperius</i>	LT986723		<i>Mesovelvia mulsanti</i>	LT986733		
	<i>Aquarius paludum</i>	LT989878		<i>Metrobates hesperius</i>	LT986732		
	<i>Cylindrostethus palmaris</i>	LT989880		DII	<i>Limnogonus franciscanus</i>	LT986710	
	<i>Gerris buenoi</i>	LT986682			<i>Metrobates hesperius</i>	LT986709	
	<i>Hydrometra stagnorum</i>	LT989889		GILT	<i>Limnogonus franciscanus</i>	LT986712	
	<i>Limnogonus franciscanus</i>	LT989890			<i>Metrobates hesperius</i>	LT986713	
	<i>Limnoporus dissortis</i>	LT989891			<i>Limnogonus franciscanus</i>	LT986741	
	ASH	<i>Mesovelvia mulsanti</i>		LT986683	ND1	<i>Mesovelvia mulsanti</i>	LT986743
		<i>Microvelia americana</i>		LT986679		<i>Metrobates hesperius</i>	LT986742
<i>Microvelia longipes</i>		LT989879	Scr	<i>Limnogonus franciscanus</i>	LT986706		
<i>Oiovelia cunucunumana</i>		LT989892		<i>Metrobates hesperius</i>	LT986707		
<i>Paravelia bullialata</i>		LT989881	Ubx	<i>Limnogonus franciscanus</i>	LT986704		
<i>Rhagovelia antilleana</i>		LT989893		<i>Metrobates hesperius</i>	LT986705		
<i>Rhagovelia obesa</i>		LT989882	12S rRNA	<i>Limnogonus franciscanus</i>	LT990042		
<i>Stridulivelia strigosa</i>		LT989894		<i>Mesovelvia mulsanti</i>	LT990044		
<i>Limnogonus franciscanus</i>		LT986734		<i>Metrobates hesperius</i>	LT990043		
COI		<i>Metrobates hesperius</i>	LT986735	<i>Limnogonus franciscanus</i>	LT990048		
	<i>Limnogonus franciscanus</i>	LT986739	16S rRNA	<i>Mesovelvia mulsanti</i>	LT990041		
COII	<i>Metrobates hesperius</i>	LT986740		<i>Metrobates hesperius</i>	LT990040		
	<i>Limnogonus franciscanus</i>	LT989872	18S rRNA	<i>Limnogonus franciscanus</i>	LT989869		
<i>Mesovelvia mulsanti</i>	LT989874	<i>Metrobates hesperius</i>		LT989870			
COIII	<i>Metrobates hesperius</i>	LT989873	28S rRNA	<i>Limnogonus franciscanus</i>	LT989871		
	<i>Oncopeltus fasciatus</i>	LT989875					

Table S1. Accession numbers of genes markers selected for phylogenetic analyses. *: 5,000 Insect Genome Project (i5k).

species	primer name	sequence 5'-3'
<i>Gerris buenoi</i>	Gbu-ASH-Fwd	AGCCAGAACATGGTGACTCAAAGG
	Gbu-ASH-Rev	TGCTGCCACCATGAGATAACGTCGA
	Gbu-ASH-Fwd1	CGAAGTTCGGCGTCCAAGAAATTG
	Gbu-ASH-Rev1	TGCCGTCGAAGCTCCTAAAG
	Gbu-ASH-Fwd-T7	TAATACGACTCACTATAGGGAGACCACAGCCAGAACATGGTGACTCAAAGG
	Gbu-ASH-Rev-T7	TAATACGACTCACTATAGGGAGACCCTGCTGCCACCATGAGATAACGTCGA
	Gbu-ASH-Fwd1-T7	TAATACGACTCACTATAGGGAGACCACCGAAGTTCGGCGTCCAAGAAATTG
	Gbu-ASH-Rev1-T7	TAATACGACTCACTATAGGGAGACCCTGCCGTCGAAGCTCCTAAAG
	Mmu-ASH-Fwd	CGACGCGATGACGTCGGTTGTAGG
	Mmu-ASH-Rev	TGCTGCCACCACGATATAACATCAA
<i>Mesovelia mulsanti</i>	Mmu-ASH-Fwd-T7	TAATACGACTCACTATAGGGAGACCCTGCTGCCACCACGATATAACATCAA
	Mmu-ASH-Rev-T7	TAATACGACTCACTATAGGGAGACCACCGACGCGATGACGTCGGTTGTAGG
	Mam-ASH-Fwd	GGTCATAAAATGGTGACACAAAGA
<i>Microvelia americana</i>	Mam-ASH-Rev	TGTTGCCACCATGATATAACATCTA
	Mam-ASH-Fwd-T7	TAATACGACTCACTATAGGGAGACCACGGTCATAAAATGGTGACACAAAGA
	Mam-ASH-Rev-T7	TAATACGACTCACTATAGGGAGACCCTGTTGCCACCATGATATAACATCTA

Table S2. List of PCR primers used in this study.

taxonomic group	Species name	abbreviation	ASH ortholog(s)	asense ortholog	
Collembola	<i>Orchesella cincta</i>	<i>Oci</i>	1	?	
	<i>Catajapyx aquilonaris</i>	<i>Caq</i>	1	1	
Palaeoptera	<i>Ephemera danica</i>	<i>Eda</i>	1	1	
	<i>Ladona fulva</i>	<i>Lfu</i>	1	?	
	<i>Blattella germanica</i>	<i>Bgi</i>	1	1	
Polyneoptera	<i>Locusta migratoria</i>	<i>Lmi</i>	?	2	
	<i>Medauroidea extradentata</i>	<i>Mex</i>	1	?	
	<i>Zootermopsis nevadensis</i>	<i>Zne</i>	?	1	
	<i>Bemisia tabaci</i>	<i>Bta</i>	1	0	
	<i>Cimex lectularius</i>	<i>Cle</i>	1	0	
	<i>Frankliniella occidentalis</i>	<i>Foc</i>	1	0	
	<i>Halyomorpha halys</i>	<i>Hha</i>	1	0	
	<i>Nilaparvata lugens</i>	<i>Nlu</i>	1	0	
	<i>Oncopeltus fasciatus</i>	<i>Ofa</i>	1	0	
	<i>Rhodnius prolixus</i>	<i>Rpr</i>	1	0	
	<i>Aquarius paladum</i>	<i>Apa</i>	1	0	
	<i>Cylindrostethus palmaris</i>	<i>Cpa</i>	1	0	
	<i>Gerris buenoi</i>	<i>Gbu</i>	1	0	
	Condylognatha	<i>Hydrometra stagnorum</i>	<i>Hst</i>	1	0
<i>Limnogonus franciscanus</i>		<i>Lfr</i>	1	0	
<i>Limnopus dissortis</i>		<i>Ldi</i>	1	0	
<i>Mesovelia mulsanti</i>		<i>Mmu</i>	1	0	
<i>Microvelia americana</i>		<i>Mam</i>	1	0	
<i>Microvelia longipes</i>		<i>Mlo</i>	1	0	
<i>Oiovelia cunucunumana</i>		<i>Ocu</i>	1	0	
<i>Paravelia bullialata</i>		<i>Pbu</i>	1	0	
<i>Rhagovelia antilleana</i>		<i>Ran</i>	1	0	
<i>Rhagovelia obesa</i>		<i>Rob</i>	1	0	
<i>Stridulivelia strigosa</i>		<i>Sst</i>	1	0	
Psocodea		<i>Pediculus humanus</i>	<i>Phu</i>	1	1
		<i>Aedes aegypti</i>	<i>Aae</i>	2	1
		<i>Anopheles gambiae</i>	<i>Aga</i>	1	1
	<i>Apis mellifera</i>	<i>Ame</i>	1	1	
	<i>Bombyx mori</i>	<i>Bmo</i>	3	1	
	<i>Ceratitis capitata</i>	<i>Cca</i>	2	1	
	<i>Dendroctonus ponderosae</i>	<i>Dpo</i>	1	1	
	<i>Drosophila melanogaster</i>	<i>Dme</i>	3	1	
Holometabola	<i>Leptinotarsa decemlineata</i>	<i>Lde</i>	1	1	
	<i>Limnephilus lunatus</i>	<i>Llu</i>	?	1	
	<i>Musca domestica</i>	<i>Mdo</i>	3	1	
	<i>Nasonia vitripennis</i>	<i>Nvi</i>	1	1	
	<i>Pogonomyrmex barbatus</i>	<i>Pba</i>	1	1	
	<i>Rhagoletis zephyria</i>	<i>Rze</i>	3	1	
	<i>Tribolium castaneum</i>	<i>Tca</i>	1	1	

Table S3. Presence/absence of *ASH* and *asense* orthologs in the species investigated in this study.