Table S1. Summary of fifteen microsatellite loci run for all samples. Universal tail sequences were bound to the 5′ tail of the forward primer following Blacket et al., (2012). Final concentration of forward (label + forward) and reverse primers in PCR was 0.3 (uM) each. The volumes reported create a 10 uM working solution from 100 uM primer stocks. Microsatellites were grouped and run together in a multiplex PCR reaction.

Locus	Size range (bp)	Forward primer tail/label	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	Multiplex group	Number of alleles	Label Volume (ul)	Forward Primer Volume (ul)	Reverse Primer Volume (ul)
GmuD55	175-220	Universal NED	GTG ATA CTC TGC AAC CCA TCC	TTG CAT TCA GAA TAT CCA TCA G	1	12	1.0	9.0	10.0
GmuD90	122-134	Universal FAM	ATA GCA GGA CAA TTA CCA CCA G	CCT AGT TGC TGC TGA CTC CAC	1	3	0.5	9.5	10.0
GmuD87	190-255	Universal VIC	AAA CCC TAA GAC ATC AGA CAG G	CAA ATC CAG TAC CCA GAA AGT C	1	10	1.0	9.0	10.0
GmuD88	115-171	Universal VIC	AAC AAT GCC TGA AAA TGC A C	AGG CTA CCT CTG AAA ATG CTG	2	12	0.5	9.5	10.0
Cp2	187-229	Universal PET	C TCT AAG GGT TGC ACT TCT CAA A	GAG GTG GCA TCA AAA CAT CAT	2	9	1.0	9.0	10.0
GmuD28	180-230	Universal NED	AGC TGT TTG TCA TCA TAC ACT CTC	TGG CCC TCA TGT TTT ATA AGT G	2	14	2.0	8.0	10.0
BTCA9	147-188	Universal FAM	TAC TCA AGA TTT GAA GCA GAT ACA	GGC TTG ATT CTA CTG TCA CTT AC	2	11	1.0	9.0	10.0
Eb19	97-110	Universal NED	AGG GCT CTG AAG CAC TAA AGT AA	GGC ACT GAA ATA AGA GAA AGT A	3	3	1.0	9.0	10.0
GmuD93	185-389	Universal VIC	AGA CTC TCT TGA CCA GAT TTT CTC	TCT GCC TTC TAT CAC TCT CCT G	3	2	1.0	9.0	10.0
GmuD107	189-209	Universal FAM	GAC AAA CAT GAA CAG GAG AAG AG	ATT AGA GAG ACA GAT AGA TAG GAC TTG	3	10	1.0	9.0	10.0
Eb17	94-117	Universal VIC	CCC ACA AAA GTA GAC ACC TAT	GGC ACT GAA ATA AGA GAA AGT A	4	6	1.0	9.0	10.0
GmuD121	138-178	Universal FAM	GGCAAA TAT CCA ATA GAA ATC C	CAA CTT CCT CGT GGG TTC AG	4	7	1.0	9.0	10.0
GmuD79	164-192	Universal PET	GCC CTG TTC CAT TCT TAT TCT G	ATC CCC TTA GTC GTC TCT TTT C	4	1	1.0	9.0	10.0
GmuD16	149-210	Universal NED	ATC CCT GAA ATT TTG TGT GTT C	TTT ACT CTA GAA GGG GCA ATC C	5	15	0.5	9.5	10.0
GmuD40	182-285	Universal PET	T TTG TCA TAT CAT CCA CTC ACC	TTT GTC ACA GAT GGG AAT TAG C	5	25	2.0	8.0	10.0

Table S2. Summary of pairwise F_{ST} (above diagonal) and D (Jost D) (below diagonal). Bold values do not include 0 in the 95% confidence intervals.

|	IN01	IN06	IN07	MI05	MI06	MI07	MI10	MI15	OH01	OH06	OH08	OH09	OH13	OH16	OH17	OH18
IN01	0	0.0284	0.0441	**0.0800**	**0.0945**	**0.0681**	0.0541	0.0695	**0.0683**	**0.1465**	**0.1727**	**0.1739**	**0.1418**	**0.1349**	**0.2093**	**0.0758**
IN06	**0.0448**	0	0.0283	0.0556	**0.0706**	**0.0548**	0.0491	0.0507	0.0652	**0.1395**	**0.1314**	**0.1446**	**0.0957**	**0.1112**	**0.1188**	**0.0543**
IN07	**0.0618**	**0.0451**	0	0.0422	**0.0683**	0.0332	0.0360	0.0281	**0.0528**	**0.0972**	**0.1133**	**0.1243**	**0.0928**	**0.1049**	**0.1494**	0.0297
MI05	**0.0786**	**0.07**	**0.062**	0	0	0.0052	0	0	0.0117	**0.0520**	**0.0492**	**0.0549**	**0.0420**	**0.0410**	**0.0914**	0
MI06	**0.0848**	**0.0844**	**0.0646**	0	0	0	-0.0035	0	0	**0.0497**	**0.0338**	**0.0322**	0.0195	**0.0441**	**0.1013**	0
MI07	**0.0676**	**0.0650**	**0.0418**	0.0084	0.0021	0	-0.0051	0	0.0031	**0.0422**	**0.0361**	**0.0258**	0.0147	**0.0284**	**0.0930**	0.0027
MI10	**0.0643**	**0.0708**	**0.0573**	0.0035	-0.0123	-0.0056	0	-0.0079	0.0038	**0.0499**	**0.0495**	0.0132	0.0112	**0.0267**	**0.0995**	0
MI15	**0.0812**	**0.0708**	**0.0534**	0.0023	0.0068	-0.001	-0.0126	0	0.0012	0.0308	0.0304	0.0187	0.0043	0.0080	**0.0595**	0
OH01	**0.0868**	**0.0689**	**0.0498**	**0.0206**	0.0013	0.0075	0.0052	0.0025	0	0.0048	0.0150	0.0185	0.0089	0.0175	**0.0597**	0.0106
OH06	**0.1266**	**0.1103**	**0.0714**	**0.0467**	**0.0344**	**0.0304**	**0.0367**	**0.0324**	0.0164	0	0	0	0	0.0089	**0.0860**	**0.0380**
OH08	**0.1257**	**0.1062**	**0.0804**	**0.0421**	**0.0251**	**0.0311**	**0.0368**	**0.0341**	**0.0177**	0.0054	0	0.0021	0.0087	0.0114	**0.0929**	0.0259
OH09	**0.1236**	**0.1121**	**0.0862**	**0.0485**	**0.0305**	**0.0304**	**0.0355**	**0.0298**	**0.0274**	-0.0011	0.0103	0	0	0.0109	**0.0638**	0.0243
OH13	**0.1048**	**0.0884**	**0.0643**	**0.0325**	**0.0275**	**0.0142**	**0.0226**	0.0096	0.0170	0.0069	0.0148	0.0076	0	0.0092	**0.0583**	0.0085
OH16	**0.1106**	**0.1034**	**0.0834**	**0.0435**	**0.0342**	**0.0246**	**0.0268**	0.0178	**0.0216**	0.0170	0.0256	0.0191	0.0114	0	**0.0680**	0.0262
OH17	**0.1488**	**0.1071**	**0.1055**	**0.0907**	**0.0798**	**0.0678**	**0.0842**	**0.0626**	**0.0615**	0.0656	0.0715	0.0582	0.0548	0.0473	0	**0.0516**
OH18	**0.0896**	**0.0661**	**0.0509**	0.0015	0.0062	0.0111	-0.0014	0.0042	0.0253	**0.0449**	0.0367	0.0461	0.0293	0.0373	0.0721	0

Number of Alleles vs Location Sample Size
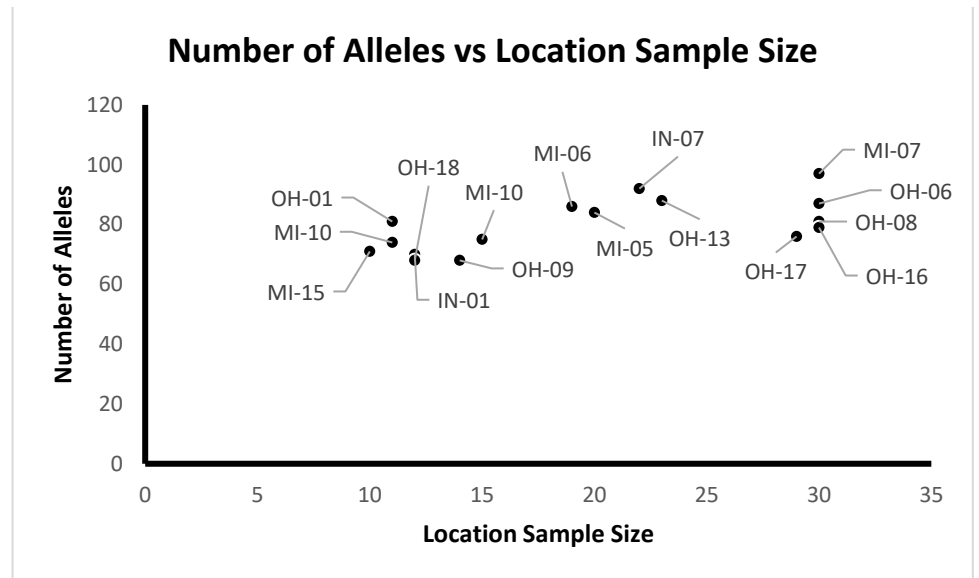
Figure S1. Number of alleles detected at locality versus sample size by locality. Number of allels vs sample size shows relative levels of genetic diversity by locality.
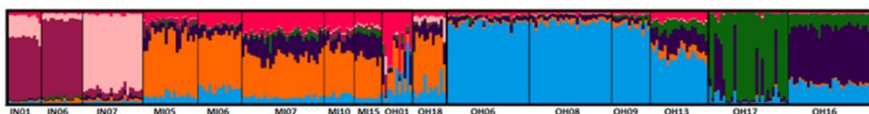
Figure S2. MedMeaK and MedMedK values reducing inclusion of extra clusters, estimated using the methods of Puechmaille (2016). Models are based on STRUCTURE using LOCPRIOR at a .5 threshold. Showing bar graphs for (a) K=6 and (b) K=7.
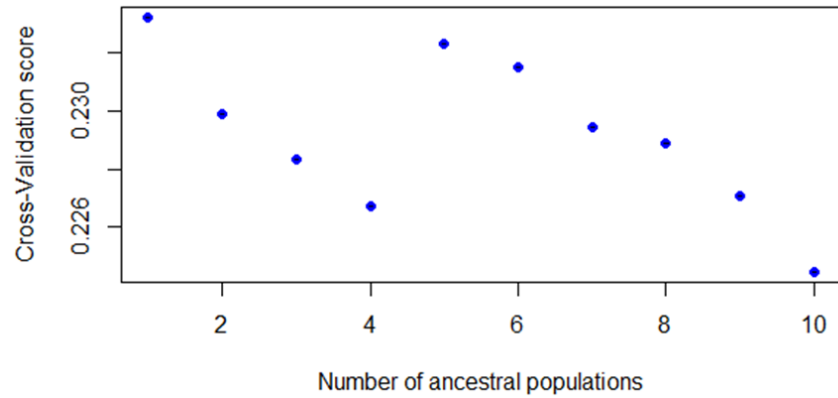

(**a**)
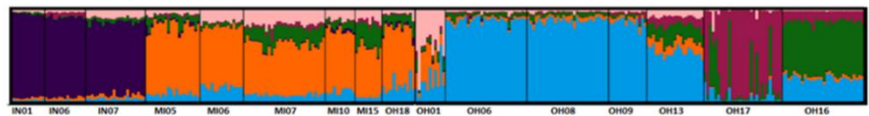
(**b**)

(**a**)

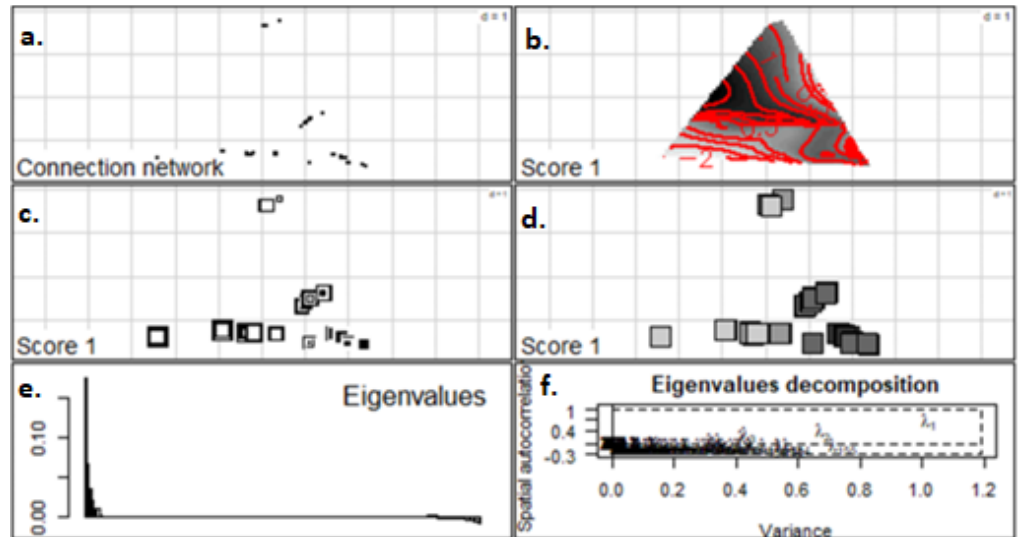Figure S3.Cross validation score for inferring number of clusters.

Figure S4. Spatial Principal Component Analysis (**a**) connection network based on overlap in home range, (**b**) local structure displayed using contour lines (closer line represent increased differentiation), (**c**) global structure displayed through color and size (smaller squares are less differentiated), (**d**) global structure displayed through grayscale, (**e**) eigenvalues, where positive represents global structure and negative values are local structure, (**f**) decomposition in eigenvalues indicating where the majority of variation is held.
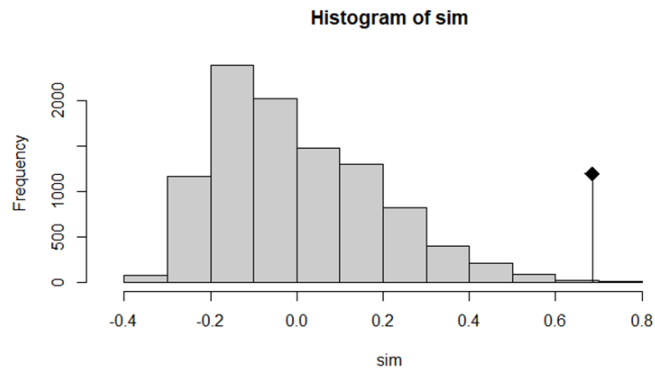
Histogram of sim

Figure S5. Histogram of Mantel test for isolation by distance (IBD).

Table S3. Locality Grouping based on Tes3r cluster assignment.

Tess3r Cluster	N	Sites
Cluster 01	49	IN01, IN06, IN07
Cluster 02	108	MI05, MI06, MI07, MI10, MI15, OH01, OH18
Cluster 03	97	OH06, OH08, OH09, OH13
Cluster 04	59	OH16, OH17

Table S4. **Mean historic migration calculated through Migrate, recent migration rate from BayesAss. Migrate indicates the mean migration rate with 95% confidence interval. BayesAss displays mean migration rate with 95% confidence interval derived from migration rate by population size.**

TESS3r Cluster pair	Historic Migration Rate	Recent Migration Rate
Cluster 01 - 02	0.0038 (±0.0035)	0.08 (±0.12)
Cluster 01 - 03	0.0087 (±0.0045)	0.08 (±0.12)
Cluster 01 - 04	0.0046 (±0.0037)	0.09 (±0.13)
Cluster 02 - 01	0.0091 (±0.0045)	0.08 (±0.12)
Cluster 02 - 03	0.0091 (±0.0047)	0.08 (±0.13)
Cluster 02 - 04	0.011 (±0.0048)	0.09 (±0.13)
Cluster 03 - 01	0.0085 (±0.0044)	0.08 (±0.13)
Cluster 03 - 02	0.0056 (±0.0052)	0.08 (±0.12)
Cluster 03 - 04	0.0094 (±0.0046)	0.09 (±0.13)
Cluster 04 - 01	0.0057 (±0.0042)	0.08 (±0.12)
Cluster 04 - 02	0.0054 (±0.0040)	0.08 (±0.13)
Cluster 04 - 03	0.0067 (±0.0039)	0.08 (±0.13)

Table S5. Total emigration, total immigration, and net emigration for mean historic (Migrate) and recent (BayesAss) migration. A positive value for net emigration suggest that the cluster is a source of migrants.

	Total Emigration		Total Immigration		Net Emigration	
	Mean Historic	Mean Reccent	Mean Historic	Mean Reccent	Mean Historic	Mean Reccent
Cluster 01	0.0017	0.25	0.0233	0.24	-0.0062	+0.01
Cluster 02	0.0292	0.25	0.0148	0.24	+0.0144	+0.01
Cluster 03	0.0235	0.25	0.0245	0.24	-0.0010	+0.01
Cluster 04	0.0178	0.24	0.0250	0.27	-0.0072	-0.03

R Code for Mantel Test:

```
install.packages("ade4")
library(ade4)
library(adegenet)

EBgp=read.genepop("EBgp.gen")
EB=genind2genpop(EBgp)
Dgen=dist.genpop(EB, method = 2)

loc=read.table("SiteLoc.csv", sep=",", header = T)
Fst=read.matrix("Fst.csv",header = FALSE,sep=",")
GeoDis=dist(cbind(loc$x,loc$y))

mant=mantel.randtest(m1=GeoDis, m2=Dgen, nrepet = 9999)
mant
plot(mant)
```

Table S6. Summary of descriptive statistics by Cluster derived from TESS3r including number of turtles sampled (N), total number of alleles (A), number of private alleles (PA), mean allelic richness (AR with SE), mean observed heterozygosity (H_O with SE), expected heterozygosity (H_E with SE), and inbreeding coefficient (F_{IS} includes 95% confidence interval).

Site	State	County	N	A	PA	AR	H_O	H_E	F_{IS}
Cluster-01	IN		49	122	19	8.37 (0.71)	0.66	0.68	0.03 (-0.02-0.08)
							(0.03)	(0.03)	
Cluster-02	MI/O		10	138	19	8.48 (0.67)	0.65	0.68	0.04 (0.01-0.08)
	H		8				(0.02)	(0.03)	
Cluster-03	OH		97	107	4	6.94 (0.52)	0.66	0.67	0.02 (-0.01-0.06)
							(0.02)	(0.02)	
Cluster-04	OH		59	92	4	6.19 (0.33)	0.69	0.68	-0.01 (-0.06-0.04)
							(0.02)	(0.02)	

Table S7. Summary of Bottleneck tests and effective population (Ne) at the .05 and .02 P_{Crit} values size estimates with 95% confidence intervals. Low p values <.05 represent heterozygosity deficiency rather than excess.

	BOTTLENECK			Effective Population Size	
Site	*Wilcoxon test*	*Sign test*	*Mode shift*	Ne 0.05 95% CI	Ne 0.02 95% CI
Cluster-01	P= 0.976	P= 0.038	none	154.4 (80.3-805.3)	180.5 (111.2-424.8)
Cluster-02	P= 0.992	P= 0.070	none	3344.9 (362.0-∞)	535.7 (282.8-3037.1)
Cluster-03	P= 0.916	P= 0.035	none	790.7 (251.4-∞)	685.2 (286.5-∞)
Cluster-04	P= 0.927	P= 0.107	none	19.7 (16.1-24.2)	25.9 (21.7-31.3)