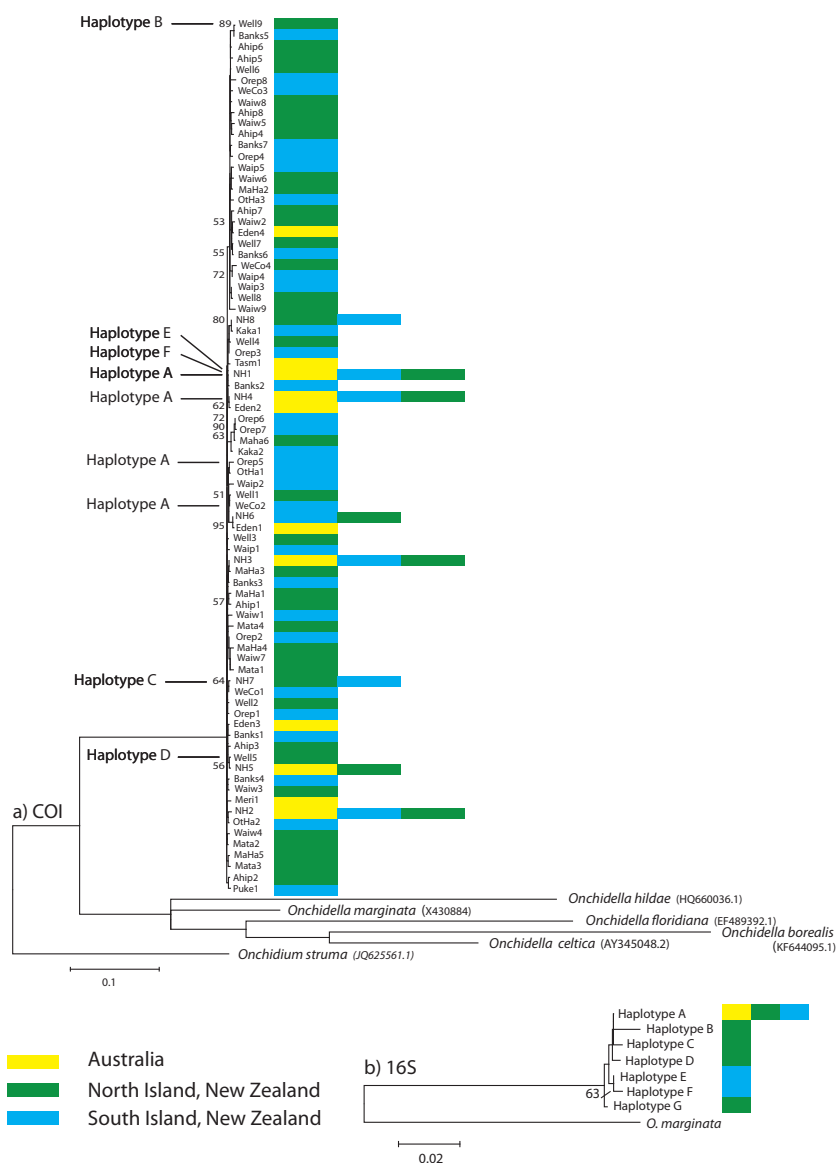


# Trans-Tasman genetic connectivity in the intertidal air-breathing slug *Onchidella nigricans*

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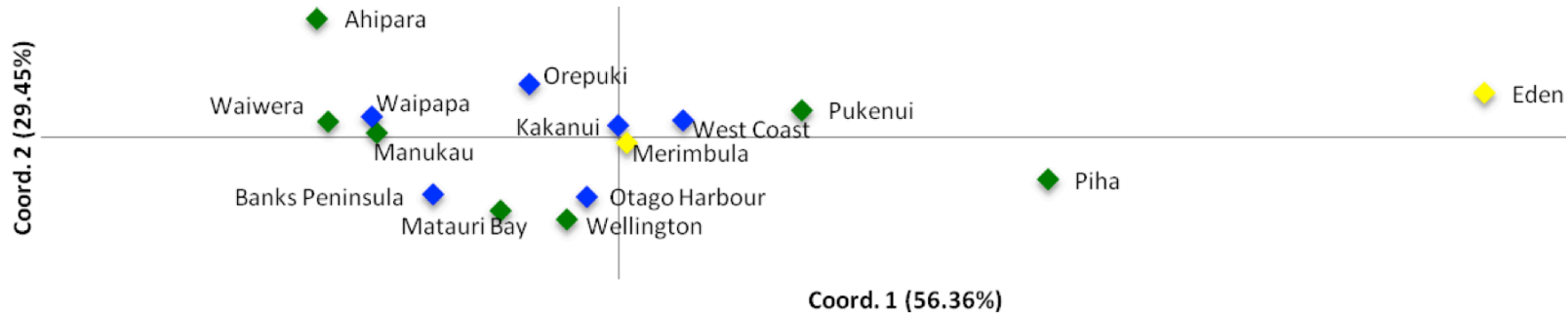
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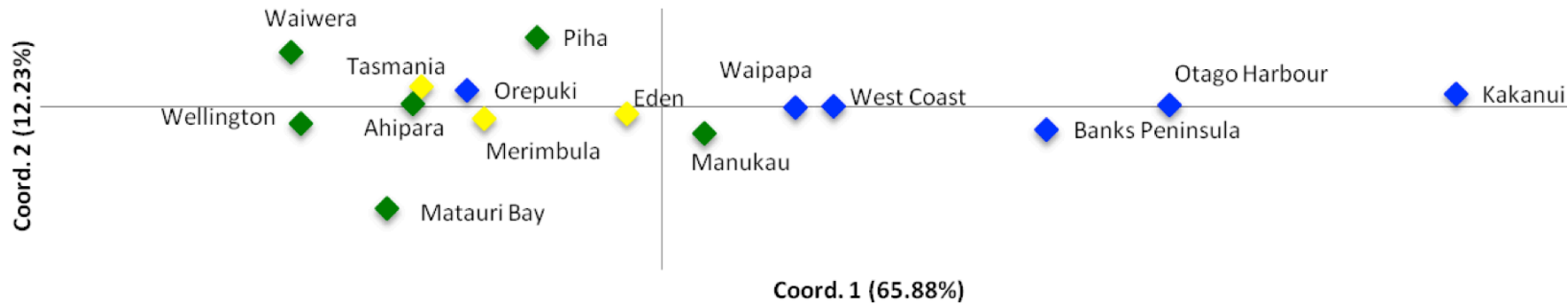


**Fig. S1** Maximum likelihood phylogenetic reconstructions of *Onchidella nigricans* mtDNA lineages based on a) 740 base pairs of COI (n = 111; plus six other *Onchidella* spp., and *Onchidium struma* as the outgroup), and b) 351 base pairs of 16S rDNA (n = 12; *O. marginata* as the outgroup). The positions of individuals with 16S haplotype data are highlighted on the COI tree (Haplotype A – G). Only bootstrap values > 50% are shown adjacent to corresponding branch. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Codon positions included were 1st+2nd+3rd+Noncoding. Colour bars indicate landmass (Australia, New Zealand’s North & South Islands). The trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

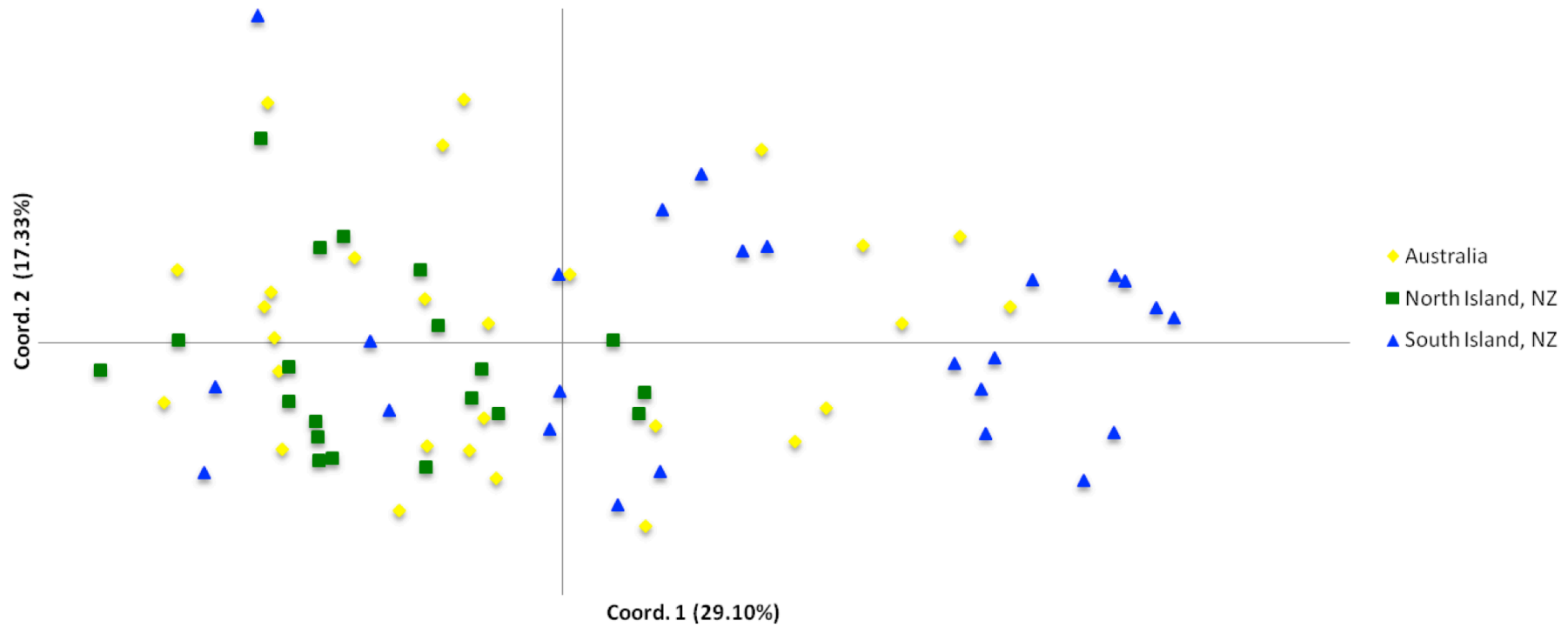
a) COI



b) AFLP



**Fig. S2** Principal coordinate plots of population level  $\Phi_{PT}$  of a) mtCOI sequence data and b) AFLP data for *Onchidella nigricans*. The percentage of variation explained by each axis is shown for each coordinate. Data points are coloured by region (Landmass): yellow = Australia; Green = North Island, New Zealand; Blue = South Island, New Zealand.



**Fig. S3** Principal coordinate plots of AFLP fingerprint distances between individual *Onchidella nigricans* slugs, grouped by landmass.

**Table S1** Sampling locations, sites and sample sizes for each marker type for *Onchidella nigricans*

<b>Region</b>	<b>Location</b>	<b>Code</b>	<b>Site</b>	<b>GPS co-ordinates</b>	<b>CO1</b>	<b>16S</b>	<b>AFLP</b>
Australia	Merimbula	NSW(m)	Boardwalk	36°53'45.49"S, 149°54'9.53"E	4	1	9
	Eden	NSW(c)	Cocora Beach	37° 4'24.48"S, 149°53'48.72"E	3		5
		NSW(q)	Quarantine Bay	37° 4'35.95"S, 149°53'3.98"E	2	1	9
		NSW(n)	Torrango Point	37° 6'7.73"S, 149°53'49.76"E	4		3
	Tasmania	Tas	Coningham	43° 4'30.04"S, 147°16'39.88"E	1		2
					14	2	28
North Island, New Zealand	Ahipara	Nth(a)	Ahipara	35°10'7.44"S, 173° 3'43.88"E	7		5
	Mataura Bay	Nth(an)	Mataura Bay	35°02'7.43"S, 173°9'14.74"E	7		3
	Bay of Islands	BI(t)	Tapeka Point	35°14'44.71"S, 174° 7'14.51"E		2	1
		BI(p)	Pukenui	34°48'53.42"S, 173° 7'8.90"E	2	1	1
	Piha	Nth(p)	Piha	36°57'34.90"S, 174°27'45.49"E	4		2
	Manukau Harbour	Nth(m)	Mill Bay	36°59'37.68"S, 174°36'18.82"E	4		1
		Nth(h)	Huia	37° 0'8.14"S, 174°33'53.84"E	6		1
	Waiwera	Nth(w)	Waiwera site 1	36°32'55.61"S, 174°42'36.03"E	5		2
		Nth(wi)	Waiwera site 2	36°32'34.31"S, 174°42'49.30"E	6		3
	Wellington	Nth(m)	Makara Beach	41°13'6.66"S, 174°42'33.29"E	11	3	3
					52	6	22
South Island, New Zealand	Banks Peninsula	Bp(o)	Te Oka Bay	43°51'3.58"S, 172°47'6.59"E	3		1
		Tm	Taylors Mistake	43°34'57.16"S, 172°46'38.65"E	4		2
		Bp(l)	Le Bons Bay	43°44'15.67"S, 173° 5'41.81"E	5		2
	Kakanui	Ot(k)	Kakanui	45°10'59.83"S, 170°54'24.21"E	4		2
	Otago Harbour	Ot(v)	Vauxhall	45°53'9.65"S, 170°31'26.70"E	3		2
		Ot(b)*	Broad Bay	45°50'52.33"S, 170°37'19.35"E	3		1
	Waipapa Point	SI(w)	Waipapa Point	46°39'41.25"S, 46°39'41.25"E	7		5
	Orepuki	SI(o)	Cosy Nook	46°19'53.26"S, 167°42'37.40"E	9	1	5
	West Coast	WC(p)	Punakaiki	42° 6'45.57"S, 171°19'50.86"E	7	3	5
						45	4
Total N					111	12	75

**Table S2** List of haplotypes with corresponding GenBank accession numbers for all *Onchidella nigricans* samples. SH = shared COI haplotype amongst > 1 location. COI haplotypes are ordered numerically and 16S rDNA alphabetically. Individuals with corresponding AFLP fingerprint or H3 sequence data are indicated\* or highlighted in bold (respectively). H3 GenBank accession number KF258007.

Haplotype COI	GenBank COI	16S Haplotype	GenBank 16S	Individual	Locations represented:
NH1 (n = 18)	KF258031	Haplotype A	KF258001	BI(P)3*	Banks Peninsula x 3
				Bp(L)13	Merimbula x 1
				Bp(o)1	Manukau Harbour x 2
				Bp(o)2	Piha x 1
				<b>NSW(m)6*</b>	Waiwera x 1
				Nth(h)45	Otago Harbour x 2
				Nth(m)42	Kakanui x 2
				Nth(p)36*	Waipapa Point x 1
				Nth(w)6	West Coast x 2
				Ot(b)6	Wellington x 2
				Ot(b)8	Pukenui x 1
				Ot(k)11*	
				Ot(k)16	
				Sl(w)22	
	Haplotype E	KF258002	WC(P)2		
	Haplotype F	KF258003	WC(P)3		
			Nth(m)6		
			Nth(m)8*		
Eden1 (n = 4)	KF258099			NSW(q)10*	Eden x 4
				NSW(n)16*	
				NSW(c)21*	
				NSW(c)20*	
NH2 (n = 4)	KF258049			TM1*	Banks Peninsula x 1
				SL(w)21*	Waipapa Point x 1
				Ot(b)9	Otago Harbour x 1
				NSW(n)18*	Eden x 1
NH3 (n = 3)	KF258036			Nth(m)5	Wellington x 1
				Nth(an)31	Mataura Bay x 2
				Nth(an)29	
NH4 (n = 3)	KF258047			<b>TM3*</b>	Banks Peninsula x 1
				Nth(p)37	Piha x 1
				NSW(M)2*	Merimbula x 1
NH5 (n = 2)	KF258070			Nth(Wi)8*	Waiwera x 1
				NSW(M)3*	Merimbula x 1
NH6 (n = 2)	KF258041			WC(P)7*	West Coast x 1
				Nth(p)32*	Piha x 1
NH7 (n = 2)	KF258037	Haplotype C	KF258006	Nth(m)4	Wellington x 1
		Haplotype A	KF258001	NSW(q)11*	Eden x 1
NH8 (n = 2)	KF258052			SL(o)18	Orepuki x 1
				Nth(p)35	Piha x 1
Mata1	KF258090			Nth(an)26*	
Banks1	KF258106			BP(L)17	
Waiw1	KF258071			Nth(wi)7*	

Haplotype COI	GenBank COI	16S Haplotype	GenBank 16S	Individual	Locations represented:
Waiw2	KF258078			Nth(W)2	
Ahip1	KF258097			Nth(A)16*	
MaHa1	KF258082			Nth(h)49	
MaHa2	KF258080			Nth(m)39	
Meri1	KF258102			NSW(m)5	
Puke1	KF258109			BI(P)4*	
Waip1	KF258051			SI(w)20	
Banks2	KF258107			<b>Bp(L)16*</b>	
Tasm1	KF258030			Tas1c*	
Eden2	KF258101			NSW(n)1b	
Well1	KF258033			Nth(m)10*	
OtHa1	KF258068			ONCH-HL	
Banks3	KF258108			Bp(L)7	
MaHa5	KF258086			Nth(h)44	
WeCo1	KF258042			WC(P)6*	
Well2	KF258040			<b>Nth(m)1</b>	
Eden3	KF258100			NSW(n)17*	
Orep1	KF258054			SL(o)16	
Ahip2	KF258093			Nth(a)22*	
WeCo2	KF258044	Haplotype A	KF258001	WC(P)4*	
Mata2	KF258091			Nth(an)25	
Well3	KF258035			Nth(m)7	
Banks4	KF258046			TM4	
OtHa2	KF258065			Ot(v)1*	
Waiw3	KF258069			Nth(wi)10*	
Orep2	KF258056			SL(O)6*	
Well4	KF258032			Nth(m)11*	
Orep3	KF258055			SL(o)15	
Kaka1	KF258067			Ot(k)14	
Mata3	KF258088			Nth(an)30*	
Well5	KF258038	Haplotype D	KF258005	Nth(m)3	
Orep4	KF258058			SI(o)4	
Orep5	KF258057	Haplotype A	KF258001	SI(o)5*	
Ahip3	KF258092			Nth(a)24*	
Waip2	KF258062			SI(W)14	
Waiw4	KF258072			Nth(wai)55	
Ahip4	KF258096			Nth(a)17	
Banks5	KF258105			Bp(l)18*	
Kaka2	KF258066			Ot(k)15*	
Waiw5	KF258076			Nth(w)5*	
Ahip5	KF258095			Nth(a)18*	
Well6	KF258034			Nth(m)9	
Waip3	KF258050			SL(w)23*	
Well7	KF258081			Nth(m)38	
Well8	KF258079			Nth(m)40*	
Ahip6	KF258087			Nth(an)28	
Waip4	KF258061			SI(W)15	
WeCo3	KF258045			WC(P)1*	

Haplotype COI	GenBank COI	16S Haplotype	GenBank 16S	Individual	Locations represented:
Ahip7	KF258098			Nth(a)12*	
Banks6	KF258104			Bp(o)3	
Eden4	KF258103			NSW(c)22*	
WeCo4	KF258043			WC(P)5*	
OtHa3	KF258064			Ot(v)3*	
Ahip8	KF258094			Nth(a)19	
Well9	KF258039	Haplotype B	KF258004	Nth(m)2	
Waip5	KF258063			SI(W)13	
Waiw6	KF258075			Nth(wai)52	
MaHa3	KF258083			Nth(h)48*	
Waiw7	KF258073			Nth(wai)54	
Waiw8	KF258077			<b>Nth(w)4*</b>	
Waiw9	KF258074			Nth(wai)53	
MaHa4	KF258085			Nth(h)46	
Mata4	KF258089			Nth(an)27*	
Orep6	KF258053			SL(o)17*	
Orep7	KF258060			SL(O)1*	
MaHa6	KF258084			Nth(h)47	
Orep8	KF258059			SL(O)3*	
Banks7	KF258048			TM2	
-		Haplotype A	KF258001	BI(t)1	
-		Haplotype G	KF258000	BI(t)2	
-				NSWc23*	
-				NSWc24*	
-				NSW(q)35*	
-				NSW(q)37*	
-				NSW(q)38*	
-				NSW(q)39*	
-				NSW(q)40*	
-				NSW(q)41*	
-				NSW(q)42*	
-				NSW(m)30*	
-				NSW(m)31*	
-				NSW(m)32*	
-				NSW(m)33*	
-				NSW(m)34*	
-				Tas2*	
-				<b>Wc(m)1</b>	

**Table S3** *Onchidella nigricans* mtDNA COI diversity statistics for each sampling locality within New Zealand (North and South Islands) and Australia

Location	n	Number of haplotypes	Polymorphic sites	Haplotype diversity ( $H$ )	Nucleotide diversity ( $\pi \times 10^{-3}$ )
<b>North Island</b>					
Ahipara	7	7	17	1.00 +/- 0.08	8.5 +/- 5.3
Manukau Harbour	10	9	22	0.98 +/- 0.05	7.75 +/- 4.61
Mataura Bay	7	6	16	0.95 +/- 0.10	6.94 +/- 4.41
Piha	4	4	8	1.00 +/- 0.18	5.71 +/- 4.3
Pukenui	2	2	2	1.00 +/- 0.50	2.86 +/- 3.5
Waiwera	11	11	28	1.00 +/- 0.04	8.75 +/- 5.1
Wellington	11	10	18	0.98 +/- 0.05	5.04 +/- 3.13
<b>Total/Average</b>	52	49	15	n/a	n/a
<b>South Island</b>					
Banks Peninsula	12	10	19	0.95 +/- 0.06	5.28 +/- 3.23
Kakanui	4	3	10	0.83 +/- 0.22	7.14 +/- 5.23
Otago Harbour	6	5	11	0.93 +/- 0.12	5.23 +/- 3.56
Waipapa Point	7	7	17	1.00 +/- 0.08	7.62 +/- 4.79
Orepuki	9	9	24	1.00 +/- 0.05	10.4 +/- 6.1
Punakaiki	7	6	17	0.95 +/- 0.10	7.76 +/- 4.87
<b>Total/Average</b>	45	40	16	n/a	n/a
<b>Australia</b>					
Eden	9	6	19	0.83 +/- 0.13	8.1 +/- 4.86
Merimbula	4	4	7	1.00 +/- 0.18	5 +/- 3.83
Coningham	1	1	n/a	n/a	n/a
<b>Total/Average</b>	14	11	13	n/a	n/a



**Table S4** *Onchidella nigricans* AFLP diversity statistics for each sampling locality within New Zealand (North and South Islands) and Australia (n = 73; 232 loci).

Location	n	Number of bands (loci)	Number of private bands	Mean expected ( $H_E$ )	SE of mean ( $H_E$ )
<b>North Island</b>					
Ahipara	5	77	5	0.084	0.010
Manukau Harbour	2	56	7	0.079	0.011
Mataura Bay	3	49	0	0.057	0.009
Piha	2	42	2	0.055	0.009
Waiwera	5	85	4	0.105	0.011
Wellington	3	54	1	0.070	0.010
<b>Total/Average</b>	<b>20</b>	<b>61</b>	<b>3</b>	<b>0.075</b>	<b>0.010</b>
<b>South Island</b>					
Banks Peninsula	5	82	5	0.103	0.011
Kakanui	2	50	0	0.062	0.010
Otago Harbour	3	72	3	0.094	0.010
Waipapa Point	5	84	4	0.110	0.011
Orepuki	5	81	3	0.100	0.011
Punakaiki	5	108	8	0.127	0.011
<b>Total/Average</b>	<b>25</b>	<b>80</b>	<b>4</b>	<b>0.099</b>	<b>0.011</b>
<b>Australia</b>					
Eden	17	152	31	0.119	0.010
Merimbula	9	102	7	0.101	0.010
Coningham	2	45	0	0.050	0.009
<b>Total/Average</b>	<b>28</b>	<b>100</b>	<b>13</b>	<b>0.090</b>	<b>0.010</b>

**Table S5** Partitioning of the genetic variation among three nested levels of spatial hierarchy for *Onchidella nigricans*: among landmasses, among localities and within localities for a) COI sequence data, and b) AFLP fingerprint data.  $\Phi_{RT} = AR / (WP + AP + AR) = AR / TOT$ ;  $\Phi_{PR} = AP / (WP + AP)$ ;  $\Phi_{PT} = (AP + AR) / (WP + AP + AR) = (AP + AR) / TOT$ ; Key: AR = Est. Var. Among Landmasses, AP = Est. Var. Among Localities, WP = Est. Var. Within Localities.

a) COI					
Source	df	SS	MS	Est. Var.	%
Among Landmasses	2	9.367	4.683	0.062	2%
Among Localities	12	31.659	2.638	0.002	0%
Within Localities	95	249.065	2.622	2.622	98%
Total	109	290.091		2.686	100%

Stat	Value	P(rand > = data)
PhiRT	0.023	0.018
PhiPR	0.001	0.449
PhiPT	0.024	0.069

b) AFLP					
Source	df	SS	MS	Est. Var.	%
Among Landmasses	2	64.451	32.225	0.536	3%
Among Localities	12	229.384	19.115	0.073	0%
Within Localities	58	1090.686	18.805	18.805	97%
Total	72	1384.521		19.413	100%

Stat	Value	P(rand > = data)
PhiRT	0.028	0.0002
PhiPR	0.004	0.366
PhiPT	0.031	0.004

**Table S6** *Onchidella nigricans* (landmass level) pairwise  $\Phi_{PT}$  estimates and their statistical significance levels (above diagonal). *P*-values still significant at alpha-level 0.05 after applying Benjamini and Hochberg False Discovery Rate correction for COI and AFLP data are highlighted.

a) COI			b) AFLP		
Australia	North Is.	South Is.	Australia	North Is.	South Is.
	<b>0.009</b>	<b>0.011</b>		0.458	<b>0.003</b>
0.058		0.374	0.003		<b>0.0001</b>
0.058	0.000		0.025	0.062	

**Table S7** *Onchidella nigricans* (locality level) pairwise  $\Phi_{PT}$  estimates (below diagonal) and their statistical significance levels (above diagonal) for COI data (n = 110). *P*-values still significant at alpha-level 0.05 after applying Benjamini and Hochberg False Discovery Rate correction are highlighted.

Eden	Merimbula	Ahipara	Manukau Harbour	Mataura Bay	Piha	Pukenui	Waiwera	Wellington	Banks	Kakanui	Orepuki	Otago	Waipapa	Punakaiki	
	0.240	<b>0.044</b>	<b>0.019</b>	0.053	0.263	0.499	<b>0.018</b>	<b>0.037</b>	<b>0.023</b>	0.238	<b>0.031</b>	0.130	0.060	0.184	Eden (9)
0.067		0.290	0.398	0.409	0.425	0.539	0.338	0.364	0.408	0.429	0.391	0.332	0.286	0.432	Merimbula (4)
0.145	0.020		0.336	0.102	0.155	0.438	0.360	0.061	0.109	0.360	0.261	0.126	0.304	0.460	Ahipara (7)
0.129	0.000	0.000		0.399	0.220	0.412	0.350	0.295	0.363	0.360	0.358	0.372	0.366	0.335	Manukau Harbour (10)
0.131	0.000	0.067	0.000		0.301	0.459	0.305	0.408	0.395	0.424	0.160	0.469	0.308	0.263	Mataura Bay (7)
0.000	0.000	0.099	0.034	0.015		0.471	0.137	0.356	0.254	0.430	0.386	0.515	0.240	0.350	Piha (4)
0.000	0.000	0.003	0.000	0.000	0.000		0.368	0.341	0.372	0.602	0.365	0.396	0.321	0.362	Pukenui (2)
0.139	0.000	0.000	0.000	0.009	0.057	0.000		0.301	0.288	0.317	0.219	0.259	0.336	0.336	Waiwera (11)
0.122	0.000	0.071	0.004	0.000	0.000	0.000	0.016		0.376	0.384	0.100	0.495	0.148	0.325	Wellington (11)
0.135	0.000	0.051	0.000	0.000	0.030	0.000	0.004	0.000		0.347	0.100	0.375	0.398	0.316	Banks (12)
0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.394	0.391	0.253	0.529	Kakanui (4)
0.125	0.000	0.022	0.000	0.043	0.006	0.000	0.018	0.053	0.048	0.000		0.219	0.394	0.425	Orepuki (9)
0.101	0.000	0.064	0.002	0.000	0.004	0.000	0.009	0.000	0.000	0.000	0.034		0.416	0.237	Otago (6)
0.128	0.000	0.000	0.000	0.016	0.046	0.000	0.000	0.018	0.000	0.000	0.005	0.000		0.401	Waipapa (7)

**Table S8** *Onchidella nigricans* (locality level) pairwise  $\Phi_{PT}$  estimates (below diagonal) and their statistical significance levels (above diagonal) for AFLP data (n = 73; 232 loci). *P*-values still significant at alfa-level 0.05 after applying Benjamini and Hochberg False Discovery Rate correction are highlighted.

Eden	Merimbula	Conningham	Ahipara	Manukau Harbour	Mataura Bay	Piha	Wellington	Waiwera	Banks	Kakanui	Orepuki	Otago	Waipapa	Punakaiki	
	0.461	0.430	0.452	0.488	0.437	0.445	0.435	0.111	0.082	<b>0.045</b>	0.275	0.091	0.454	0.443	Eden (17)
0.000		0.456	0.298	0.426	0.423	0.441	0.450	0.142	<b>0.024</b>	<b>0.018</b>	0.218	<b>0.004</b>	0.174	0.192	Merimbula (9)
0.000	0.000		0.328	0.331	0.297	0.334	0.500	0.192	0.094	0.333	0.473	0.104	0.426	0.427	Tasmania (2)
0.000	0.011	0.011		0.240	0.311	0.337	0.219	0.317	<b>0.007</b>	<b>0.048</b>	0.479	<b>0.018</b>	0.445	0.102	Ahipara (5)
0.000	0.000	0.000	0.035		0.610	0.666	0.395	0.143	0.240	0.330	0.288	0.203	0.284	0.523	Manukau Harbour (2)
0.000	0.000	0.042	0.021	0.000		0.100	0.499	0.142	0.102	0.101	0.237	0.105	0.407	0.313	Mataura Bay (3)
0.000	0.000	0.000	0.034	0.000	0.106		0.201	0.236	0.147	0.335	0.334	0.192	0.471	0.425	Piha (2)
0.000	0.000	0.000	0.029	0.057	0.000	0.041		0.399	0.056	0.097	0.381	0.101	0.249	0.460	Wellington (3)
0.028	0.029	0.024	0.018	0.080	0.067	0.028	0.000		<b>0.008</b>	<b>0.049</b>	0.227	<b>0.017</b>	0.100	0.147	Waiwera (5)
0.031	0.063	0.075	0.100	0.039	0.081	0.063	0.100	0.156		0.379	<b>0.029</b>	0.497	0.448	0.474	Banks (5)
0.104	0.171	0.232	0.229	0.102	0.289	0.224	0.292	0.267	0.000		<b>0.046</b>	0.403	0.241	0.379	Kakanui (2)
0.010	0.021	0.000	0.000	0.051	0.029	0.018	0.013	0.031	0.078	0.181		<b>0.016</b>	0.367	<b>0.047</b>	Orepuki (5)
0.048	0.115	0.126	0.123	0.036	0.143	0.068	0.151	0.194	0.000	0.000	0.128		0.471	0.468	Otago (3)
0.000	0.028	0.000	0.007	0.040	0.004	0.000	0.034	0.063	0.000	0.036	0.007	0.001		0.501	Waipapa (5)
0.000	0.020	0.000	0.028	0.000	0.018	0.000	0.000	0.046	0.000	0.000	0.047	0.000	0.000		Punakaiki (5)