

Integrative Taxonomy of Two Peruvian Strains of *Brachionus plicatilis* Complex with Potential in Aquaculture

Pedro Pablo Alonso Sánchez-Dávila^{1*}, Giovanna Sotil², Araceli Adabache-Ortiz³, Deivis Cueva² and Marcelo Silva-Briano³

¹ Instituto del Mar del Perú - IMARPE, DGIA, AFIA, BGOA, Esquina Gamarra y Gral. Valle s/n, Callao, Peru.
² Instituto del Mar del Perú - IMARPE, DGIA, Laboratorio de Genética Molecular, Esquina Gamarra y Gral. Valle s/n, Callao, Peru.
³ Universidad Autónoma de Aguascalientes. Centro de Ciencias Básicas. Edificio 202. Laboratorio No. 1, de Ecología. Departamento de Biología. México.
* Correspondence: psanchez@imarpe.gob.pe

Supplementary Materials

Table S1. List of GenBank accession numbers for COI and ITS1 sequences obtained in this study, from organisms of strains isolated from Peru.

Collection site	Strain code	Number of organisms	Sex	GenBank accession number	
				COI	ITS1
Santo Domingo, Ica	IMP-BG-Z018-SD	1	Female	MK534737	MZ569584
		1	Female	MZ662909	MZ695037
		1	Male	MZ662910	-
		1	Female	-	MZ695038
		2	Females	MZ662911	MZ695039
		2	Females	MZ662912	MZ695040
		3	Females	MZ662913	MZ695041
		3	Females	MZ662914	MZ695042
		10	Females	MZ662915	MZ695043
		10	Females	MZ662916	MZ695044
Ventanilla, Callao	IMP-BG-Z010-VL	1	Female	MK534738	MZ569507
		1	Female	MZ662901	MZ695046
		1	Male	MZ662902	-
		1	Female	-	MZ695047
		2	Females	MZ662903	MZ695048
		2	Females	MZ662904	MZ695049
		3	Females	MZ662905	MZ695050
		3	Females	MZ662906	MZ695051
		10	Females	MZ662907	MZ695052
		10	Females	MZ662908	MZ695053

Table S2. Measures from parthenogenetic females of the strains IMP-BG Z010-VL and Z018-SD.

	Lorica length (a)		Distance between lateral spines (b)		Lorica width (c)		Distance between central spines (d)		Dorsal sinus depth (e)		Eggs length		Eggs width	
	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD
	n=120	n=125	n=120	n=125	n=120	n=125	n=48	n=125	n=48	n=125	n=48	n=75	n=48	n=75
Mean	248.65	186.21	131.53	95.88	190.31	144.97	26.18	19.45	23.86	19.39	146.61	100.07	94.88	86.74
Minimum	194.08	145.30	80.49	63.89	130.54	96.28	13.06	10.24	15.02	13.22	102.53	81.57	61.13	61.69
Maximum	295.16	226.87	156.78	127.78	238.68	189.49	37.91	27.39	32.19	26.80	175.59	121.72	145.67	98.62
Standard Deviation	16.18	17.41	12.03	16.48	21.04	20.48	5.39	3.08	3.34	2.99	18.04	7.47	21.74	8.20

Table S3. Measures from parthenogenetic females of the strains IMP-BG Z010-VL and Z018-SD used for statistical comparison (n=60).

	Lorica length		Distance between lateral spines		Lorica width		Distance between central spines		Dorsal sinus depth		Distance between central and medial spines		Medial spine length		Head aperture		Lateral spine length	
	(a)		(b)		(c)		(d)		(e)		(f)		(g)		(h)		(i)	
	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD	VL	SD
Mean	251.1	193.3	124.3	88.13	184.3	141.7	24.0	16.0	19.6	20.1	27.2	20.9	11.8	10.2	12.7	12.2	139.5	96.23
	7	6	6		6	8	3	3	9	0	7	1	4	0	0	6	9	
Minimum	235.0	180.4	105.1	72.76	157.3	101.7	18.8	10.2	14.6	13.6	21.3	13.9	9.28	6.05	8.31	7.09	121.0	84.03
	3	0	9		3	3	5	4	9	5	0	5					2	
Maximum	273.7	241.6	140.5	109.8	211.1	184.9	31.1	21.2	28.6	25.8	37.4	25.9	14.7	14.4	16.4	16.4	163.4	118.5
	5	3	2	2	2	8	0	3	8	1	8	6	9	7	6	1	2	5
Standard Deviation	9.92	10.62	7.56	7.49	10.96	12.92	2.71	2.32	3.23	2.53	3.31	2.40	1.46	1.76	1.85	1.90	9.17	8.00

Table S4. Measures from parthenogenetic females of the reference strains L (*B. plicatilis* s.s.) and SS (*B. rotundiformis*) used for statistical comparison (n=60).

	Lorica length (a)		Distance between lateral spines (b)		Lorica width (c)		Distance between central spines (d)		Dorsal sinus depth (e)		Distance between central and medial spines (f)		Medial spine length (g)		Head aperture (h)		Lateral spine length (i)	
	L	SS	L	SS	L	SS	L	SS	L	SS	L	SS	L	SS	L	SS	L	SS
Mean	263.1	147.8	115.4	82.49	174.6	116.8	27.8	16.5	26.6	18.9	26.6	18.6	15.1	13.5	15.5	14.9	126.5	81.80
	4	0	8		2	8	0	7	1	7	0	3	3	8	8	9	7	
Minimum	248.3	130.5	88.92	18.56	149.9	88.74	19.8	10.1	21.4	11.2	18.9	14.1	10.0	9.25	11.4	10.2	105.8	61.81
	4	1			5		7	8	3	6	8	7	7		7	2	8	
Maximum	296.8	170.9	140.8	105.0	200.8	141.3	36.4	29.5	34.0	25.4	32.8	23.8	18.8	19.2	19.2	22.7	151.0	102.5
	6	7	0	1	5	8	6	3	0	7	3	0	8	5	1	3	9	4
Standard Deviation	10.86	9.88	11.52	13.11	11.56	10.82	3.76	3.02	2.44	3.26	2.65	2.46	1.76	2.37	1.88	2.5	11.06	10.01

Table S5. Record of production parameter of both parthenogenetic females of the strains IMP-BG Z010-VL and Z018-SD. Min = minimum, Max = maximum, SD = Standard deviation. The units of each parameter are indicated in parenthesis. Data obtained from the F1 with less than 24 hours after hatching. (n=48).

Production parameters	Strain IMP-BG Z010-VL					Strain IMP-BG Z018-SD				
	Mean	SD	Min	Max	Median	Mean	SD	Min	Max	Median
Longevity (days)	19.2	3.9	29	12	18.5	14.4	3.9	23	7	13
Progeny (number of offspring)	15.5	6.6	31	0	15	7.8	3.9	16	0	7
Production (number of eggs)	18	5.7	31	7	17	9	4.2	19	2	8
Maximum load (number of eggs)	2.5	0.9	6	1	2	1.6	0.6	3	1	2
Pre-reproductive Age (days)	4.4	1	7	3	5	4.5	2.8	10	2	3.5
Reproductive Age (days)	12	4.6	22	0	12	8	3.2	17	2	8
Post-reproductive Age (days)	2.5	2	11	0	2	1.9	1	5	0	2

Table S6. Nucleotide composition registered in mtDNA COI sequences (570 bp) of L4 and SM2 organisms from different origins, considered for phylogenetic analysis.

Morphotype	Locality	Number of samples	Nucleotide composition (%)			
			T	C	A	G
L4	Peru	9	38.40	19.50	25.10	17.00
	Chile	27	38.57	19.24	24.99	17.19
	Mexico	6	41.22	16.82	23.52	18.38
	USA	7	41.06	17.21	23.67	18.10
	China	1	41.60	16.50	23.50	18.40
	Japan	5	40.40	18.10	23.90	17.70
	Australia	1	40.40	18.10	23.90	17.70
	France	5	40.74	17.52	23.84	17.88
SM2	Peru	9	45.40	15.60	20.70	18.20
	USA	7	46.09	15.30	21.84	16.79
	Spain	3	46.10	15.37	21.20	17.27
	Cayman Islands	3	46.00	15.40	21.80	16.80
	Philippines	1	46.00	15.30	21.80	17.00
	South Korea	5	44.80	15.78	21.38	18.08
	Turkey	1	43.30	16.30	21.60	18.80
	Italy	1	43.00	17.00	20.70	19.30

Table S7. Nucleotide composition registered in rDNA ITS1 sequences (331 bp) of L4 and SM2 organisms from different origins, considered for phylogenetic analysis.

Morphotype	Locality	Number of samples	Nucleotide composition (%)			
			T	C	A	G
L4	Peru	9	38.40	15.90	31.40	14.30
	Chile	23	38.49	15.90	31.27	14.30
	Mexico	4	38.40	15.90	31.40	14.30
	USA	3	38.40	15.90	31.40	14.30
	Japan	4	38.40	15.90	31.40	14.30
	Australia	1	38.40	15.90	31.40	14.30
SM2	Peru	9	40.10	13.70	32.50	13.70
	Spain	2	39.80	13.90	32.60	13.70
	Cayman Islands	3	39.80	13.70	32.80	13.70
	Philippines	1	40.10	13.70	32.50	13.70
	South Korea	5	40.10	13.70	32.56	13.64
	USA	7	39.80	13.70	32.80	13.70
	Turkey	1	39.80	13.70	32.80	13.70
	Italy	1	40.50	13.60	32.30	13.60

Table S8. Summary of the outputs of the R-Studio programme. They showed low correlation expressed by Monte-Carlo Test on the sum of eigenvalues of a co-inertia analysis (RV).

Ventanilla Strain_L4_IMP-BG-Z010	Santo Domingo Strain_SM2_IMP-BG-Z018
Coinertia analysis Class: coinertia dudi Call: coinertia(dudiX = pca_production, dudiY = pca_measurement, scannf = FALSE, nf = 2) Total inertia: 3.318 Eigenvalues: Ax1 Ax2 Ax3 3.20013 0.09382 0.02357 Projected inertia (%): Ax1 Ax2 Ax3 96.4613 2.8281 0.7106 Cumulative projected inertia (%): Ax1 Ax1:2 Ax1:3 96.46 99.29 100.00 Eigenvalues decomposition: eig covar sdX sdY corr 1 3.20012568 1.7888895 1.250093 1.728665 0.8278092 2 0.09382405 0.3063071 0.853485 1.273372 0.2818421 Inertia & coinertia X (pca_production): inertia max ratio 1 1.562733 1.764605 0.8855994	Coinertia analysis Class: coinertia dudi Call: coinertia(dudiX = pca_production, dudiY = pca_measurement, scannf = FALSE, nf = 2) Total inertia: 5.113 Eigenvalues: Ax1 Ax2 Ax3 5.029053 0.082265 0.001413 Projected inertia (%): Ax1 Ax2 Ax3 98.36334 1.60902 0.02765 Cumulative projected inertia (%): Ax1 Ax1:2 Ax1:3 98.36 99.97 100.00 Eigenvalues decomposition: eig covar sdX sdY corr 1 5.02905337 2.2425551 1.5000611 1.857256 0.8049379 2 0.08226479 0.2868184 0.8060476 1.163411 0.3058532 Inertia & coinertia X (pca_production): inertia max ratio 1 2.250183 2.397150 0.9386910

12 2.291170 2.593068 0.8835748 Inertia & coinertia Y (pca_ measurement): inertia max ratio 1 2.988282 3.184835 0.9382849 12 4.609759 4.898301 0.9410935 RV: 0.4317608	12 2.899896 2.905029 0.9982332 Inertia & coinertia Y (pca_ measurement): inertia max ratio 1 3.449400 3.506581 0.9836934 12 4.802926 4.841645 0.9920029 RV: 0.5245938
Total inertia: 3.318 / RV: 0.4317608	Total inertia: 5.113 / RV: 0.5245938