Morphological novelty emerges from pre-existing phenotypic plasticity

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1   Supplementary Information for

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9   Table S1. Trophic traits associated with carnivore-omnivore polyphenism and their functions.

Trait (abbreviation)	Function	Reference
Denticle rows (DR)	rasps food off surfaces; more DR favored for smaller prey	[55,56]
Keratinized mouthparts (MP)	grasps prey; larger, more serrated MP favored for larger prey	[55,56]
Orbitohyoideus muscle (OH)	opens mouth; larger OH favored for larger prey	[56-58]
Gut coils (GC)	used to digest food; more GC favored for more plants or bacteria in diet	[12,29]

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11   Table S2. Results from morphological experiment using *Sc. holbrookii* reared on alternative

12   diets. Values under 'Shrimp' and 'Detritus' indicate the mean ($\pm$ s.e.m.) of each trait on the

13   respective diet. Bolded rows indicate that there was a significant difference between diets

14   according to a likelihood ratio test.

Variable	Shrimp	Detritus	χ^2	*P* value
SVL (mm)	**8.11 (0.16)**	**8.96 (0.11)**	**32.79**	**1.60×10^{-8}**
Gosner Stage	31.97 (0.35)	33.03 (0.21)	1.05	0.0810
GC	**5.50 (0.07)**	**6.53 (0.13)**	**5.33**	**0.0082**
DR	**13.30 (0.31)**	**16.68 (0.35)**	**22.96**	**1.30×10^{-6}**
MP	**1.23 (0.06)**	**1.78 (0.08)**	**6.05**	**0.0065**
LMP	**2.05 (0.13)**	**2.62 (0.13)**	**4.14**	**0.0131**
Size-corrected OH	-0.002 (0.01)	0.002 (0.01)	0.05	0.1911

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16 Table S3. Summary statistics from an ANOVA (with type III sum of squares) on various trophic

17 traits in *Sc. holbrookii* reared on alternative diets. Every trait had a significant difference between

18 families and/or a significant family*diet interaction (bolded values). Diet dependence of trait

19 expression (i.e., plasticity) corroborates our findings in Table S2.

Snout-vent length					Denticle rows				
Variable	Sum of Squares	DF	*F*	*P* value	Variable	Sum of Squares	DF	*F*	*P* value
Family	**22.95**	**1**	**41.2**	**0.000**	**Family**	**30.80**	**1**	**4.98**	**0.028**
Diet	5.27	1	9.47	0.003	Diet	156.80	1	25.35	0.000
Family*Diet	1.87	1	3.36	0.069	Family*Diet	0.70	1	0.11	0.742
Gosner developmental stage					Mouthpart score				
Variable	Sum of Squares	DF	*F*	*P* value	Variable	Sum of Squares	DF	*F*	*P* value
Family	**96**	**1**	**68.54**	**0.000**	**Family**	**3.75**	**1**	**14.08**	**0.000**
Diet	0	1	0	1.000	Diet	11.27	1	42.30	0.000
Family*Diet	**34**	**1**	**24.3**	**0.000**	**Family*Diet**	**3.01**	**1**	**11.29**	**0.001**
Size-corrected OH					Gut length				
Variable	Sum of Squares	DF	*F*	*P* value	Variable	Sum of Squares	DF	*F*	*P* value
Family	0.02	11	3.69	0.057	**Family**	**29.40**	**1**	**76.78**	**0.000**
Diet	0.01	1	1.95	0.165	Diet	1.67	1	4.34	0.039
Family*Diet	**0.03**	**1**	**4.87**	**0.029**	**Family*Diet**	**14.70**	**1**	**38.29**	**0.000**

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22   Table S4. The genes whose expression was examined in this study. 'Bias' indicates the biased

23   expression pattern described by [30].

Gene	Symbol	Bias	Functional Group
basic transcription factor 3[ap]	*btf3*	Carnivore	Gene regulation
T-box transcription factor TBX15-like[ap]	*tbx15*	Carnivore	Gene regulation
peptidase M20 domain containing[ap,wc]	*pm20d2*	Carnivore	Metabolism
collagen, type II, alpha 1[ap]	*col2a1*	Carnivore	Structural
pancreatic triacylglycerol lipase-like[ap,wc]	*pnlip*	Omnivore	Metabolism
amylase, alpha 2A (pancreatic)[ap,wc]	*amy2a*	Omnivore	Metabolism
peptidoglycan recognition protein 1[ap]	*pglyrp1*	Omnivore	Immunity
transferrin[ap]	*tf*	Omnivore	Immunity
beta (β)-actin[ap]	*actb*	Control	Structural
glyceraldehyde-3-phosphate dehydrogenase[wc]	*gapdh*	Control	Metabolism

24   ap = genes used in the ancestral plasticity experiment; wc = genes used the wild-caught *Spea* experiment

25   Table S5. Summary statistics from analysis of diet-dependent gene expression plasticity in *Sc. holbrookii*. Five genes had diet-

26   dependent expression plasticity 'Bias' refers to morph-biased expression in *Spea*. Values below 'RQ$_{Shrimp}$' and 'RQ$_{Detritus}$' are the

27   mean expression levels (± s.e.m.) of tadpoles reared on each diet. logRatio refers to our measure of plasticity (see Methods). We called

28   the expression of a gene as plastic (Y) if its RQ between diets was significantly different (*P* value < 0.05) and if its logRatio (± 99%

29   CI) did not overlap with 0, otherwise it was called non-plastic (N).

Gene	Bias	RQ$_{Shrimp}$ (± s.e.m.)	RQ$_{Detritus}$ (± s.e.m.)	χ^2	*P* value	logRatio	Lower 99% CI	Upper 99% CI	Plasticity
btf3	Carnivore	0.019 (0.005)	0.354 (0.124)	13.6013	0.0004	-2.949	-3.991	-1.951	Y
col2a1	Carnivore	0.051 (0.015)	0.537 (0.171)	10.2575	0.0025	-2.346	-3.402	-1.309	Y
tbx15	Carnivore	0.043 (0.012)	0.746 (0.337)	6.0612	0.0244	-2.848	-4.227	-1.607	Y
pm20d2	Carnivore	0.008 (0.002)	0.389 (0.140)	17.3107	0.0003	-3.843	-5.011	-2.736	Y
tf	Omnivore	0.037 (0.011)	0.372 (0.105)	15.9796	0.0003	-2.316	-3.108	-1.505	Y
pnlip	Omnivore	0.314 (0.222)	0.244 (0.103)	0.4017	0.4884	0.251	-1.959	3.014	N
pglyrp1	Omnivore	0.349 (0.202)	0.320 (0.120)	0.1785	0.5497	0.088	-1.960	2.478	N
amy2a	Omnivore	0.281 (0.157)	0.254 (0.114)	0.048	0.6000	0.099	-2.330	2.682	N
actb		---	---	---	---	-2.619	-5.230	0.154	N

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31   Table S6. Results from morphological experiment using *Sp. multiplicata* reared on alternative

32   diets. Values under 'Shrimp' and 'Detritus' indicate the mean (± s.e.m.) of each trait on the

33   respective diet. Bolded rows indicate that there was a significant difference between diets

34   according to a likelihood ratio test.

Variable	Shrimp	Detritus	χ^2	*P* value
SVL (mm)	14.04 (0.11)	14.08 (0.07)	0.046	0.398
Gosner Stage	38.62 (0.16)	38.65 (0.10)	0.001	0.427
Mass	322.91 (7.45)	324.68 (5.03)	0.006	0.437
Size-corrected OH	**0.01 (0.009)**	**-0.01 (0.006)**	**5.63**	**0.016**
MP	**1.40 (0.05)**	**1.08 (0.02)**	**4.98**	**0.020**
DR	**12.15 (0.27)**	**15.41 (0.26)**	**56.87**	**7.21×10^{-14}**
GC	**7.61 (0.19)**	**10.76 (0.16)**	**81.44**	**6.82×10^{-16}**

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37   Table S7. Results from Fisher's exact test on the number of individuals with ('Yes') and without

38   ('No') detectable levels of gene product for each gene.

	pm20d2		*pnlip*		*amy2a*	
	Yes	No	Yes	No	Yes	No
Carnivores	11	1	0	12	1	11
Omnivores	2	8	8	2	9	1
P value	0.0015		0.0001		0.0003	

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41 Table S8. Results from our likelihood ratio test to evaluate if morph influences the expression of

42 each gene in wild-caught *Sp. multiplicata*.

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amy2a	Model	AIC value	χ^2	Df	*P* value
	Morph	101.66	37.263	1.00	<0.001
	Null	136.92	---	---	---
pm20d2	Model	AIC value	χ^2	Df	*P* value
	Morph	100.45	4.909	1.00	0.0267
	Null	103.36	---	---	---
pnlip	Model	AIC value	χ^2	Df	*P* value
	Morph	96.898	40.313	1.00	<0.001
	Null	135.212	---	---	---

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45   Table S9. Summary statistics from our comparison of the palate spike among wild-caught *Spea*

46   and *Scaphiopus*. Overall, there was a significant effect of lineage on extent of spike formation

47   (top), but not all groups significantly differed from each other (bottom). On the bottom, effect

48   sizes (*Z* scores) are above the diagonal and *P* values are below the diagonal.

Model	AIC value	χ^2	Df	*P* value
Null	710.73	---	---	---
Full	657.51	41.219	3	< 0.0001
	Sb-S	Sc	Sm-C	Sm-O
Sb-S	---	14.77	11.3	15.69
Sc	0.0001	---	3.49	-0.67
Sm-C	0.0001	0.0034	---	3.29
Sm-O	0.0001	0.6969	0.01	---

Sb-S = Sympatric *Sp. bombifrons*; Sc = *Sc. couchii*; Sm-C = *Sp. multiplicata* carnivores;
Sm-O = *Sp. multiplicata* omnivores

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50   Table S10. Estimated family variance (V_{AF}) and total estimated variance (V_{total}) for fitness and

51   morphological parameters. The 95% confidence intervals (*CI*) for broad-sense heritabilities (H^2)

52   were calculated using 500 bootstrap replicates of the original data. If H^2 confidence intervals of

53   alternative diets were not overlapping for a particular trait, the H^2 of that trait was considered to

54   be significantly diet-dependent. Greater H^2 on a shrimp diet is indicate of the release of cryptic

55   genetic variation when fed that diet.

Species	Trait	Diet	V_{AF}	V_{total}	H^2	*CI*	H^2 diet dependent?
Sc. couchii	log(SVL)	Detritus	0.001	0.025	0.05	(-0.12 ,0.09)	Y
		Shrimp	0.005	0.022	0.50	(0.34 ,0.65)	
	Gosner Stage	Detritus	0.000	1.888	0.00	(-0.13, 0.00)	Y
		Shrimp	0.486	2.857	0.33	(0.20, 0.49)	
	Gut length	Detritus	0.003	0.046	0.12	(-0.04, 0.25)	Y
		Shrimp	0.006	0.043	0.28	(0.13, 0.40)	
	log(OH)	Detritus	0.001	0.009	0.19	(0.02, 0.33)	N
		Shrimp	0.002	0.018	0.19	(0.03, 0.33)	
Sp. multiplicata	log(SVL)	Detritus	0.001	0.004	0.46	(-0.05, 0.57)	N
		Shrimp	0.002	0.012	0.26	(-0.28, 0.48)	
	Gosner stage	Detritus	0.113	1.468	0.14	(-0.62, 0.36)	N
		Shrimp	0.931	2.976	0.48	(-0.05, 0.71)	
	Gut length	Detritus	0.005	4.124	0.00	(-0.50, 0.03)	N
		Shrimp	1.208	4.031	0.46	(0.03, 0.56)	
	log(OH)	Detritus	0.001	0.008	0.27	(-0.21, 0.36)	N
		Shrimp	0.003	0.028	0.19	(-0.38, 0.36)	
	Denticle rows	Detritus	0.563	9.772	0.11	(-0.34, 0.17)	N
		Shrimp	2.559	8.268	0.47	(0.01, 0.59)	
	Mouthpart score	Detritus	0.000	0.078	0.00	(-0.54, 0.11)	N
		Shrimp	0.000	0.343	0.00	(-0.46, 0.11)	

56   *Sc. couchii* data are from [26]

57   Table S11. Concentration and purity values from RNA extractions of *Sc. holbrookii* (left) and

58   wild-caught *Sp. multiplicata* (right). For *Sc. holbrookii*, the sample ID denotes family-individual-

59   diet. For *Sp. multiplicata*, the sample ID denotes population-morph-individual.

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Sample	Mean concentration (ng/µL)	260/280	260/230	Sample	Mean concentration (ng/µL)	260/280	260/230
1.1s	881.5	2.11	2.21	SC1	724.8	1.96	2.00
1.2s	1171.4	2.06	1.82	SC2	652.9	1.99	2.00
1.3s	1303.9	2.07	1.96	SC3	766.9	1.98	2.00
1.4s	1339.5	2.07	2.06	SC4	1168.8	2.00	1.94
1.5s	966.5	2.07	2.04	SC5	871	2.05	1.99
1.6s	1147.8	2.09	2.08	SO1	532.1	1.58	1.41
1.7s	1073.9	2.06	1.96	SO2	399.9	1.68	1.55
1.1d	501	2.05	1.24	SO3	627.6	1.65	1.54
1.2d	434.4	2.06	1.83	SO4	468.4	1.71	1.51
1.3d	315.2	2.07	1.44	SO5	486.1	1.66	1.48
1.4d	414.9	2.10	2.01	PC1	742.9	1.91	1.75
1.5d	377.03	2.06	1.67	PC2	943.5	1.98	1.85
1.6d	808	2.07	2.05	PC3	1149.2	2.01	2.00
1.7d	499.9	2.06	1.64	PC4	1148.9	1.99	1.87
2.1s	1177.8	2.08	2.01	PC5	729.3	2.10	2.12
2.2s	918.6	2.06	2.07	PO1	555.6	1.84	1.54
2.3s	1091.6	2.08	2.11	PO2	827.8	1.89	1.50
2.4s	1217.9	2.06	2.04	PO3	671.4	1.80	1.73
2.5s	968.1	2.08	2.07	PO4	683.5	1.84	1.68
2.6s	989	2.06	2.04	PO5	946.4	2.02	2.05
2.7s	1227	2.06	2.00	BC1	894.8	2.00	2.03
2.1d	746.1	2.07	2.12	BC2	715.6	1.98	2.01
2.2d	791.8	2.06	1.88	BO1	907.6	1.81	1.57
2.3d	899.2	2.08	1.62	BO2	504.1	1.70	1.52
2.4d	949.2	2.08	2.06				
2.5d	370.9	2.04	1.49				
2.6d	849.7	2.08	1.94				
2.7d	619.2	2.07	2.10				

61   s = shrimp diet; d = detritus diet; S =Silver Creek; P = Peach Orchard Rd.; B = Observatory; C = carnivore; O =
62   omnivore

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64   Table S12. Reaction components for qPCR.
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Component	Volume (µL)	Final Concentration
iTaq^{TM} universal SYBR® Green supermix (2x)	10	1X
Forward and reverse primers	1 (each)	500 nm
cDNA template	2	300 pg
H$_2$O	6	---

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68   Table S13. Reaction conditions for qPCR.

| Setting | Polymerase Activation and DNA Denaturation at 95°C, sec | Amplification | | Cycles | Melt Curve Analysis |
		Denaturation at 95°C, sec	Annealing/Extension and Plate read at 60°C, sec		
Standard	30	15	60	40	Instrument default setting

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71 Table S14. Genbank accessions and qPCR primer sequences for each gene.

Gene	Accession	Left Primer Sequence (5'-3')	Right Primer Sequence (5'-3')
btf3^h	JN639644	ACAGCCACTGCTGATGACAA	CAGCCAAGGAAGCCTGTACT
tbx15^h	JN639816	GACTCTGGAAGGAACCGGAC	CTGCCACCTTGCTGTTTCTG
pm20d2^h	JN639773	GGTGCATTTCAGGGGATGGA	TGGATATGCAGCAGCATGAGA
pm20d2^m	JN639770	AATCTGCAGCTGAGGCAACT	AACCGCAAAGCTCACATTTC
col2a1^h	JN639671	TGGTGGACAAGCAAGAGCAA	TGGGATGCATCAGTGGACAG
pnlip^h	JN639778	TGGACTTCTTCCCCAATGGC	GGTGTTGGCGTAGTGATGC
pnlip^m	JN639775	TCCCCAGTTTAGGTTTTGGA	AGAACAGGGGTAGCTGACGA
amy2a^h	JN639636	TGGACGTGGAAACAAAGGGT	GAGTGCCTTCTTTCTGCCCA
amy2a^m	JN639633.1	TGGGTCAACCAAATCTGTCA	GAGTGGCGTCCATATTCCAT
tf^h	JN639821	GACCATGCCAAAGCAGGCAAA	TTAGCAATCAGGCCGACAGG
actb^h	NM_213719.1	TGCTCTGGACTTTGAGCAGG	GCTGGAAGAGTGCTTCTGGA
gapdh^m	BC075438.1	CTGTGAAAGCGTGGACAGTG	GTTGGTGTGAACCACGAGAA

72   h = primers for *Sc. holbrookii*; m = primers for *Sp. multiplicata*
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74   Table S15. Results for comparison of control gene expression between diets (for *Sc. holbrookii*)

75   and morphs (for *Sp. multiplicata*). A Wilcoxon test was performed to assess if expression varied

76   by treatment. Neither *actb* nor *gapdh* significantly differed between treatments.

| Gene	Species	Z	*P* value
actb	*Sc. holbrookii*	1.63114	0.1029
gapdh	*Sp. multiplicata*	1.94517	0.0518

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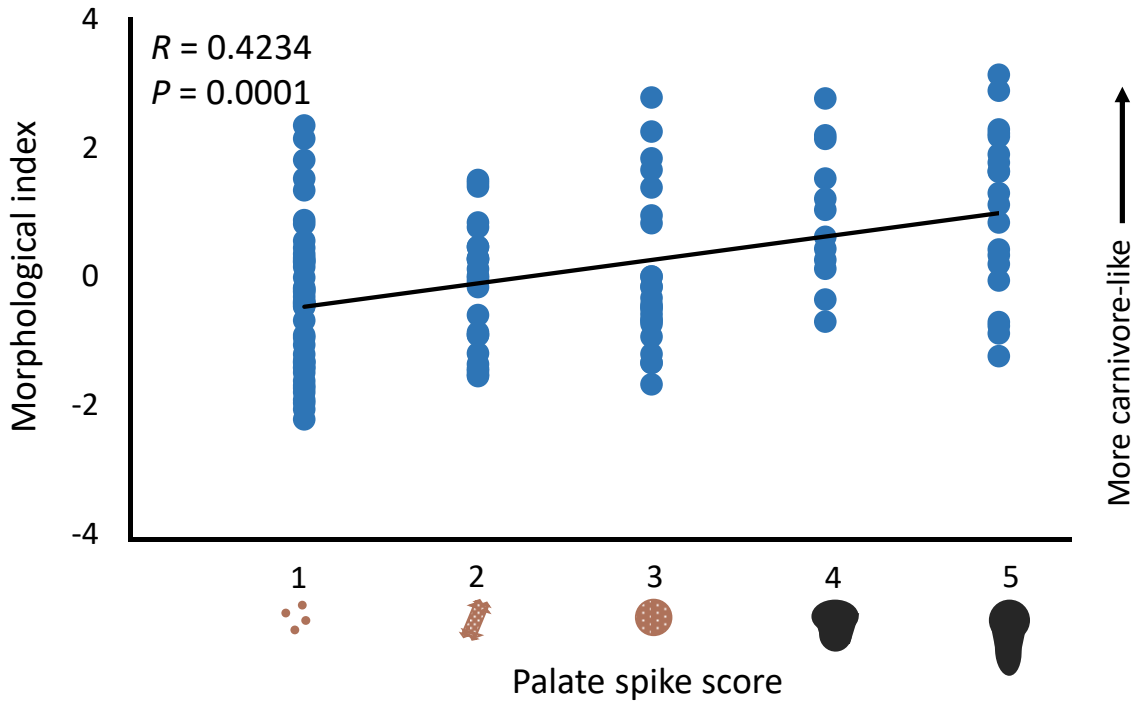
Fig. S1. Relationship (Pearson correlation) between palate spike score and morphological index in wild-caught *Sp. multiplicata* and *Sp. bombifrons* carnivores (n = 127). A more extensive palate spike was significantly positively associated with more carnivore-like tadpoles. Regression line is for visualization purposes only.

84
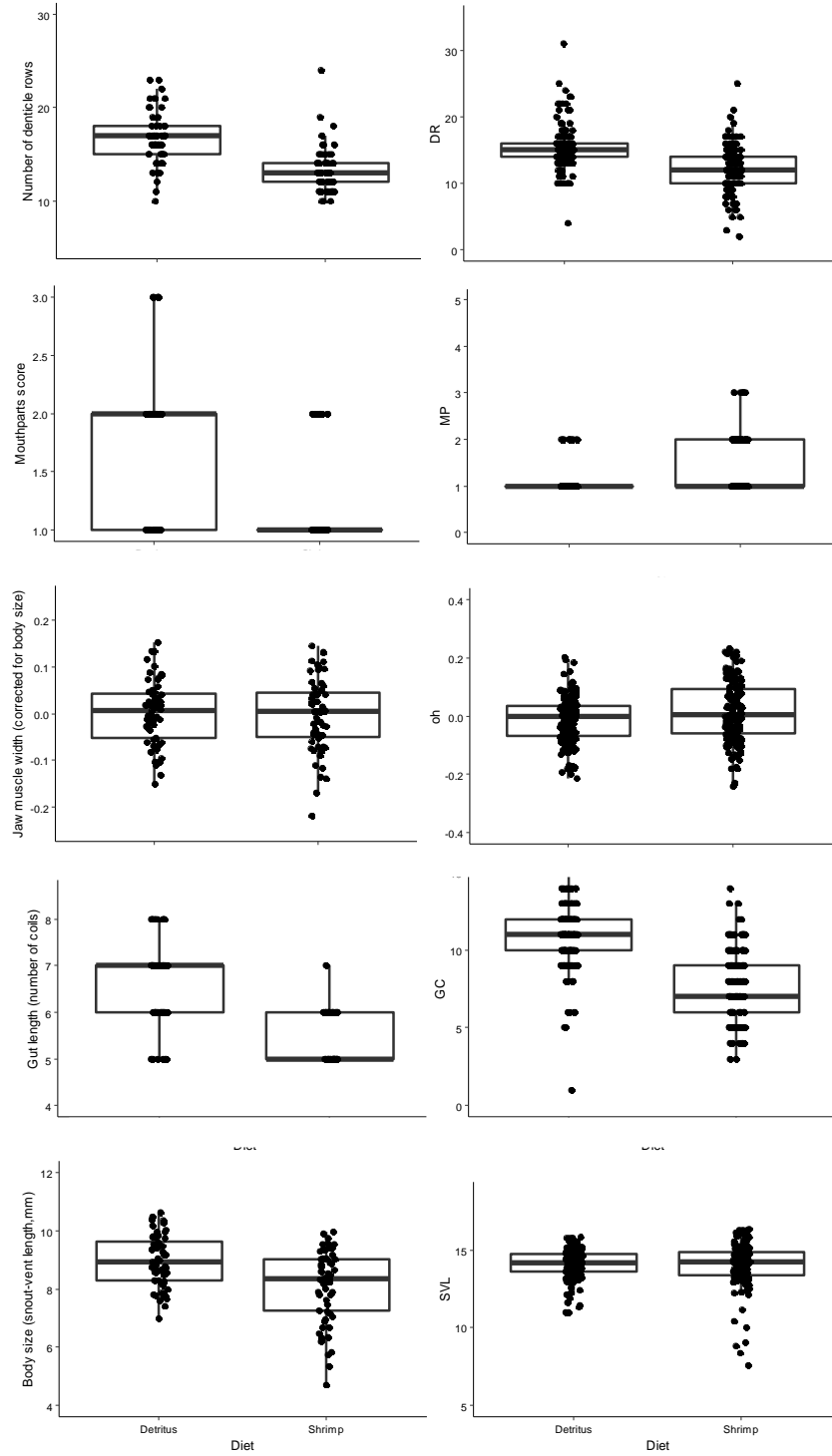
Fig. S2. Diet-induced morphological plasticity in *Sc. holbrookii* reared on detritus or shrimp (left

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panels) and *Sp. multiplicata* reared on detritus or shrimp (right panels). Points: values for

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individual tadpoles (for mean ± s.e.m. plots, see Fig. 2).

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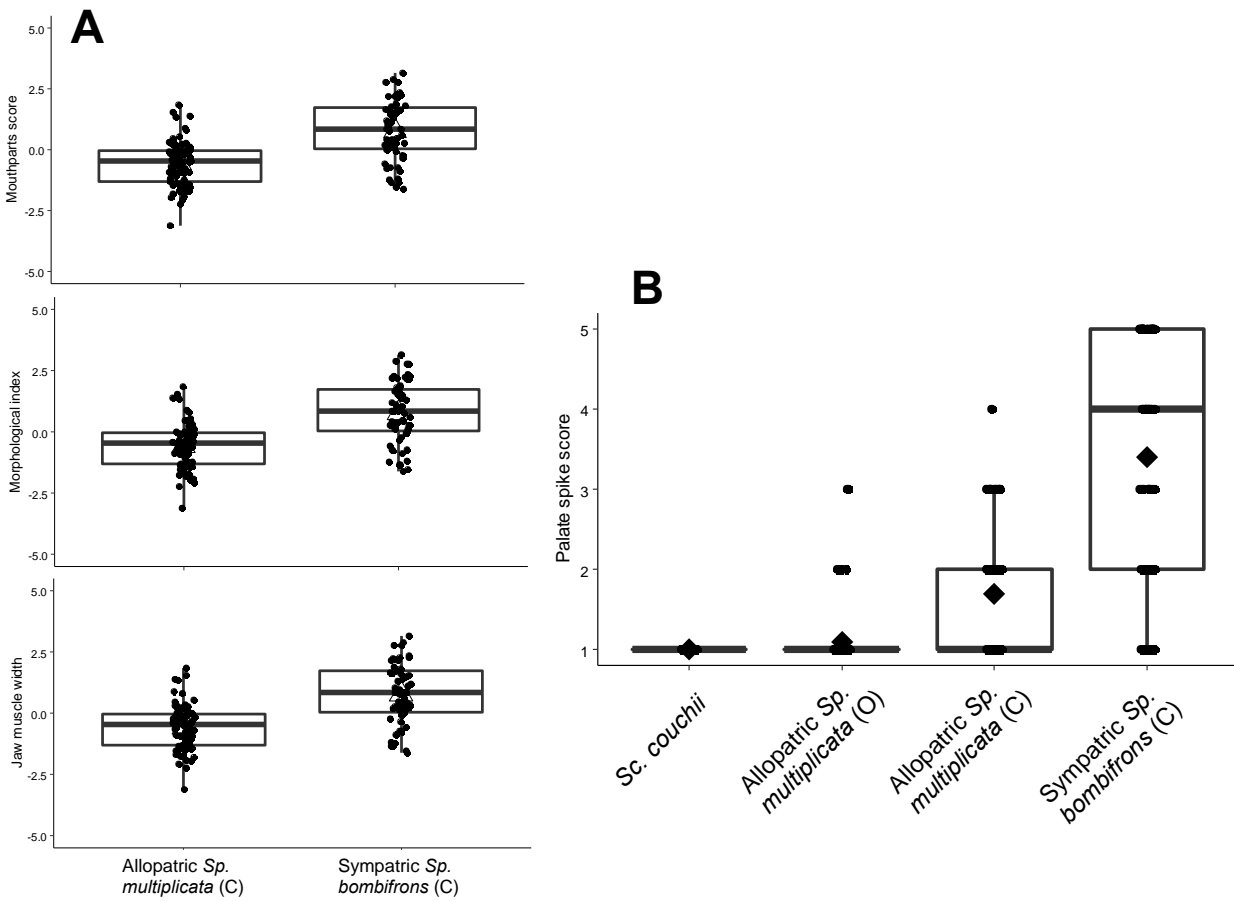
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90   Fig. S3. **A,** Differences in magnitude of expression of trophic traits of carnivores in a lineage in

91   which carnivores are produced at low-intermediate frequencies as part of a polyphenism

92   (allopatric *Sp. multiplicata*) versus a lineage in which carnivores are produced at high

93   frequencies (sympatric *Sp. bombifrons*). **B,** Differences in magnitude of expression of a novel

94   palate spike in lineages/morphs that differ in likelihood of expressing carnivore features.

95   Diamonds: mean values. In all panels, points are values for individual tadpoles (for mean ±

96   s.e.m. plots, see Fig. 5).