

SUPPORTING MATERIAL

Table S1. Name, acronym and geographic coordinates of the sampling sites. Locations 1 to 5 were collected in this study. Geographic information of the locations 6 and 8 were obtained from [6].

Location	Location code	State	Collecting date	Latitude N	Longitude W	Elevation (masl)
1. Cuitzeo lagoon, limnetic	CUI	Michoacán	25/02/2014	19.8894	-100.9439	1836
2. Santa Teresa dam, littoral	STE	Michoacán	17/08/2014	19.8886	-100.1722	2307
3. Yuriria dam, littoral	YUR	Guanajuato	27/09/2014	20.2211	-101.1361	1736
4. Tepatitlan-Yahualica pond	TYA	Jalisco	22/08/2015	21.0850	-102.8694	1780
5. Huapango dam, littoral	HUA	State of Mexico	17/08/2014	19.9483	-99.7144	2619
6. Ignacio Ramirez dam, limnetic	IGR	State of Mexico	22/06/2011	19.46	-99.777	
7. Timilpan pond	TIM	State of Mexico	22/06/2011	19.887	-99.739	
8. Aguada Kohunlich	KOH	Quintana Roo	03/02/2011	18.447	-88.825	

Table S2. PCR reaction mix and cycle profile for ITS1 nuclear marker.

PCR reagents	PCR profile
Volumes for one reaction (13.5 μL)	Initial step: 95°C for 5 min
DNA template 3 μ L	36 cycles of the following profile:
Trehalose 10% 6.25 μ L	
ddH ₂ O 2 μ L	
10X PCR buffer 1.25 μ L	
50 mM MgCl ₂ 0.625 μ L	
0.01 mM Primer 1 0.125 μ L	Denaturing step: 95°C for 50 s
0.01 mM Primer 2 0.125 μ L	Annealing step: 54°C for 50 s
0.05 mM dNTP mix 0.0625 μ L	Extending step: 72°C for 1 min
Taq polymerase 0.06 μ L	Final step: 72°C for 5 min

Table S4. Percentages of uncorrected genetic distances of COI gene. On diagonal are the genetic distances within the ESUs under study, and below the diagonal are the genetic distances among the ESUs discriminated by the species delineation methods.

	ESU1	ESU2	ESU3	ESU4	ESU5	ESU6
ESU1	0					
ESU2	4.4	0.2				
ESU3	15.7	17.2	0.5			
ESU4	14.9	15.6	13.1	0.1		
ESU5	15.6	15.8	14.3	11.9	0.5	
ESU6	19.2	20.1	17.5	17.2	17.7	0.8

Table S5. Percentages of uncorrected genetic distances of ITS1 marker. On diagonal are the genetic distances within the ESUs under study, and below the diagonal are the genetic distances among the ESUs discriminated by the species delineation methods.

	ESUI	ESUII	ESUIII	ESUIV
ESUI	0			
ESUII	9.0	0.5		
ESUIII	5.8	9.4	0.1	
ESUIV	3.8	10.8	5.8	0

Table S6. Measurements of *Keratella* specimens from Mexico. Measurements are in microns. TL = Total length, LL = Lorica length, LW= Lorica width, RPS= Right posterior spine, LPS= Left posterior spine, AMS= Anteromedian spine, AIS= Antero intermediate spine, ALS= Antero lateral spine.

CUITZEO		TL	LL	LW	RPS	LPS	AMS	AIS	ALS
	1	306	130	80	148	44	26	18	16
	2	284	130	76	128	44	26	18	16
	3	274	128	76	120	42	25	17	15
	4	270	124	76	120	44	26	18	16
	5	304	132	76	146	50	26	18	16
	6	314	136	74	152	56	26	18	16
	7	304	134	74	144	54	26	18	16
	8	260	134	74	100	62	26	18	16
	9	304	132	76	146	54	26	18	16
	10	298	126	74	146	44	26	18	16

11	275	130	75	118	45	27	18	15
12	306	137	76	142	52	27	18	15
13	306	137	75	142	55	27	18	16
14	301	135	75	140	50	26	18	16
15	296	140	75	130	50	26	18	16

SANTA TERESA	TL	LL	LW	RPS	LPS	AMS	AIS	ALS
1	266	126	80	106	58	34	21	19
2	260	128	80	96	68	36	22	20
3	264	128	80	100	70	36	22	20
4	264	128	80	100	58	34	21	19
5	270	130	82	104	48	36	22	20
6	274	132	82	106	70	36	22	20
7	264	128	74	100	68	36	22	20
8	264	126	80	102	56	36	22	20
9	264	128	84	100	68	36	22	20
10	266	130	84	100	66	36	22	20

HUAPANGO	TL	LL	LW	RPS	LPS	AMS	AIS	ALS
1	194	108	64	54	40	30	19	17
2	200	112	68	56	48	32	20	18
3	204	116	68	56	44	32	20	18
4	202	114	64	56	44	32	20	18
5	200	114	68	54	44	32	20	18
6	194	110	66	52	40	30	19	17
7	204	114	68	58	46	32	20	18
8	202	112	66	58	50	32	20	18
9	208	116	72	60	58	32	20	18
10	204	116	66	56	44	32	20	18
11	197	110	65	55	40	32	19	17
12	205	117	63	57	47	32	20	18
13	202	112	64	58	50	32	19	17
14	205	117	64	57	49	33	20	18
15	199	112	63	55	45	32	19	17

YURIRIA	TL	LL	LW	RPS	LPS	AMS	AIS	ALS
1	248	118	68	100	30	28	19	18
2	246	116	66	100	50	30	20	20
3	254	120	70	100	48	34	22	20
4	242	118	70	90	44	34	24	20
5	248	114	68	100	46	34	22	20
6	230	110	70	90	38	30	20	20
7	210	100	70	80	30	28	19	18
8	242	114	68	98	40	30	20	18
9	264	110	70	118	48	36	24	20

10	250	118	70	98	46	34	22	20
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TEPATITLAN-YAHUALILCA	TL	LL	LW	RPS	LPS	AMS	AIS	ALS
1	196	92	48	80	28	24	20	20
2	184	90	50	72	14	22	20	20
3	200	98	60	78	26	24	22	20
4	202	98	56	80	26	24	20	20
5	194	94	56	76	24	24	20	20
6	188	94	56	70	8	24	20	20
7	180	90	50	66	22	24	20	20
8	168	92	54	54	34	22	18	18
9	196	96	54	80	20	20	18	18
10	174	90	54	64	16	20	18	18

IGNACIO RAMIREZ	TL	LL	LW	RPS	LPS	AMS	AIS	ALS
1	314	152	87	137	57	25	25	17
2	332	150	87	147	50	35	30	25
3	324	152	85	135	57	37	25	20
4	304	147	87	125	55	32	25	17
5	316	147	87	132	50	37	25	22
6	314	152	87	130	57	32	25	20
7	309	142	85	137	40	30	25	20
8	317	150	77	132	45	35	27	22
9	322	157	85	130	57	35	27	22
10	309	152	87	125	52	32	27	20
11	297	145	82	120	30	32	25	20
12	297	150	82	115	42	32	25	20

KOHUNLICH	TL	LL	LW	RPS	LPS	AMS	AIS	ALS
1	275	125	77	105	62	45	25	22
2	259	112	75	102	57	45	22	20
3	264	112	75	107	57	45	22	20
4	271	112	75	112	65	47	25	22
5	259	107	75	107	57	45	20	17
6	254	107	75	105	62	42	22	20
7	271	107	75	122	55	42	22	20
8	240	105	67	95	52	40	22	20
9	247	105	72	102	57	40	22	20
10	252	107	75	100	55	45	25	22
11	259	112	77	105	62	42	22	20
12	242	105	75	95	57	42	22	20