

Table S1 Coordinate of 288 sites on Hainan island

site	rep	longitude	latitude
baisha	1	109.444094	19.22307007
baisha	2	109.45559	19.22394146
baisha	3	109.4637401	19.22692614
baisha	4	109.5081314	19.26196407
baisha	5	109.4864875	19.22656266
baisha	6	109.4864264	19.21092889
baisha	7	109.486872	19.19789457
baisha	8	109.489405	19.182284
baisha	9	109.436402	19.21794058
baisha	10	109.4370352	19.21323176
baisha	11	109.4309794	19.20568916
baisha	12	109.4298171	19.21695702
baisha	13	109.4256377	19.22077007
baisha	14	109.4207046	19.21801799
baisha	15	109.4173209	19.2139298
baisha	16	109.4093235	19.21084268
baoting	1	109.66239	18.66244404
baoting	2	109.6944908	18.63942676
baoting	3	109.7291388	18.63631371
baoting	4	109.7636752	18.62425128
baoting	5	109.7838744	18.61245518
baoting	6	109.8120893	18.60542095
baoting	7	109.8291111	18.60305381
baoting	8	109.851971	18.59534635
baoting	9	109.7941667	18.62645277
baoting	10	109.794169	18.64337637
baoting	11	109.7995035	18.65483136
baoting	12	109.8136643	18.65463949
baoting	13	109.785609	18.65779861
baoting	14	109.7737944	18.66773344
baoting	15	109.7623331	18.67449541
baoting	16	109.7530419	18.69463775
changjiang	1	109.0472901	19.25568905
changjiang	2	109.053252	19.25110582
changjiang	3	109.057991	19.24467687
changjiang	4	109.0726085	19.24616534
changjiang	5	109.085303	19.24502808
changjiang	6	109.0922606	19.25394093
changjiang	7	109.0715631	19.23633891
changjiang	8	109.0762288	19.22458323
changjiang	9	109.0404676	19.26083821

changjiang	10	109.0293382	19.26238954
changjiang	11	109.0172222	19.26243193
changjiang	12	109.0049811	19.25538406
changjiang	13	109.0023977	19.23966252
changjiang	14	108.9819791	19.24459415
changjiang	15	108.9472015	19.22234142
changjiang	16	108.8908262	19.23008818
chengmai	1	110.0115439	19.72666369
chengmai	2	110.017611	19.72001383
chengmai	3	110.0276965	19.71551736
chengmai	4	110.0397449	19.72479578
chengmai	5	110.043895	19.70968899
chengmai	6	110.047425	19.74580863
chengmai	7	110.076619	19.74900551
chengmai	8	110.1030502	19.72926674
chengmai	9	109.9863272	19.72030841
chengmai	10	109.9738324	19.71211686
chengmai	11	109.9854394	19.69260046
chengmai	12	109.9699986	19.68746054
chengmai	13	109.9550006	19.69833293
chengmai	14	109.9769644	19.67053176
chengmai	15	109.9657892	19.67440003
chengmai	16	109.9839268	19.66006136
danzhou	1	109.5602727	19.50562703
danzhou	2	109.5529277	19.50243922
danzhou	3	109.5439198	19.50629811
danzhou	4	109.5340272	19.50514299
danzhou	5	109.5226393	19.49937022
danzhou	6	109.5097061	19.50030676
danzhou	7	109.541055	19.49125299
danzhou	8	109.5301027	19.5230948
danzhou	9	109.6090284	19.53236287
danzhou	10	109.6174976	19.53353659
danzhou	11	109.624214	19.52644543
danzhou	12	109.6350722	19.5300263
danzhou	13	109.6432356	19.54102831
danzhou	14	109.6524812	19.53976543
danzhou	15	109.6519998	19.55698255
danzhou	16	109.6389109	19.5515003
dingan	1	110.3291137	19.68022398
dingan	2	110.32075	19.66769933
dingan	3	110.3264829	19.653987
dingan	4	110.3185297	19.63108941
dingan	5	110.3415708	19.7007701

dingan	6	110.3665147	19.7080937
dingan	7	110.3408508	19.72107043
dingan	8	110.3164224	19.71232581
dingan	9	110.38292	19.71505056
dingan	10	110.3949252	19.70346415
dingan	11	110.3960129	19.68977517
dingan	12	110.399692	19.67336887
dingan	13	110.4040792	19.662223
dingan	14	110.3836795	19.65871868
dingan	15	110.3800844	19.64581875
dingan	16	110.386544	19.62612211
dongfang	1	108.6863732	19.05317308
dongfang	2	108.7053506	19.0605214
dongfang	3	108.7207073	19.06592378
dongfang	4	108.7271356	19.08932392
dongfang	5	108.7552365	19.06295837
dongfang	6	108.7855228	19.09071934
dongfang	7	108.8072923	19.11066598
dongfang	8	108.8508069	19.09563516
dongfang	10	108.6834604	19.04728259
dongfang	11	108.6927733	19.04652828
dongfang	12	108.7036641	19.04619056
dongfang	13	108.7080109	19.05130501
dongfang	14	108.7196377	19.04996637
dongfang	15	108.7339322	19.04167296
dongfang	16	108.7497221	19.0424024
haikou	3	110.3510585	19.98725056
haikou	4	110.3818203	19.97216116
haikou	5	110.3287991	19.97159515
haikou	1	110.3482861	19.98160873
haikou	2	110.341063	19.97562581
haikou	6	110.3736297	19.96355952
haikou	7	110.3797849	19.96815766
haikou	8	110.3261973	19.96275668
haikou	9	110.3532483	19.96184856
haikou	10	110.3640027	19.96125185
haikou	11	110.3938698	19.95290993
haikou	12	110.3211356	19.9399819
haikou	13	110.2571399	19.97418672
haikou	14	110.3115511	19.9655684
haikou	15	110.234584	19.98002614
haikou	16	110.3404615	19.92332854
ledong	1	108.9881833	18.43505368
ledong	2	108.9976152	18.44985862

ledong	3	109.0076125	18.46845868
ledong	4	109.0359943	18.46742337
ledong	5	109.0592097	18.45849667
ledong	6	108.929613	18.4760142
ledong	7	108.8989942	18.48611368
ledong	8	108.9023219	18.50368937
ledong	9	108.9158796	18.52024128
ledong	10	108.9426424	18.5311759
ledong	11	108.974021	18.53595477
ledong	12	109.0023177	18.55427715
ledong	13	109.0253735	18.57426917
ledong	14	109.0454786	18.56639859
ledong	15	109.0714601	18.60755608
ledong	16	109.0934255	18.55831596
lingao	1	109.6940229	19.91874059
lingao	2	109.700593	19.9290337
lingao	3	109.7095595	19.94063392
lingao	4	109.7180076	19.93538291
lingao	5	109.7262378	19.95216357
lingao	6	109.7117734	19.95654873
lingao	7	109.6902781	19.95783899
lingao	8	109.6934634	19.94519011
lingao	9	109.675415	19.90268639
lingao	10	109.6719024	19.897122
lingao	11	109.6613816	19.89569517
lingao	12	109.6752034	19.88904636
lingao	13	109.6859886	19.89134994
lingao	14	109.689743	19.87838477
lingao	15	109.6764133	19.87124918
lingao	16	109.6848676	19.86936319
lingshui	1	110.0254965	18.52569987
lingshui	2	110.0139032	18.53002926
lingshui	3	109.997521	18.52761019
lingshui	4	109.9986785	18.53736001
lingshui	5	109.9734615	18.53926942
lingshui	6	109.9571525	18.54826883
lingshui	7	109.9800331	18.55469039
lingshui	8	109.956552	18.58462683
lingshui	9	110.0371766	18.5553822
lingshui	10	110.0274279	18.57970405
lingshui	11	110.0321402	18.60419791
lingshui	12	110.0324097	18.62721737
lingshui	13	110.0130333	18.64707045
lingshui	14	110.0238219	18.66539345

lingshui	15	110.0520642	18.6742816
lingshui	16	109.9457972	18.61366223
qionghai	1	110.4804949	19.23309694
qionghai	2	110.4889511	19.22925641
qionghai	3	110.4961797	19.22271068
qionghai	4	110.487519	19.20876275
qionghai	5	110.4897077	19.1887976
qionghai	6	110.4602252	19.20924663
qionghai	7	110.4247221	19.247284
qionghai	8	110.37852	19.28859756
qionghai	9	110.4471443	19.3014216
qionghai	10	110.4549888	19.28652347
qionghai	11	110.4452613	19.2630424
qionghai	12	110.4911472	19.27233962
qionghai	13	110.4897097	19.29461102
qionghai	14	110.5749009	19.28347612
qionghai	15	110.5735953	19.31459144
qionghai	16	110.5097469	19.34177661
qiongzhang	1	109.834738	19.03192987
qiongzhang	2	109.843142	19.03068998
qiongzhang	3	109.8519171	19.03360567
qiongzhang	4	109.8638711	19.03517539
qiongzhang	5	109.8598415	19.04305076
qiongzhang	6	109.8698578	19.04127925
qiongzhang	7	109.86769	19.03384952
qiongzhang	8	109.8590458	19.03693494
qiongzhang	9	109.8580419	19.04513738
qiongzhang	10	109.8584749	19.05125355
qiongzhang	11	109.8609582	19.05862883
qiongzhang	15	109.9128974	19.08179082
qiongzhang	16	109.9587171	19.09386671
qiongzhang	13	109.9243298	19.05967299
qiongzhang	12	109.8806009	19.11020764
qiongzhang	14	109.935035	19.11033261
sanya	1	109.4798805	18.3245759
sanya	2	109.4787309	18.33396369
sanya	3	109.4711029	18.35098799
sanya	4	109.4818692	18.36620337
sanya	5	109.5159113	18.35558627
sanya	6	109.5293812	18.36140672
sanya	7	109.4371558	18.34414747
sanya	8	109.4248041	18.35121894
sanya	9	109.5439071	18.31225469
sanya	10	109.5519114	18.31451577

sanya	11	109.5586828	18.32015442
sanya	12	109.5618864	18.32858284
sanya	13	109.5691438	18.33572345
sanya	14	109.5560948	18.33254003
sanya	15	109.5655484	18.34337267
sanya	16	109.5892231	18.34566001
tunchang	1	110.0736061	19.35226105
tunchang	2	110.0882252	19.34791834
tunchang	3	110.1145546	19.34438413
tunchang	4	110.1168062	19.32470359
tunchang	6	110.1480431	19.3248384
tunchang	8	110.052032	19.31073438
tunchang	10	110.1451774	19.40290836
tunchang	11	110.1655523	19.40041416
tunchang	12	110.1856587	19.39473527
tunchang	15	110.1913113	19.37539716
tunchang	16	110.1703342	19.34882372
tunchang	13	110.1537935	19.33983313
tunchang	14	110.1324513	19.33769348
tunchang	7	110.1450078	19.33249863
tunchang	5	110.1224244	19.33300818
tunchang	9	110.1881429	19.36128231
wanning	1	110.3796893	18.82444574
wanning	2	110.3614144	18.82332553
wanning	3	110.3493349	18.79895353
wanning	4	110.362546	18.77587161
wanning	5	110.315488	18.78113122
wanning	6	110.2728044	18.77330952
wanning	7	110.343347	18.76876062
wanning	8	110.3357359	18.75860056
wanning	8	110.3076183	18.77786647
wanning	9	110.249487	18.77125499
wanning	10	110.2285796	18.7641745
wanning	11	110.2276533	18.77719143
wanning	12	110.2187318	18.78525072
wanning	13	110.2177754	18.75731926
wanning	14	110.2401821	18.76141636
wanning	15	110.2396401	18.75772807
wanning	16	110.2450819	18.75168131
wenchang	1	110.7668401	19.62421173
wenchang	2	110.75206	19.6352641
wenchang	3	110.7478943	19.64042979
wenchang	4	110.7537541	19.64732029
wenchang	5	110.7446605	19.64532837

wenchang	6	110.7378271	19.65106631
wenchang	7	110.7273189	19.6562829
wenchang	8	110.7200869	19.65617575
wenchang	9	110.7298707	19.66315114
wenchang	10	110.7383611	19.66556824
wenchang	11	110.7629617	19.68000756
wenchang	12	110.7413783	19.67097746
wenchang	13	110.7458332	19.67539191
wenchang	14	110.7486277	19.68432664
wenchang	15	110.7712854	19.67781304
wenchang	16	110.8030927	19.62731769
wuzhishan	1	109.5288089	18.77039715
wuzhishan	2	109.5435339	18.75906253
wuzhishan	3	109.5573701	18.74478385
wuzhishan	4	109.5786342	18.7415928
wuzhishan	5	109.6031492	18.74389898
wuzhishan	6	109.5779342	18.72234347
wuzhishan	7	109.5629901	18.73132723
wuzhishan	8	109.6330215	18.75457154
wuzhishan	9	109.4958805	18.76958322
wuzhishan	10	109.4812044	18.78067665
wuzhishan	11	109.4593729	18.79095263
wuzhishan	12	109.4392582	18.8000065
wuzhishan	13	109.4028129	18.80377036
wuzhishan	14	109.4879505	18.75159524
wuzhishan	15	109.4801938	18.72958926
wuzhishan	16	109.4744554	18.71799049

Table S2 order assigned using NCBI database

id	order_name	reads
1	Primates	1755899
2	Bacteria	9000726
3	Unassigned	9710092
4	Cypriniformes	1401692
5	Fusobacteriales	357208
6	Gobiiformes	253438
7	Pseudomonadales	1465415
8	Euglenales	126191
9	Synechococcales	482857
10	Siluriformes	84710
11	Galliformes	27466
12	Anura	168280
13	Bacillales	77751

14	Anabantiformes	45364
15	Anseriformes	59382
16	Xanthomonadales	16323
17	Chroococcales	114963
18	Squamata	49309
19	Burkholderiales	158112
20	Beloniformes	20460
21	Testudines	27605
22	Rhizobiales	15351
23	Deinococcales	2053
24	Mugiliformes	8629
25	Scombriformes	4685
26	Artiodactyla	6428
27	Cichliformes	16753
28	Pleuronectiformes	1857
29	Flavobacteriales	472
30	Oscillatoriales	2849
31	Gruiformes	145
32	Desulfovibrionales	1738
33	Synbranchiformes	92
34	Cyprinodontiformes	3529
35	Pasteurellales	458
36	Candidatus Nanopelagicales	1714
37	Petrotogales	1324
38	Clupeiformes	81
39	Alteromonadales	1036

TABLE S3 Species occurrences, expressed as the percentage of sites where each species was found using eDNA metabarcoding or traditional methods

Species	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
<i>Duttaphrynus melanostictus</i>	74.31	50.69
<i>Fejervarya kawamurai</i>	1.04	0.00
<i>Fejervarya multistriata</i>	69.79	68.75
<i>Hoplobatrachus rugulosus</i>	80.21	18.40
<i>Hyla simplex</i>	90.97	16.32
<i>Kalophrynus pleurostigma</i>	2.78	0.35
<i>Kaloula pulchra</i>	1.04	0.00
<i>Limnonectes fragilis</i>	1.74	0.00
<i>Microhyla butleri</i>	1.74	0.00
<i>Microhyla ornata</i>	83.68	76.74
<i>Microhyla pulchra</i>	2.78	2.43
<i>Micryletta inornata</i>	1.04	0.00
<i>Occidozyga martensii</i>	1.04	0.00

<i>Polypedates megacephalus</i>	4. 86	3. 13
<i>Sylvirana guentheri</i>	5. 56	2. 78
