Supplementary Materials

Table S1: Primers used in this study

I. Primer for amplification of mitochondrial control region (CR)

Name	Sequence (5'-3')	Purpose
Noto_Cytb_F	GTTATTCTCACCTGAAT TGGTGGCATG	Amplify the region across Cytb and $tRNA^{Glu}$ (first half of CR) in *T. bernacchii*, *T. newnesi*, *T. eulepidotus*, *P. borchgrevinki*, *P. antarcticum*, *N. coriiceps*, *N. rossii*, *N. angustata*, *N. microlepidota*, *L. squamifrons*, *H. antarcticus*, *H. velifer*, *P. cerebropogon*, *P. scotti*, *R. glacialis*, *C. rastrospinosus*, *C. myersi*, *C. aceratus*, *C. esox*
Noto_Glu_R	ACGATGGCTTTTCATGT CTTTAGTC	
Noto_Glu_F	GACTAAAGACATGAAA AGCCATCGT	Amplify the region across $tRNA^{Glu}$ and 12SrRNA (2nd half of CR) *in N. angustata*, *N. coriiceps*, *H. antarcticus*, *P. scotti*, *R. glacialis*, *C. myersi and C. aceratus*, *C. rastrospinosus*, and entire CR in *P. urvillii*
Noto_12S_R	CTTGGGCTAAGATTGTG CCTGATA	
Pa_12S_R	GATCCTCCTAGAGGAGC CTGTTC	Pair with Noto_Cytb_F to amplify the region across Cytb and 12SrRNA (entire CR) in *P. antarcticum*
Na_CR_R	GCTCTGGCACTGGGCGA CTAATG	Pair with Noto_Cytb_F to amplify the gap between two parts of CR in *N. angustata*
Ha_CR_R	TCTCTCTGAGTGGAGGG GGGCTA	Pair with Noto_Cytb_F to amplify the gap between two parts of CR in *H. antarcticus*
CaCmCr_CR_R	GGAGTTAGCGGTGGGA GTTAAAATCT	Pair with Noto_Cytb_F to amplify the gap between two parts of CR in *C. aceratus*, *C. myersi* and *C. rastrospinosus*
Nc_CR_R	TTTATGGGGCGGCACAG AGTAGT	Pair with Noto_Cytb_F to amplify the gap between two parts of CR in *N. coriiceps*

Ps_CR_R	AGAAACTGCCAAGAAA ACCAAAT	Pair with Noto_Cytb_F to amplify the gap between two parts of CR in *P. scotti*
Rg_CR_R	GGGCTTTAAGGGTTCCT GTAGTTGA	Pair with Noto_Cytb_F to amplify the gap between two parts of CR in *R. glacialis*
Em_Cytb_F	CGCATCCGTCCTCTACT TCTCTATTTT	Pair with Em_12S_R to amplify the entire CR in *E. maclovinus*
Em_12S_R	AACAAGGAARGGTGAG GTTGAACGGG	
Bv_ Cytb_F	GCAAAYGTTGYTATTCT YACCTGAAT	Pair with Noto_12S_R to amplify the entire CR in *B. variegatus*

II. Primer for amplification of mitochondrial ND5-Cytb

Name	Sequence (5'-3')	Purpose
EleND5-extFor[a]	CTMAAYAWGCCCCTKA TYACAACC	Amplify the region across ND5 and Cytb in *N. angustata*
EleCytb-extRev[a]	ATTTTTARGAGGGGGTG TGTTTY	
Rg_ND5_F	TCTTTATATTAASCCTCG CCCTTGT	Amplify the region across ND5 and Cytb in *R. glacialis*
Rg_Cytb_R	GGTAAGACGTATCCGAC GAAAGC	
PaNcPu_3tRNA _F	AGGAACCAAAAACTCTT GGTGCAAATCCAA	Pair with PaNc_Cytb_R to amplify the region across ND5 and Cytb in *P. antarcticum* and *N. coriiceps*, and pair with Noto_Glu_R to amplify ND5 and ND6 in *P. urvillii*
PaNc_Cytb_R	ATGTGAGGAGGGGTTAC AAGGGG	Pair with PaNcPu_3tRNA_F to amplify the region across ND5 and Cytb in *P. antarcticum* and *N. coriiceps*
Pa_Cytb_F	GCGACAAGATTCCATTC CACCCA	Amplify Cytb in *P. antarcticum*

Pa_Cytb_R	AGAGAAGTACAAGACG GATGCGA	

III. Primer for amplification of mitochondrial ND6 gene and for priming 1st strand ND6 cDNA

Name	Sequence (5'-3')	Purpose
Em_ND6_F	TCTTTTAACTGACCGAA GAGGCCCTCGGC	Prime the cDNA 1st strand synthesis of ND6 gene in *E. maclovinus*
Em_ND6_R	ATGGCATACTTTGTGTA TTTGTTTATGGTGG	Pair with Em_ND6_F in RT-PCR to amplify ND6 gene in *E. maclovinus*
Tn_ND6_F	CCTTTTGATAGGCCGGA CCGG	Prime the cDNA 1st strand synthesis of ND6 gene in *T. newnesi*
Tn_ND6_R	ATGTCATACTGCGGAGA CTTACTTATACTGG	Pair with Tn_ND6_F in RT-PCR to amplify ND6 gene in *T. newnesi*
Hv_ND6 _F	CCTTTTAATTGGCCGTA CAGGCC	Prime the cDNA 1st strand synthesis of ND6 gene in *H. velifer*
Hv_ND6_R	CTTATGTTGGGCGTAGT GGTGG	Pair with Hv_ND6_F in RT-PCR to amplify ND6 gene in *H. velifer*
Rg_ND6 _F	CCTTTTAATTGGCCGCA CCG	Prime the cDNA 1st strand synthesis of ND6 gene in *R. glacialis*
Rg_ND6 _R	GTTATACTTTGGGTATC TAGTTATGTTGGGCG	Pair with Rg_ND6_F in RT-PCR to amplify ND6 gene in *R. glacialis*
Na_ND6 _F	CTACTCAATTGGCCGCA CTGTC	Prime the cDNA 1st strand synthesis of ND6 gene in *N. angustata*
Pb_ND6 _F	CCTTTTGATAGGGCGGA CCG	Prime the cDNA 1st strand synthesis of ND6 gene in *P. borchgrevinki*
Ha_ND6 _F	CCTTTTAATTGACCGTA CCGGCC	Prime the cDNA 1st strand synthesis of ND6 gene in *H. antarcticus*
Ca_ND6 _F	CCTTTTAATTGGCCGCA CCGG	Prime the cDNA 1st strand synthesis of ND6 gene in *C. aceratus*

Noto_ND6_R	ATGYTATAYTGTGGRW ATTTACTTATGYTGGG	Pair with Na_ND6 _F, Pb_ND6 _F, Ha_ND6 _F and Ca_ND6 _F in RT-PCR to amplify ND6 gene in *N. angustata*, *P. borchgrevinki*, *H. antarcticus* and *C. aceratus*, respectively

[a] Papetti et al., 2007

Supplementary Tab. S2
Table S2
Pairwise similarity percentages* of ND6 gene nucleotide and deduced amino acid sequences of 22 Notothenioid species

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*B.variegatus*		61	58	47	47	48	48	49	50	46	52	50	51	45	51	52	52	50	47	50	49	49
2	*P.urvillii*	64		62	53	53	52	56	52	54	52	55	57	56	50	53	52	52	54	52	54	54	53
3	*E.maclovinus*	63	68		50	46	47	50	49	48	48	49	48	50	45	49	50	49	50	50	51	50	50
4	*L.squamifrons*	35	58	59		81	78	76	79	83	69	84	86	85	70	73	73	73	70	71	72	70	71
5	*N.angustata*	59	63	61	73		88	84	89	79	72	81	81	80	70	77	76	77	75	74	74	77	75
6	*N.coriiceps*	58	62	61	73	84		86	97	80	77	83	83	83	75	82	81	82	79	78	78	78	79
7	*N.microlepidota*	59	62	63	72	85	84		85	77	72	78	79	77	74	80	78	79	76	77	77	79	78
8	*N.rossii*	36	61	61	74	84	89	83		79	78	81	81	83	73	83	82	83	79	78	78	78	79
9	*P.borchgrevinki*	33	62	60	82	73	74	72	76		69	92	94	92	71	76	76	76	72	73	74	76	73
10	*P.antarcticum*	59	59	59	70	72	73	72	76	70		70	70	70	67	74	72	74	69	69	70	67	70
11	*T.bernacchii*	37	60	62	83	73	76	73	77	92	71		97	94	73	76	76	76	73	72	75	75	74
12	*T.eulepidotus*	37	61	63	84	74	75	73	77	93	72	94		96	74	76	76	76	73	73	74	75	74
13	*T.newnesi*	37	62	61	84	73	75	73	76	89	72	90	92		72	75	75	75	74	74	74	75	75
14	*H.antarcticus*	59	64	62	70	74	74	74	76	73	71	73	73	72		82	82	82	78	76	77	75	77
15	*H.velifer*	58	63	64	69	76	79	77	80	72	75	73	72	73	84		97	98	85	81	81	81	82
16	*P.cerebropogon*	58	64	64	70	76	79	77	81	73	74	74	73	73	85	96		98	85	81	82	82	82
17	*P.scotti*	59	63	64	71	76	79	76	80	73	74	74	73	74	84	96	98		85	82	82	82	82
18	*R.glacialis*	61	65	66	72	76	77	78	78	73	72	74	74	75	80	83	83	84		88	89	86	89
19	*C.myersi*	59	61	62	70	75	77	76	78	73	71	73	74	74	79	81	81	81	85		95	89	96
20	*C.aceratus*	59	62	65	69	75	77	76	77	72	72	72	73	73	79	81	81	81	88	93		90	96
21	*C.esox*	59	63	63	70	76	76	77	79	73	71	73	73	72	77	79	80	80	84	86	87		90
22	*C.rastrospinosus*	59	61	64	70	76	77	77	78	73	71	72	72	72	78	82	82	82	86	95	94	87	

Note: upper right = Amino acid similarity; Lower left = Nucleotide similarity
* calculated by ClustalW2 with gap parameters set to default: open = 10, extend = 0.05

Supplementary Fig. S1

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                                     cTAS           TAS       cTAS          TAS
                                                              |→ETAS
B.variegatus_CR       -----TGACTGATTTTGCATACTAATACATTATATGTATTATCACCATACATTTATATT 55
P.urvillii_CR         -CAAATAGTATATATGTATTTTTTA-TACATG-TATGTATTATCACCATAAATTTATAAT 57
E.maclovinus_CR       -TAAACCTTCATTGCATGTCCACGAC-ATAC--TATGTATTATCACCATTAATATATATT 56
P.antarcticum_CR1     GCGCCCCCCAAAAATGTACTTTTAAGTACAT--TATGTATTATCAACACTCATTTATATT 58
P.antarcticum_CR2     GCGCCCCCCAAAAATGTACTTTTAAGTACAT--TATGTATTATCAACACTCATTTATATT 58
N.angustata_CR1       --CCGCCCATAGAATGTACTTATAATCATATC-TATGTACTATCACCATACATTTATATT 57
N.angustata_CR2       --CCGCCCATAGAATGTACTTATAATCATATT-TATGTATTATCACCATACATTTATATT 57
N.coriiceps_CR1       --CCGCCCCATAAATGTACTTA-GAGTATATC-TATGTATTATCAACATACATTTATATT 56
N.coriiceps_CR2       --CCGCCCCATAAATGTACTTAGAGT-ATATC-TATGTATTATCAACATACATTTATATT 56
N.microlepidota_CR    --CCGCCCATAG-ATGTACGTACAAACATATC-TATGTATTATCACCATACATTTATATT 56
N.rossii_CR           --CCGCCCCATAAATGTACTTATAAGTACATC-TATGTACTATCACCATACATTTATATT 57
H.antarcticus_CR      ----------TATATGTACTTTTAC--ATGAT-CATGTATTATCAGCATTAATTTATATT 47
R.glacialis_CR1       --CAGCCCTA-AAATGTACCTGAAC--ACATC-TATGTATTACCCCCATAAATTTATATT 54
R.glacialis_CR2       --CAGCCCTA-AAATGTACCTGAAC--ACATC-TATGTATTACCCCCATAAATTTATATT 54
P.scotti_CR           --------CCTATATGTAC-TGTAA--GTGTA-TATGTATTATCAGCAAACATTTATATT 48
C.aceratus_CR         ------CCTATGTCCCTAAATGCAG--GTATG-TATGTATTATCAGCATTCATCTATATT 51
C.myersi_CR           -CCCCCCCTATGTCCGAATAAGCAG--GTATG-TATGTATTATCAGCATTCATCTATATT 56
C.rastrospinosus_CR   GCCCCCCTTATGTCCGAAAATGCAG--GTATG-TATGTATTATCAGCATTCATCTATATT 57
                                                       ***** ** *  **   ** **** *

                ETAS←|      cTAS                       cTAS
B.variegatus_CR       CAACATATCTTGA---TTTCAAGAACA--TATATGTTAATCCACATTAAATTATTTCAA 110
P.urvillii_CR         AACCA-TTAATATCAATGTTTTAAGCACATAAATGTTAAATC--ACCATTAATAATTTAA 114
E.maclovinus_CR       AACCATATATGTA--CATTCAAGGACA--TATTATGTATAATCAACATAAATTGACTCAA 112
P.antarcticum_CR1     AACCACTTAATGG-GCATTCGTGGACA-GGATTTGATTTTAGG-ACAAAATTTGACTCGA 115
P.antarcticum_CR2     AACCACTTAATGG-GCATTCGTGGACG-GGATTTGATTTTAGG-ACAAAATTTGACTCGA 115
N.angustata_CR1       AACCATTTAATAA-GCATTCATGAGCA-ATGTTTGATTATTTT-ACAAAACTTGACTTGC 114
N.angustata_CR2       AACCATTTAATAA-GCATTCATGAGCA-ATGTTTGATTATTTT-ACAAAACTCGACTTGC 114
N.coriiceps_CR1       AACCATTTAATGA-GCATTCGTGGGCA-ATATTTAATTAATTT-ACAAAACTTGCTTCAC 113
N.coriiceps_CR2       AACCATTTAATGA-GCATTCGTGGGCA-ATATTTAATTAATTT-ACAAAACTTGCTTCAC 113
N.microlepidota_CR    AACCATTTAATGA-GCATTCGTGGGCA-ATATTTGATTATTTT-ACAAAACTTGGTTCAC 113
N.rossii_CR           AACCATTTAATGC-GCATTCATGGGCA-ATATTTGATTAATTT-ACAAAACTTGTTTTAA 114
H.antarcticus_CR      AAACATTTAATGG-ACATTCGTGAGCA-ATATTTGATTAATTT-ACAAATCATGTTTAAA 104
R.glacialis_CR1       AATCATTTCGTAG-GAACTCATGAACA-ATGTTTGACAGATTTTACAAATCTTGATTCAC 112
R.glacialis_CR2       AATCATTTCGTAG-GAACTCATGAACA-ATGTTTGACAGATTTTACAAATCTTGATTCAC 112
P.scotti_CR           AACCATTTAACAGATTATCCATGAGTA-ATCTTGGTTATTT--ACAAAATTTGGTTTTC 105
C.aceratus_CR         AACCATTTAACAG-TCATTCACGGGCA-ATACTTGATTAATTA-ACAAATATTGACACTC 108
C.myersi_CR           AAACATTTAAGTC-TCATCCATGGGCA-ATACTTGATTAATTA-ACAAATCTTGACACTC 113
C.rastrospinosus_CR   AAACACTTAAGTC-TCATTCAAGGGCA-ATACTTGATTAATTA-ACAAATCTTGACACTC 114
                        * **   *

                                                      TAS          TAS
B.variegatus_CR       ACCCTCACATAGCACCAAA--GAGAGGAAAAAATACATAAAGCAT-TAATTAAAGTTTGA 167
P.urvillii_CR         -ACCCTACAGG-AACGACA-TTACCAGAAAAGAC-CATGAAGCATACATATACGTCTAAA 170
E.maclovinus_CR       CCCCTATCATA-CGTCAAAAGCACTATTAGAGGTACACGAAGCATACATGAAGATTCAAA 171
P.antarcticum_CR1     CAC-ACAAATA-TATCAGAATTAACGGGTGATATACGCAAAGCAT-TTGAGAAGCCTCA- 171
P.antarcticum_CR2     CAC-ACAAATA-TATCAGAATTAACGGGTGATATACGCAAAGCAT-TTGAGAAGCCTCA- 171
N.angustata_CR1       CACCAGGCAGA-TATCACAACTAACAGTAGGTATACATTAAGCAT-TACATAAGTCTCC- 171
N.angustata_CR2       CACCAGGCAGA-TATCACAACTAACAGTAGGTATACATTAAGCAT-TACATAAGTCTCC- 171
N.coriiceps_CR1       CACCAGGCAGA-TATCACTACTAACAGTAGGTATACATTAAGCAT-TTGATAAGTCTCC- 170
N.coriiceps_CR2       CACCAGGCAGA-TATCACTACTAACAGTAGGTATACATTAAGCAT-TTGATAAGTCTCC- 170
N.microlepidota_CR    CACCAGGCAGA-TATCACAACTAACAGTAGGTATACATTAAGCAT-TAAATAAGTCTCC- 170
N.rossii_CR           CACTAGACAGG-TATCACTACTAACAGTAGGTATACATTAAGCAC-TTCACAAGTCTCC- 171
H.antarcticus_CR      CTCCA-ACACA-TATCAAAACTAATAAAGGGTGTACATTAAGCAT-CAGAAAAGTCTCC- 160
R.glacialis_CR1       CGCCA-ACACA-CATCAAAACTAACAGCAGGTATACATTAAGCAT-TAAATAAG-TCCC- 167
R.glacialis_CR2       CGCCA-ACACA-CATCAAAACTAACAGCAGGTATACATTAAGCAT-TAAATAAG-TCCC- 167
P.scotti_CR           --TTG-GCAGT-TTCTAAA--CATCAAAGATTATACATTAAGCAT-CAAAGAAATCTCCT 158
C.aceratus_CR         CATAA-CCATA-TGTCAAAATTAACAGTAGGTATACATTAAGCAT-TTAAGAAGACTCC- 164
C.myersi_CR           CATAA-ACAGG-TAACAAA-TTAACAGTAGGTATACATTAAGCAT-TTAAGAAGTTTCC- 168
C.rastrospinosus_CR   CATAA-ACAGG-TAACAAA-TTAACAGTAGGTATACATTAAGCAT-TTAAGAAGATTCC- 169
                                 *       *     *           *   *****       *
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B.variegatus_CR          TCCAATAAAAAGATATTAACCAAGCAAGTTTGTTAGCAAACAGCAGCCTGCCAATTTAAT 227
P.urvillii_CR            TATAATG-AACTCAGGGATTAGCGAAA-CTTAAGACCGAACTCATAAT---CCATATGTC 225
E.maclovinus_CR          TACACTAAAATTCTAATACTAGCGAAACTTATAGTCCCAACATAATAC---TCATTAGTT 228
P.antarcticum_CR1        CATAATCCAATTTAATGACAGGCGAAA-TTTAAGACCGAACTCTAACC---TCATTGGTT 227
P.antarcticum_CR2        CATAATCCAATTTAATGACAGGCGAAA-TTTAAGACCGAACTCTAACC---TCATTGGTT 227
N.angustata_CR1          CTTCACCCAATTTAAGGGCAAGCGAGA-TTTAAGACCGAACACTCAAC---TCATAAGTT 227
N.angustata_CR2          CTTCACCCAATTTAAGGGCAAGCGAGA-TTTAAGACCGAACACTCAAC---TCATAAGTT 227
N.coriiceps_CR1          CATAGTCCTATTCCAGGGCAGGCGAAA-CTTAAGACCGAGCGCCAGAT---TCATAAGTT 226
N.coriiceps_CR2          CATAGTCCTATTCCAGGGCAGGCGAAA-CTTAAGACCGAGCGCCAGAT---TCATAAGTT 226
N.microlepidota_CR       CACACCTGAATTTAAGGGCAGGCGAAA-TTTAAGACCGATCACTAAAC---TCATAAGTT 226
N.rossii_CR              CATAATACTATTCCAGGGCAGGCGAAA-TTTAAGACCGAACTCTAAAC---TCATAGGTT 227
H.antarcticus_CR         CACACCTCTATTTAAGGACAGGCGAAAGTTTAAGACCGAACACTCAGA---TCATTAGTT 217
R.glacialis_CR1          ACTAATATGATTTAACGGCAGACGAAA-TTTAAGACCGATCACTAGAAA--TCATTGGTT 224
R.glacialis_CR2          ACTAATATGATTTAACGGCAGACGAAA-TTTAAGACCGATCACTAGAAA--TCATTGGTT 224
P.scotti_CR              ACACTTTTGATCTAAAAGCAGGCGAAA-CTTAAGACCGAGCACCAACA---TCATTAGCT 214
C.aceratus_CR            ACTCACCCAATTTAACAACAGACGAAA-TTTAAGACCGAATACTTAAAT--CCATAAGTT 221
C.myersi_CR              ACTCGCCCAATTTAAGAACAGACGAAA-CTTAAGACCGAATACTTAAAT--CCATAAGTT 225
C.rastrospinosus_CR      ACTCGCCCAATTTTAGAACAGGCGAAA-ATTAAGACCGAACACTCTAAT--CCATAAGTT 226
                                  *             *  *  *      * *              **

                                                                        cTAS  CSB-F
B.variegatus_CR          AAATTAAATAATTCTACCAACAAACTTTTTAACCCAAAAGATTTA-GGCAGGGCGAGAAC 286
P.urvillii_CR            AAGTTATACCATTAC-TCAAACTCC-CGCCAATTCTCAAATCTTT-AATGTAGTAAGAGC 282
E.maclovinus_CR          AAGTTATACCTTTAC-TCAAAATCCCTGCAAAT---AAAAATCTT-AATGTAGTAAGAAC 283
P.antarcticum_CR1        AAGTTATACCTTTAT-CCAACTTCC-TTGCAGTTTACAGATTCTT-AATGTAGTAAGAGC 284
P.antarcticum_CR2        AAGTTATACCTTTAT-CCAACTTCC-TTGCAGTTTACAGATTCTT-AATGTAGTAAGAGC 284
N.angustata_CR1          AAGTTATACCTTTAT-CCACCATCT-CGCCAATTCCCAGATTTTT-AATGTAGTAAGAGC 284
N.angustata_CR2          AAGTTATACCTTTAT-CCACCATCT-CGCCAATTCCCAGATTTTT-AATGTAGTAAGAGC 284
N.coriiceps_CR1          AAGTTATACCTTTAT-CCACCATCT-CGTCAATTCTCAGATTCTT-AATGTAGTAAGAGC 283
N.coriiceps_CR2          AAGTTATACCTTTAT-CCACCATCT-CGTCAATTCTCAGATTCTT-AATGTAGTAAGAGC 283
N.microlepidota_CR       AAGTTATACCTTTAC-CCACCATCT-CGCCAATTCTCAGATTTTT-AATGTAGTAAGAGC 283
N.rossii_CR              AAGTTATACCTTTAT-CCACCATCT-CGCCAATTCTCAGATTTTT-AATGTAGTAAGAGC 284
H.antarcticus_CR         AAGTTACACGTTTAT-TCTGCATCC-CATCAACTCTCACGTTTAA-AGCGCAGTAAGAGC 274
R.glacialis_CR1          AAGTTATACGTTTAT-CCAACATCT-CGCCAAG-CCCACACTTTACAGCGCAGTAAGAGC 281
R.glacialis_CR2          AAGTTATACGTTTAT-CCAACATCT-CGCCAAG-CCCACACTTTACAGCGCAGTAAGAGC 281
P.scotti_CR              AAGTTGTAGATTTAT-CCAACATCTACGTCGACTCTCACATTCTA-AGCGCAGTAAGAGC 272
C.aceratus_CR            AAGTTATACGTTTAC-TTAACATCT-TGTCAGCTCTCACATTTTG-AGCGCAGTAAGAGC 278
C.myersi_CR              AAGTTATACGTTTAC-TTAACATCT-CGTCAAATCTCACATTTTG-AGCGCAGTAAGAGC 282
C.rastrospinosus_CR      AAGTTATACGTTTAC-TTAACATCT-CGCCAAATCTCACATTTTG-AGCGCAGTAAGAGC 283
                         ** **  *   **                        *              *  *** *

                         CSB-F                             CSB-E
B.variegatus_CR          CTACCAA-AAGCG-ATTCCTAAATGTACCTGACTAATGATGGTGAGGTGCAAGAATT-GT 343
P.urvillii_CR            CTACC-ATCAGTTGATTCCTGAATGATAACGGTTATTGAGGGTGAGGGACAAGTATTTGT 341
E.maclovinus_CR          CTACC-ATCCCTATATTACTTAATGCTAACGTTTATTGAAGATGAGGGGCAAGAATT-GT 341
P.antarcticum_CR1        CGACCAACAAGCTCATATCTTAAGGCTCACGGTTATTGAGGGTGAGGGACAAGTAAT-GT 343
P.antarcticum_CR2        CGACCAACAAGCTCATATCTTAAGGCTCACGGTTATTGAGGGTGAGGGACAAGTAAT-GC 343
N.angustata_CR1          CGACCAACCAGCACATATCTTAAGGCTAACGGTTATTGAAGGTGAGGGACAACTATC-GT 343
N.angustata_CR2          CGACCAACCAGCACATATCTTAAGGCTAACGGTTATTGAAGGTGAGGGACAACTATC-GT 343
N.coriiceps_CR1          CGACCAACAAGCACATATCTTAAGGCTAACGGCTAATGAGGGTGAGGTGCAACCATT-GT 342
N.coriiceps_CR2          CGACCAACAAGCACATATCTTAAGGCTAACGGCTAATGAGGGTGAGGTGCAACCATT-GT 342
N.microlepidota_CR       CGACCAACCAGCACATATCTTAAGGCCAACGGTTATTGAAGGTGAGGGACAACCCTC-GT 342
N.rossii_CR              CGACCAACCAGCACATATCTTAAGGCCAACGGCTAATGAGGGTGAGGTGCAATTATT-GT 343
H.antarcticus_CR         CGACCTACAAGCACATATCTTAAGGTCAACGGTTATTGAAGGTGAGGTGCAATTATC-GT 333
R.glacialis_CR1          CGACCAACCAGCACATATCTTAAGGTCAACGGTTATTGATGGTGAGGGACAAGGATT-GT 340
R.glacialis_CR2          CGACCAACCAGCACATATCTTAAGGTCAACGGTTATTGATGGTGAGGGACAAGGATT-GT 340
P.scotti_CR              CGACCAACAAGCACATATCTTAAGGTCAACGGTTATTGAGGGTGAGGGACAATCATC-GG 331
C.aceratus_CR            CGACCTACAAGCACATATCTTAAGGTCAACGGTTATTGAGGGTGAGGGACAATTAAC-GT 337
C.myersi_CR              CGACCTACAAGCACATATCTTAAGGTCAACGGTTATTGAGGGTGAGGGACAATTAAT-GT 341
C.rastrospinosus_CR      CGACCTACAAGCACATATCTTAAGGTCAACGGTTATTGAGGGTGAGGGACAATTAAT-GT 342
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                                         CSB-D
B.variegatus_CR       GGGGGTTACACTCAGTGAATTATTCCTGACATTTGGTTCCTACTTCAGGAGCTCACGGTG 403
P.urvillii_CR         GGGGGTTTCACAGGGTGAACTATTCCTGGCATTTGGTTCCTACTTCAGGGCCATGTATTG 401
E.maclovinus_CR       GGGGGTTATACAGAGTGCACTATTCCTGACATTTGGTTCCTACTTCAGGTTCACCGATTG 401
P.antarcticum_CR1     GGGGGTTTCACATGGTGAACTATTCCTGGCATTTGGTTCCTACTTCAGGGCCAATGATTG 403
P.antarcticum_CR2     GGGGGTTTCACATGGTGAACTATTCCTGGCATTTGGTTCCTACTTCAGGGCCAATGATTG 403
N.angustata_CR1       GGGGGTTTCACACAGTGAATTATTCCCGGCATTTGGTTCCTATTTCAGGGCCAATGATTG 403
N.angustata_CR2       GGGGGTTTCACACAGTGAATTATTCCCGGCATTTGGTTCCTATTTCAGGGCCAATGATTG 403
N.coriiceps_CR1       AGGG-TTTCACACAGTGAACTATTCCTGGCATTTGGCTCCTATTTCAGGGCCAATGATTG 401
N.coriiceps_CR2       AGGG-TTTCACACAGTGAACTATTCCTGGCATTTGGCTCCTATTTCAGGGCCAATGATTG 401
N.microlepidota_CR    GGGGGTTTCACACAGTGAATTATTCCCGGCATTTGGTTCCTACTTCAGGGCCAATGATTG 402
N.rossii_CR           AGGG-TTTCTAACAGTGAACTATTCCTGGCATTTGGCTCCTATTTCAGGGCCAATGATTG 402
H.antarcticus_CR      AGGG-TTACCCAGGGTGAATTATTCCCGGCATTTGGCTCCTATTTCAAGAACAATGATTG 392
R.glacialis_CR1       AGGG-TAACCCACAGTGAATTATTCCTAGCATTTGGCTCCTACTTCAGGAACAATAACTG 399
R.glacialis_CR2       AGGG-TAACCCACAGTGAATTATTCCTAGCATTTGGCTCCTACTTCAGGAACAATAACTG 399
P.scotti_CR           ACAG-TAACATACAGTGCACTATTCCTGACATTTGGCTCCTACTTCAAGGGCAATAGTTG 390
C.aceratus_CR         AGGG-TAACACACTGTGCACTATTCCTGGCATTTGGTTCCTACTTCAGGAACAATGGGTG 396
C.myersi_CR           AGAG-TGACCCATGGTGAATTATTCCTGGCATTTGGCTCCTACTTCAGGAACAATGGGTG 400
C.rastrospinosus_CR   AGAG-TGACCCATGGTGAATTATTCCTGGCATTTGGCTCCTACTTCAGGAACAATGGGTG 401
                         * *        *** * ******   ******* ***** **** *  *      **

B.variegatus_CR       G-GTCATTCCACATACGTTCATCGACGCTAACATAAGTTAATGGTGGAAT-ACATACTCC 461
P.urvillii_CR         ATATTATTCCTCACACTTTCATCGACGCTTACATAAGTTAATGTTTGGAT-ACATACTCC 460
E.maclovinus_CR       ATATTATTCCTCACACTTTCATCGACGCTTGCATAAGTTAATGCTTGTATTACATACTCC 461
P.antarcticum_CR1     GTGTCATCCCCCGCACTTTTACCGACGCTTACATAAGTTAATGTTTGTATTACATACTCC 463
P.antarcticum_CR2     GTGTCATCCCCCGCACTTTTACCGACGCTTACATAAGTTAATGTTTGTATTACATACTCC 463
N.angustata_CR1       GTATCATCCCCCGCACTTTTATCGACGCTTACATAAGTTAATGTTTGTATTACATACTCC 463
N.angustata_CR2       GTATCATCCCCCGCACTTTTATCGACGCTTACATAAGTTAATGTTTGTATTACATACTCC 463
N.coriiceps_CR1       GTAACACCCACCGCACTTTTATCGACGCTTACATATATGAATGTTTTTATTACATACTCC 461
N.coriiceps_CR2       GTAACACCCACCGCACTTTTATCGACGCTTACATATATGAATGTTTTTATTACATACTCC 461
N.microlepidota_CR    GTGTCATCCCCCGCACTTTTATCGACGCTTACATAAGTTAATGTTTGGATTACATACCCC 462
N.rossii_CR           GTATCACCCACCGCACTTTTATCGACGCTTACATAAATCAATGTTTGTATTACATACTCC 462
H.antarcticus_CR      CTATCATTCCCCTCACTTTTACCGACGCTTACATAGGTTAATGCTCTAATAGCATACTCC 452
R.glacialis_CR1       G-GTCACTCACCTCACTTTCACCGACGCTTACATATGTCATGGTTGAGTC--CATACTCC 456
R.glacialis_CR2       G-GTCACTCACCTCACTTTCACCGACGCTTACATATGTCATGGTTGAGTC--CATACTCC 456
P.scotti_CR           GTATCATTCCCTGCACTTTCATCGACGCTTACATTTCTCATGGTTTGTCG--CATACTCC 448
C.aceratus_CR         GTATCATTCCCTCCACGTCCCCTAACGCTTACATTTCTCATGTTTTTAAT--CTTTGTAC 454
C.myersi_CR           GTGTCATCCCCCTCACGTCCCATAACGCTTACATTTGTCATGTTTTTAAT--CCGTGCGC 458
C.rastrospinosus_CR   GTGTCATCCCCCTCACGTCCCATAACGCTTACATTTGTCATGTTTTTAAT--CAATTGAC 459
                           *  *     ** *      *****  ***   * *    *       *      *

B.variegatus_CR       TCGTTACCCACCAAGCCGGGCGTTCACTCCATAGGGTCACTGGTTCTTTTTTTTCTCTTT 521
P.urvillii_CR         TCGTTACCCAGCAAGCCGAGCGTTCACTCCAGCGAGCAAGGGGTTCTCTTTTTTTTTTTT 520
E.maclovinus_CR       TCGTTACCCACCAAGCCGAGCGTTCACTCCACAGGGGCAGCTGGTTCTCTTTTTTTTTTT 521
P.antarcticum_CR1     TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTC-CTTTTTTTTTTTC 522
P.antarcticum_CR2     TCGTTACCCAGCAGGCCGGGCGTTCACTCCAGCGAGCCAGGGGCTCTCTTTTTTTTTTTC 523
N.angustata_CR1       TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTC-TTTTTTCTTTT 522
N.angustata_CR2       TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTC-TTTTTTTTTTT 522
N.coriiceps_CR1       TCGTTACCCAGCA-GCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTCTTTTTTTCTTTT 520
N.coriiceps_CR2       TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTCCTTTTTTTTTTT 521
N.microlepidota_CR    TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTC-TTTTTCTTTTT 521
N.rossii_CR           TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTCTTTTTTTATTT- 521
H.antarcticus_CR      TCGTTACCCACCAAGCCGGGCGTTCACTCTAACGAGCTAGGGGTTCCTTTTTTTTTTC-- 510
R.glacialis_CR1       TCGTTACCCAGCAAGCCGGGCGTTCTCTCCAGCGAGCCAGGGGTTCTCTTTTTTTTT--- 513
R.glacialis_CR2       TCGTTACCCAGCAAGCCGGGCGTTCTCTCCAGCGAGCCAGGGGTTCTCTTTTTTTTTT-- 514
P.scotti_CR           TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTCTTTTTTTTTTTT 508
C.aceratus_CR         TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTCCTTTTTTTTTTT 514
C.myersi_CR           TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGCGAGCCAGGGGTTCTCCTTTTTTTTTT- 517
C.rastrospinosus_CR   TCGTTACCCAGCAAGCCGGGCGTTCACTCCAGGGAGCCAGGGGTTCTCCTTTTTTTTTT- 518
                      ********** ** **** ****** *** *  * *  *   * *    *****
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B.variegatus_CR          CC-TTTCATTTTACTTTTCAGAGTGCACACTCCAATAACTAAGGAAG-TTGAACATTTCC 579
P.urvillii_CR            CC-TTTCACTTGACATTTCAGAGTGCACACGGCTTTAACAGACAAGGTATGAGCATTTTT 579
E.maclovinus_CR          CCTTTTCACTTTGCCCTTCAGAGTGTAAGCCGGCTTAACAGACAAGG-GTGTACATTTCC 580
P.antarcticum_CR1        CTTTCCC-CTTGCATTT-CAGAGTGCGCGCGGTTTTAACTAACAAGC-GTGAGCACTTTT 579
P.antarcticum_CR2        CTTTCTCACTTGCATTTACAGAGTGCGCGCGGTTTTAACTAACAAGC-GTGAGCACTTTT 582
N.angustata_CR1          CCTTTCCTCTGGCATTT-CAGAGTGCACACGGGTTTAACAGACAAGC-GAGAGCATTTTC 580
N.angustata_CR2          CCTTTCCTCTGGCATTT-CAGAGTGCACACGGGTTTAACAGACAAGC-GAGAGCATTTTC 580
N.coriiceps_CR1          CCTTTCCTCTGGCATTT-CAGAGTGCACACGGGTTTAACAGACAAGC-GTGAGCATTTTT 578
N.coriiceps_CR2          CCTTTCACTTGGCATTT-CAGAGTGCACACGGGTTTAACAGACAAGC-GTGTACATTTTT 579
N.microlepidota_CR       CCTTTCCTCTTGCATTT-CAGAGTGCACACGGGTTTAACAGACAAGC-GAGAGCATTTTC 579
N.rossii_CR              CCTTTCCTCTGGCATTT-CAGAGTGCACACGGGTTTAACAGACAAGC-GTGAGCGCTTTC 579
H.antarcticus_CR         -CTTTTCCTCTGGCATTTCAGAGCGCACACGGGTTTAACAGACAAGC-GAGCGCATTTTC 568
R.glacialis_CR1          -CCCTTCAGTGGGCTTTTCAGAGTGCGCACGGGTCTAACAAACAAGA-GGGAGCAGCTTC 571
R.glacialis_CR2          -CCCTTCAGTGGGCTTTTCAGAGTGCGCACGGGTCTAACAAACAAGA-GGGAGCAGCTTC 572
P.scotti_CR              CCTTTTCGGTGGACTTTTCAGAGCGCACACGCATTTAACAAACAAGA-GGGGACACTTAA 567
C.aceratus_CR            -CTTTTCACTTGGCTTTTCAGAGTGCGCACGGTTTTAACAGACAAGG-GGGAGCATATTG 572
C.myersi_CR              -CTTTTCACTTGGCTTTACAGAGTGCGCACGGTTCTAACAGACAAGG-GTGAGCATATTG 575
C.rastrospinosus_CR      -CTTTTCACTTGGCTTTACAGAGTGCGCACGGTCCTAACAGACAAGG-GGGAGCATATTG 576
                                         * ***** *    *     ****  *  *     *  *   *

B.variegatus_CR          TTGGT-TTTATATATTATTTTTTTGAGT--TATAAAGACTTTTCCAAAAGGTTTTC---- 632
P.urvillii_CR            CTTG--CCCCCAAAAATACGTTATGAATTATATTTAGACTTTCTTTTAATAATTGC---- 633
E.maclovinus_CR          TT----GCCCGGCAAA-ATAGTATGAGTTATATATAGATATTATTTTATAATTACA---- 631
P.antarcticum_CR1        CTTG--CTTGAGAGAGAATAGTCTGATCTATATTAAGACTCCG-CTTGCGGTTTTAAT-T 635
P.antarcticum_CR2        CTTGG-CTTGAGAGAGAATAGTCTGATCTATATTAAGACTCCGACTTGCGGTTTTAAT-T 640
N.angustata_CR1          CTTG--CTTTGGTAAAAATGATTAGAGT-ATTTAAGACCCTTGATTTTTTTTTTTTTA-T 636
N.angustata_CR2          CTTG--CTTGAGAGAGAATAGTTTAAACAATATTAAGACTTCATACTCTAAGTTTC-A-T 636
N.coriiceps_CR1          CTTG--CTTGGGTAA---TAAATTGAGTTATGTTAAGACCTCCTGATCTTTTTTTT-G-T 631
N.coriiceps_CR2          CTTG--CTTGGGAGA--ATAGTCTGAACAATATAAAGACTTCTACTTTTATATTTC-A-T 633
N.microlepidota_CR       CTTG--CCTTGGGAAAAAGAATTAGAGC-ACACAAGACCCTCC-TCCTTTTTTTTTTA-T 634
N.rossii_CR              CTTG--CTTGAGTAA---TAACTTGAGTTATATATAGACCTCCCATTCTTTTTTTTTA-T 633
H.antarcticus_CR         TC--TTGCCCGCCCAAGGAAATTCCAATTTTTTGAAGACCTCTTAAGCTTTTTTTTGG-- 624
R.glacialis_CR1          C---TTGCTCGACAAAAG--GCCTGAGTCACATCAAGACTTCCTTCCTTTTTTTGTTT-- 624
R.glacialis_CR2          C---TTGCTCGACAAAAG--GCCTGAGTCACATCAAGACTTCCTTCCTTTTTTTGTTT-- 625
P.scotti_CR              TCGGTCCCAAGAAGGAAATAATCTGAACAACATAAAGACTTTTAAGATCTTTTTTTTGTT 627
C.aceratus_CR            TAA-TTGCTCCGCAAATTTTGTCTGTGTCATATAAAGACTTATTTATTTTTTTTTTGT-T 630
C.myersi_CR              TAG-TTGCTCCGCAAATTTTGTCTGTGTAAAATAAAGACTATTTAAATTTTTTTTT--G 632
C.rastrospinosus_CR      TAA-TTGCCCCGCAAATTTTGCCTGTGTAAA-TAAAGACTATTTAATTTTTTTTTTTTTG 634
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                                    CSB-1
B.variegatus_CR          ATTTTTGGAATTCAAGGACATATAGGTCAAAAATCACTAAGGTAGTTTCAG--ATCTTTT 690
P.urvillii_CR            ATTTTATAATCTCAAGAGCATAAAGGATAAAAAAAGTTATCCTAAAATCTC-TAACAAAT 692
E.maclovinus_CR          -TTTTTTTTTTTCAAGGACATAAGTAGTGAAAACTCTTATCCTAAAATATT-CAATGAAA 689
P.antarcticum_CR1        TTTTTTTTT--TTCTAGGGCATAAAGTA-CCTAACCTCTCACCTGAGTATTA-TAAGAGAT 691
P.antarcticum_CR2        TTTTTTTTTTTTCTGGGGCATAAAGTA-CCTAACCTCTCACCTGAGTATTA-TAAGAGAT 698
N.angustata_CR1          TTTTTTTT--TTCAAGAGCATAAGACAACATAAAGCCTTTCGTAAATCTTATAGTGAGAG 694
N.angustata_CR2          TTTTTTT---TTCTAGGACATAAGGTA-TCCAAGATCTTACCTAAATTTCC-TGCGAGAA 691
N.coriiceps_CR1          TTTTTTTT--TTCCCGGGCATAGGGTA-GGTA-AATCTTTCCTAAATTTTA-TGCGAGAA 686
N.coriiceps_CR2          TTTTTTTT--TTCTAGGGCATAGGGTA-GGTAA-ATCTTTCCTAAATTTTA-TGCGAGAA 688
N.microlepidota_CR       TTTTTTTT--TTCAAGAGCATAAGGCCCCGGAAAGTCTT-CGTAAGTTTTA-AGTGGGAA 690
N.rossii_CR              TTTTTTTA--TTCTAGGGCATAAGGTACGATAGAATCTTCCCTAAATTTTA-TGTGAGAA 690
H.antarcticus_CR         CTTTTTTTTTTCCCAGAGCATACGCACTTGGAAA--TTTTCCTAAACTCGA-TGCGAAAG 681
R.glacialis_CR1          TTTTTTATATTCCAAGAGCATAATG--CCATAAAACCTCCCCTAGGTCTTA-TGCGATAA 681
R.glacialis_CR2          TTTTTTATATTCCAAGAGCATAATG--C-ATAAAACCTCCCCTAGGTCTTA-TGCGATAA 681
P.scotti_CR              TTTTTTTTTTATCAAGAGCATAAGGGTACATGAAGTCTTACCCAGACTTGA-TGCGAGAA 686
C.aceratus_CR            TTTTTTTTATTCTGAAAGCATAAGGGGTCGAAAAGTCTCTCCTAGATTTTA-TGTGATAA 689
C.myersi_CR              CTTTTTTTTTTTTGCAGGCATAAAGGGTCGTAATGTCTTCCCTAAATCTTA-GGTTGTAA 691
C.rastrospinosus_CR      CTTTTTTTTTTCTAAAGGCATAAGGGGTCGTAACGTCTTACCTAGATCTTA-GGTTGTAA 693
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CSB-2

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B.variegatus_CR           TTTTTAGTGAGATCTCCTTACTAAA-TCCCCCCTACCCCCCTTACTTTCCCGACA----A 745
P.urvillii_CR             TTTTTAAGGGGTTTTCCCCCT-----TACCCCCAAACTCCTAAAATAGTTAACATTTTCG 747
E.maclovinus_CR           TTTGTAAAGG-TTTATTTTCGTAAA-CCCCCCCTACCCCCCCACTTTCCTAAAGCA---T 744
P.antarcticum_CR1         ATTTT-AACGCCTCTTAGTCGAGAACCCCCCCCCACCCCCTTACTTCCCTGAAA----TC 746
P.antarcticum_CR2         ATTTT-AACGCCCTTTAGTCGAGAACCCCCCCCCACCCCCTTACTTCCCTGAAA----TC 753
N.angustata_CR1           ATTATGAAAG--TTTTTATCGAAG--ATCCCCCTACCCCCTTACTGCCCTAACA----TT 746
N.angustata_CR2           ATTATGAGTGATTTTTTACGGAAAA-ATCCCCCTACCCCCTTACTCTCGTGACT----TA 746
N.coriiceps_CR1           ATTATGATCG--TTTT-ATCGAAG--ATCCCCCTACCCCCTTACTTCCGTAACA----TG 737
N.coriiceps_CR2           ATTATGATCG--TTTTTATCGAAG--ATCCCCCTACCCCCTTACTCTCGTTACT----TA 740
N.microlepidota_CR        ATTATGAATG--TTTTTATCGAAG--ATCCCCCTACCCCCTTACTTCCCTAGTA----TG 742
N.rossii_CR               ATTATGAATG--TTTTTATCGAAG--ATCCCCCTACCCCCTTACTTCCGTAATG----TA 742
H.antarcticus_CR          GTTATAAATG-TCTTTCATCG-AA-AATCCCCCTACCCCCTTGCCCCAACAG-------C 731
R.glacialis_CR1           ATTATAAGTG-T-TTTCATCG-AA-AATCCCCCCACCCC-TTACTCCCCTAATA-----T 731
R.glacialis_CR2           ATTATA-GTG-T-TTTCATCG-AA-AATCCCCCCACCCC-TTACTCCCGTAACC-----A 730
P.scotti_CR               ATTATAAGTG-TTTTTCATCGGAA-AATCCCCCTACCCC-TTGCCCCCCCTTAAATAATT 743
C.aceratus_CR             ATTATCACTG-T-ATCCATCG-GA--AACCCCCCCCCCC-TTACTCCCGTGTAG-----T 738
C.myersi_CR               ATTATCACCG-T-TTTCCTCG-GA-AACCCCCCCCCCCC-TTACTTCCCTACAA-----T 741
C.rastrospinosus_CR       ATTATCACCG-T-CTTCCTCG-GA--AACCCCCCCCCCC-TTACTTCCCTACAA-----T 742
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CSB-3

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B.variegatus_CR           GCCTAACACTGAAAATACCTTAAAAAAATGGCACCAATTTTGAGACAAAATTCAAGCTTA 805
P.urvillii_CR             CGGACTAGTCAAAAGACTCACAAGTCTCGAGTATCACACGAAAAACTCCTAACATCA-CC 806
E.maclovinus_CR           CTGATGTTCCTGTAAACCCCCCCGAAAACAGGCAAGCCTCGAAAGCAGAAAAAAAAA-CC 803
P.antarcticum_CR1         CATAAAGACTACAAGAGACACTACGA-ACAAGAACGCCCTTACAACCCCGTTTGGA--G- 802
P.antarcticum_CR2         CATAAAGACTACAAGAGACACTACGA-ACAAGAACGCCCTTACAACCCCGTTTGGA--G- 809
N.angustata_CR1           TCTAAGAACTAAAGCAAACCTGAAG--TAAAATGAACCCAAGAACAATAGGTCCC---A- 800
N.angustata_CR2           TTTAACAGTTGCATTTGATTAAAGTGATAAAGAAAGCCCAGAAAACGCCGTGAAAC--A- 803
N.coriiceps_CR1           TCTAAGACCTAATACAAACCTAAAC--TTTAGTGAGCCCTAACGGGCTCGGCCCC---A- 791
N.coriiceps_CR2           TCTAACAACCACACTTAGTTTCAATAGTTAAGGACGTCGAGAAGTCCCCGTAAAAT--AT 798
N.microlepidota_CR        CCTAAGACCTAGAACGAGCCT-AAG--TAAAATAAGCCC--------------------- 778
N.rossii_CR               CCTAAGACCAAAAACAA-CCTAAGC--TTAAATAAGCCCGAACGGACTCGGCCCCC--A- 796
H.antarcticus_CR          GCGGACCCCCTGTTGGGGCAAGAAAAAATGTGAGG---CTAGCATCTGCTTCCTCCA-AC 787
R.glacialis_CR1           GCCCTAGGCC-------CACCAAA--ACCGAAAGC---C-CCGGGGAAAATGAGTC--AC 776
R.glacialis_CR2           ACTAAAGATTACTTTTATTCCCTC--CCCGAAAGC---C-CCAGTAAACACTACTCG-AC 783
P.scotti_CR               AAGAAAAACCTTTAACTCTACACGAACAAACAAGCGTCCCCTGGCACAAATTTATAT-AC 802
C.aceratus_CR             ACCTAAGGCCTCACAAGCCACAAGTACCTAAAAAC---TACTTGTACGTACTGGAG--CC 793
C.myersi_CR               ACCTAAGACTTTTCAAGCCTCAAA-AGCTAAAAAC---TACTTGTACGTACTAGCA--CG 795
C.rastrospinosus_CR       ACCTAAGACTTTTCACGCCCCAAA-AGCTAAAAAC---TACTTATACGTACTAGTG--CC 796
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Fig. S1. ClustalW2 alignment of L-strand nucleotide sequence of mitochondrial control region (CR) from 14 notothenioid species. The three basal non-Antarctic notothenioid species are *B. variegatus*, *P.urvillii*, and *E. maclovinus* (top three lines), and the rest belong to the five Antarctic families. *P. antarcticum*, *N. angustata*, *N. coriiceps*, and *R. glacialis* have two copies of CR labeled as CR1, CR2. The sequences start at the 5' end of the CR or CR copy and covers through ~800 nt that encompass the conserved mitochondrial regulatory sequence elements, and where significant similarities are present to allow confident alignment. Inferred regulatory sequence elements are highlighted in colored text or colored blocks and labeled: TAS - termination associated sequences; cTAS - complementary termination associated sequences; ETAS - extended termination associated sequence (green underline); CSBs - conserved sequence blocks, which include CSB-F, CSB-E, CSB-D, CSB 1, CSB 2, and CSB 3 (found in *E. maclovinus* only). Dashes are gaps introduced by ClustalW. Asterisks indicate nucleotide identity in the column.

Supplementary Material

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(A)                   Start codon
N.angustata           ATGTTTTATTATGGGTATTTACTTATGCTTGGTGTAGTGGTGGGGATGGCGGCGGTCGCC 60
N.microlepidota       CTGCTATATTACGGGTATTTACTTATGTTTGGTGTAGTGGTGGGGATAGCGGCGGTCGCC 60
N.coriiceps           ATGTTATATTGTGGGTACTTACTTATGTTTGGTGTGGTGGTGGGGATGGCGGCAGTTGCC 60
N.rossii              ATGCTATATTGTGGATATTTACTTATGTTTGGAGTGGTAGTGGGGATAGCGGCGGTTGCC 60
T.bernacchii          ATGATATACTGTGGAAACTTACTTATGCTGGGTGTGGTAGTGGGGACAGCGGCAGTTGCC 60
T.eulepidotus         ATGTTATACTGTGGAAACTTACTTATGCTGGGTGTGGTAGTAGGGACAGCGGCAGTTGCC 60
P.borchgrevinki       ATGATATACTGTGGAAACTTACTTCTGCTGGGCGTGGTTGTAGGGACAGCGGCAGTTGCC 60
T.newnesi             ATGTCATACTGCGGAGACTTACTTATACTGGGTGTGGTAGTAGGGACAGCGGCAGTTGCC 60
L.squamifrons         ATGTTATACTATGGATACTCACTTATACTAGGTGTGGTAGTAGGGATAGCGGCGGTTGCC 60
P.antarcticum         GTGTTGTATTGAGGATATTTATTTATATTGGGTATAGTAGTAGGAATAGCGGCGGTCGCC 60
P.cerebropogon        ATGCTATACTGTGGGTATTTACTTATGTTGGGCGTAGTGGTGGGAATAGCGGCAGTTGCT 60
P.scotti              ATGTTATACTGTGGGTATTTACTTATGTTGGGCGTAGTGGTGGGAATAGCGGCAGTTGCT 60
H.velifer             CTGCTATACTGTGGGTATTTACTTATGTTGGGCGTAGTGGTGGGAATAGCGGCAGTTGCT 60
H.antarcticus         ATGCTATACTGTGGGTATTTACTTATGTTGGGCGTAGTGGTGGGGGTAGTGGCGGTTGCC 60
C.myersi              ATGTTATACTGCGGGTATTTACTTATGTTGGGCGTGGTGGTGGGGATGGTGGTGGTTGCT 60
C.rastrospinosus      ATGTTATACTGCGGGTATTTACTCATGTTGGGCGTGGTGGTGGGGATGGTGCTGGTTGCT 60
C.aceratus            ATGTTATACTGCGGGTATTTACTTATGTTGGGCGTGGTGGTAGGAATGGTGGTGGTTGCT 60
C.esox                ATGATATACTGTGGGTATTTACTTATGTTGGGCGTGGTGGTGGGGATAGTGGTGGTTGCC 60
R.glacialis           ATGTTATACTTTGGGTATCTAGTTATGTTGGGCGTAGTGGTGGGGATAGTAGTGGTTGCT 60
B.variegatus          ATGACTTATTTTGTGTCTTTTTCTTTATTGGGTTTAGTACTGGGGTTAGTTGCTGTTGCT 60
P.urvillii            ATGGGATATCTTATGTCTTTGTTTATGTTGGGCTTAGTTTTAGGGATAGTGGCAGTTGCA 60
E.maclovinus          ATGGCATACTTTGTGTATTTGTTTATGGTGGGCCTGGTGCTAGGAATGGCAGTAGTTGCA 60
                       **   **                 *  * **  * **  * **    *     ** **

N.angustata           GCTAATCCTTCCCCCTTTTATGCGGCATTGGGGCTAGTTATGGTTGCAGCAATGGGGTGT 120
N.microlepidota       GCTAACCCCTCCCCCTTATATGCGGCACTTGGGCTAGTTTTGGTTGCAGCAATGGGGTGT 120
N.coriiceps           GCCAACCCTTCACCTTTTTATGCGGCTCTTGGGCTCGTTATGGTTGCAGCAATGGGGTGC 120
N.rossii              GCCAACCCCTCTCCTTTTTATGCAGCACTTGGGTTAGTTATGGTCGCAGCAATGGGGTGT 120
T.bernacchii          GCAAACCCCTCCCCCTTTTATGCGGCCCTCGGGTTGGTTGTGGTCGCGGCAATGGGGTGC 120
T.eulepidotus         GCAAATCCCTCCCCCTTTTATGCGGCCCTCGGGTTAGTCATGGTCGCGGCAATGGGGTGC 120
P.borchgrevinki       GCAAATCCCTCCCCCTTTTATGCGGCCCTCGGGTTGGTCACGGTCGCGGCAATGGGGTGC 120
T.newnesi             GCAAATCCCTCCCCCTTTTATGCGGCCCTAGGATTGGTTATGGTCGCGGCAATGGGGTGC 120
L.squamifrons         GCTAATCCCTCGCCCCTTTATGCGGCCCTTGGATTGGTCATGGTCGCAGCAATAGGGTGC 120
P.antarcticum         TCTAATCCTCCGCCTTTTTATGCGGCTCTTGGGTTGGTTGTAGTCGCAGCAATGGGGTGT 120
P.cerebropogon        GCTAACCCCTCCCCTTTCTATGCGGCTCTGGGGGTGGTTGTGGTCGCAGCAGCGGGGTGC 120
P.scotti              GCTAACCCCTCCCCTTTCTATGCGGCCCTGGGGGTGGTTGTGGTCGCAGCAGCGGGGTGC 120
H.velifer             GCTAACCCCTCCCCTTTCTATGCGGCTCTGGGGGTGGTTGTGGTTGCCGCAGCGGGGTGT 120
H.antarcticus         GCTAGCCCCGCCCCTTTCTATGCAGGCCTTGGGGTGGTGGGGGCTGCAGCAGCGGGGTGC 120
C.myersi              GCTAACCCCTCCCCCTTTTATGCGGCACTGGGGGTAGTCATGGTTGCAGCAGCGGGGTGT 120
C.rastrospinosus      GCTAACCCCTCCCCCTTTTATGCGGCACTGGGGGTAGTCATGGTTGCAGCAGCGGGGTGT 120
C.aceratus            GCTAACCCCTCCCCCTTTTATGCGGCACTGGGGGTAGTTATGGTTGCAGCAGCGGGGTGT 120
C.esox                GCTAATCCCTCTCCCTTTTATGCAGCACTGGGGGTCGTTATAGTCGCAGCAGCGGGGTGT 120
R.glacialis           GCTAACCCCTCCCCCTTTTATGCGGCACTTGGGGTGGTTATGGTCGCAGCAGCGGGGTGT 120
B.variegatus          TCTAATCCCTCCCCCTACTTCGCAGCCTTGGGCTTGGTGGTGGTAGCAGGCATGGGATGT 120
P.urvillii            GCCAACCCATCTCCGTACTTTGCAGCTTTTGGGTTGGTCATAGTTGCGGGCATGGGGTGT 120
E.maclovinus          GTTAATTTGTCCCCGTATTTCGCGGCCTTTGGTTTGGTTGTGGTTGCGGGCATGGGGTGT 120
                         *      * **    *  ** *   * **  * **    *  ** *     ** **

N.angustata           GGGTTTATTATGTGTTTAGGGGGTACTTTTTCATGTCTAGTTTTATTTCTAATCTATTTA 180
N.microlepidota       GGGTTCATTATGTGCTTGGGGGGTACTTTTTCATGTCTAGTTTTATTCTTAATCTATTTA 180
N.coriiceps           GGATTCATTATGTGCTTGGGGGGCACTTTTTCATGCCTAGTTTTATTCCTAATCTATCTA 180
N.rossii              GGGTTTATTATGTGCTTAGGGGGTACTTTCTCATGTCTAGTTTTATTCTTAATTTATTTA 180
T.bernacchii          GGGTTTATCATGTGTTCAGGGGGCACTTTTTTGTGCTTAATTCTGTTCTTAATTTATCTA 180
T.eulepidotus         GGGTTTATCATGTGTTCGGGGGGCACTTTTCTGTGCTTAATTCTGTTCTTAATTTATTTA 180
P.borchgrevinki       GGGTTTATCATGTGTTCAGGGGGCACTTTTTTGTGCTTAATCTTATTCTTAATTTATTTA 180
T.newnesi             GGGTTTATCATGTGTTCGGGGGGCACTTTTTTATGCCTGATTCTGTTCTTAATTTACTTA 180
L.squamifrons         GGGTCTATCATCTGTTCCGGGGGTACTTTTTTGTGCCTAGTTCTGTTCTTAATTTACTTA 180
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P.antarcticum         GGGTTGATCTTGTCTCTGGGGGGTACTTTTTCATGTCTAGTACTGTTCTTAATCTATCTT 180
P.cerebropogon        GGGCTTATTATGTGTTTTGGGGGTACTTTTTTATGTTTAGTTTTGTTCTTAATTTACCTA 180
P.scotti              GGGCTTATTATGTGTCTTGGGGGTACTTTTTTATGTTTAGTTCTGTTCTTAATTTACCTA 180
H.velifer             GGGCTTATTATGTGTCTTGGGGGGACTTTTTTATGTTTAGTTCTGTTCTTAATTTACCTT 180
H.antarcticus         GGGCTTATTATGTGTTTAGGGGGCACCTTTTTATGTTTAGTTTTGTTCTTAATTTACTTG 180
C.myersi              GGGTTTATCATGTGTGTAGGGGGAACTTTTTTATGCCTAGTTTTATTTTTAATTTACCTT 180
C.rastrospinosus      GGGTTTATCATGTGTGTAGGGGGTACTTTTTTATGTTTAGTTTTATTTTTAATTTATCTT 180
C.aceratus            GGGTTTATCATGTGTGTAGGGGGCACTTTTTTATGTTTAGTTTTATTTTTAATTTATCTT 180
C.esox                GGGTTCATCATGTGTGTAGGAGGTACTTTTTTATGCTTAGTTTTATTCTTAATCTATCTT 180
R.glacialis           GGGTTTATCATGTGTGTAGGGGGCACTTTTTTATGTTTAGTTCTTTTCTTAATTTATCTA 180
B.variegatus          GGGGTTATCATGGGTCATGGGGGCTCGTTTTTGTCACTAGTTCTTCTGTTAATCTACTTA 180
P.urvillii            GGGGTGGTAATAGGCTATGGGGGCGCTTTTTTGTCTTTAGTCTTATTTTTAATTTATTTA 180
E.maclovinus          GGGGTAATTATTAGCTTCGGGGGCTCTTTTCTTTCTTTAGTCCTGTTTTTAATTTATTTG 180
                      **     *  *       ** **  * **    *   *  *  *  *  **** **  *

N.angustata           GGGGGAATGTTGGTGGTGTTTGCCTATTCGGTAGCGCTGTGTGCGGACGCTCTTCCAACA 240
N.microlepidota       GGGGGAATGTTGGTGGTGTTTGCTTATTCGGTAGCTCTGTGTGCAGAGGATCTTCCGACA 240
N.coriiceps           GGGGGAATGTTGGTGGTGTTTGCTTACTCGGTAGCACTGTGTTCGGATGCGCTTCCAACA 240
N.rossii              GGGGGAATGTTGGTGGTGTTTGCTTACTCAGTAGCATTATGTGCGGATGCTCTTCCAACA 240
T.bernacchii          GGGGGTATATTGGTTGTATTCGCTTATTCGGTGGCATTGTGTGCAGACCCCTTTCCCACG 240
T.eulepidotus         GGGGGTATATTGGTTGTATTCGCTTATTCAGTGGCATTATGTGCAGACCCCTTTCCCACA 240
P.borchgrevinki       GGGGGTATGTTGGTCGTATTCGCTTATTCGGTGGCATTATGTGCAGACCCCTTCCCCACA 240
T.newnesi             GGGGGTATATTGGTTGTATTCGCTTATTCGGTGGCACTATGTGCAGACCCCTTTCCCACA 240
L.squamifrons         GGGGGGGTACTGGTTGTGTTCGCTTATTCGGTAGCATTGTGTGCAGACCCCTTTCCAACA 240
P.antarcticum         GGAGGGATGTTGGTTGTATTTGCTTACTCAGTAGCATTGTGTGCTGGTGCTCCTTCAACA 240
P.cerebropogon        GGGGGTATGCTGGTTGTGTTTGCTTACTCGGCAGCGCTATGTGCGGAAACCCATCCAACA 240
P.scotti              GGGGGTATGCTGGTTGTGTTTGCTTACTCGGCAGCGCTATGTGCGGAAACCCATCCAACA 240
H.velifer             GGGGGAATGCTGGTTGTGTTTGCTTACTCGGCAGCGCTATGTGCGGAAACCCACCCAACA 240
H.antarcticus         GGGGGGATGATGGTTGTGTTTGCTTACTCAGCGGCGTTATGTTCGGAGACCCGCCCAACA 240
C.myersi              GGGGAAATGCTGGTTGTGTTTGCTTACTCAGCAGCTTTATGTGCTGAGGTTCACCCGACA 240
C.rastrospinosus      GGGGGAATGCTGGTCGTGTTTGCTTACTCAGCAGCTTTATGTGCTGAGGTTCACCCGACA 240
C.aceratus            GGGGGAATGCTGGTTGTGTTTGCTTACTCAGCAGCTTTATGTGCTGAGGTTCACCCGACA 240
C.esox                GGGGGGATATTGGTTGTGTTTGCTTATTCAGCAGCGTTATGTGCGGAGAGTCACCCGACA 240
R.glacialis           GGGGGAATGCTGGTTGTGTTCGCTTATTCGGCAGCACTATGTGCTGAGGTTCATCCAACA 240
B.variegatus          GGAGGGATATTGGTAGTATTTGCGTACTCTGCGGCTCTTGCCGCAGAGCCTTTTCCGGAG 240
P.urvillii            GGGGGGATACTAGTTGTATTTGCATATTCTGCAGCATTGGTTGCAGAACCGTATCCTGAA 240
E.maclovinus          GGGGGTATACTGGTTGTATTTGCGTACTCGGCAGCATTAGCTGCTGAGCCTTTTCCTGAA 240
                      ** *   *  * ** ** ** ** ** ** *  **  *     * *         *

N.angustata           GGTCTGGCTGAGGGGTGGGTGCTTAAATCTATGGTAGGGTATCTTGGGCTAACAGGAGGG 300
N.microlepidota       GGGTTGGCTGAGGGGTTGGTGTTTAAAGCTATAGTGGGGTACCTTGGGGTGACAGGAGGA 300
N.coriiceps           GGTTTGGCTGAGGGGTCGGTGCTTAAGTCTATAGTGGGTTATCTTGGGCTTACAGGGGTA 300
N.rossii              GGCTTGGCTGAGGGGTCGGTGCTTAAGTCCATAGTGGGTTATCTTGGGCTGACAGGGGTA 300
T.bernacchii          GGCTTGGCGGGAGGGTCAGTGGCTAAGTACATAACGGGATACCTAGTACTAACAGGAGGG 300
T.eulepidotus         GGTTTAGCGGGGGGGTCAGTGGCTAAGTACATAGTGGGGTACCTAGTACTAACAGGAGGG 300
P.borchgrevinki       GGTTTAGCGGAGGGGTCAGTGGCTAAGTACATAGCGGGTTACCTATTACTAACAGGAGGG 300
T.newnesi             GGTTTAGCGGGGGGGTCCGTGGCTAAGTATATAGTAGGCTACCTAGCACTAACAGGGGTG 300
L.squamifrons         GGCTTAGCGGGAGAGTCCGTGGCTAAATGCATACTAGGGTACCTAGTACTAACAGGAGGA 300
P.antarcticum         GGTTTGGGGGAGGAGTCGGTGTTGAAGTGTATAGCCGGGTATCTAGGGGTGACTGGTGTT 300
P.cerebropogon        GGCCTGGCTGAGGGGTCGGTGCTCAAGTGTATAGGGGGATACTTGGCGGGGGTGGCGGGG 300
P.scotti              GGCCTGGCTGAGGGGTCGGTGCTCAAGTGTATAGGGGGATACTTGGCGGGGGTGGCGGGG 300
H.velifer             GGTTTGGCTGAGGGGTCGGTGCTCAAGTGTATGGGGGGATACTTGGCGGGGGTGGCGGGG 300
H.antarcticus         GGCTTGGCTGAGGGGTCGGTGCTTAAATGTATAGGGGGGTACTTTGTGGTGGCTGCGGGG 300
C.myersi              GGTTTGTCAGAGGGGTCGGTTCTTAAGTGTATAGTGGGTTGTTTCGGGGTAGTGTCGGGG 300
C.rastrospinosus      GGTTTGTCAGAGGGGTCGGTTCTTAAGTGTATAGTGGGTTATTTCGGGGTAGTGTCGGGG 300
C.aceratus            GGTTTGTCAGAGGGGTCAGTTCTTAAGTGTATAATGGGGTATTTCGGGGTAGTGCTGGGG 300
C.esox                GGGCTGACTGAGGGGTCTGTTCTTAAAGCTATAGTGGGGTATCTCGGGGTGGTGTTTGGG 300
R.glacialis           GGTTTGGCGGAGGGGTCAGTGCTCAAGTGTATAGTGGGGTACTTCGCGGTGGTAGCGGGG 300
B.variegatus          AGTTGAGGGAGTGGGTCTGTTATGCTGTATATATTTCTGTACTTAATAGCTACTGGGGTA 300
P.urvillii            GGTTTGGGAAGTGCGCCTGTTTTAGTATATGTGGTCGGCTTCTTGGGTATAATTGGAGGA 300
E.maclovinus          AGTTTTGGAACCACAGGGGCAGTGTTGTACTTTATGGGGTTAGTGGGAGTGGTAGGAGTT 300
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N.angustata           ATGGCTTTTTCCCAGTGAAGTTTGGAGGCGCCTCTCTTTTGGTGATTTATGGGGCAGGAG 360
N.microlepidota       GTGGCCCTGTTTCAGTGAAGTCCAGAGGCCCCTCTTTTTTGGTGATCTGTGGGAGAGGAG 360
N.coriiceps           GTGGCCTTGTTCCAGCAAAGCTCAGAGGCCCCCCTTTTTTGGTGATTTGTGGGCCAGGAG 360
N.rossii              ATGGCTTTGTCCCAGCAAAGCTCAGAGGCCCCTCTCTTTTGGTGATTTGTGGGGCAGGAG 360
T.bernacchii          GTGGCC------CAGTGTACTACACTGGGCCCCCCCTTTTGGTGATCTGTGGGCCAGGAA 354
T.eulepidotus         GTAGCC------CAGTGTACTACACTGGGCCCCCCCTTTTGGTGATCTGTGGGCCAGGAA 354
P.borchgrevinki       GCGGCC------CAGTGTACTACACTGGCCCCCCCCTTTTGATGATCTGTGGGCCAGGAA 354
T.newnesi             GTGGCC------CAGTGTACTACACTGGGCCCCCCCTTTTGATGATCTGTAGGTCAGGAA 354
L.squamifrons         ATGGCCCTGTCCCAGTGAAATATAGATAGCCCCCCTTTTTGATGGTCTGTGGGCCAGGAA 360
P.antarcticum         GTAGGCTTTTTACAAGGAAGTTCAGAAGGCCCCGCTTTCTGGTGGTTTGCAGGGGGAGAG 360
P.cerebropogon        GCGGCGTTGTTTCAGGGAGGCTCGGAGGCCCCTCTGTTTTGATGGCTTGCGGGGGAGGAG 360
P.scotti              GCGGCGTTGTTTCAGGGAAGCTCGGAGGCCCCCCTGTTTTGATGGCTTGCGGGGGAGGAG 360
H.velifer             GCGGCGTTGTTTCAGGGAAGCTCGGAGGCCCCTCTCTTTTGATGGCTTGCGGGGGAGGAG 360
H.antarcticus         GTGGCTTTATACCAAGGAGGTATGGAGGGTCCCCCCTTTTGATGGTTTGCAGGGGAGGAG 360
C.myersi              GTGGCACTGTTACAAGGGAGCCCTGAGGCTCCCCTATATTGATGATTTGTGGGGGAGGAG 360
C.rastrospinosus      GTGGCACTGTTACAGGGGAGCCCGGAGGCCCCCCTCTACTGATGGTTTGTGGGGGAGGAG 360
C.aceratus            GTGGGATTATTACAGGGGAATCCTGAGGCTCCCCTGTACTGATGATTTGTGGGGGAGGAG 360
C.esox                GCAGCTGTATTTCAGGGAACCGCGGAGGCCCCACTGTTTTGATGATTTGTGGGGGAGGAA 360
R.glacialis           GTGGCGGTATTTCAGGGAAGTTCAGAGGCCCCCGTGTATTGGTGATTTATGGGTGAGGAG 360
B.variegatus          GTGGGGAGCATACTTTTTGAGGGGTGGTATGAGTTCTCTTGGGGGGTGGTTGATGAGTTC 360
P.urvillii            GTTGGAATCGTGTGCTGAGAGGGTTGGGATGAAGCGGTTTGATTTTCAACTGAGTGTACA 360
E.maclovinus          TTCGCTATCCTCTATATTGAGGAGGGAAGCGGGCTGGGGGGATGTTCTTCCGACGAAGTT 360
                         *                                    *          *

N.angustata           TTAGAGATGAGTGCGATGCAGGGGGAAACTGAAGGGGTGTCGGAAGTATACGGAAGTGGG 420
N.microlepidota       TCCGAGATAAGTGTGATGCAGGGGGAAATTGAGGGGGTGTCGGAGGCATATGGAAGCGGC 420
N.coriiceps           TCAGAGATAAGTGTGATGCAGGGGGAGACTGAGGGGGTGTCAGAGGCATATGGGAGTGGT 420
N.rossii              TCCGAGATAAGTGTGATGCAGGGGGAGACCGATGGGGTGTCAGAAGCATATGGGAGTGGT 420
T.bernacchii          GCAGACATAAGTGCAATACAAGGGGAGACCGAGGGGGTGTCGGAAGCATACGGGAGCGGG 414
T.eulepidotus         GCAGACATAAGTGCAATACAAGGGGAGGCCGAGGGGGTGTCGGAAGCATACGGGGGCGGG 414
P.borchgrevinki       GCAGACATAAGTGCAATACAAGGGGAGGCCGAGGGGGTGTCGGAAGCATACGGAGGCGGG 414
T.newnesi             GCAGACATAAGTGCAATACAAGGGGAGACCGAGGGGGTGTCGGAAGCATACGGGGGTGGG 414
L.squamifrons         GCAGACATAAGTGCAATGCAAGGAGAGACCGAGGGGGTCTCGGAGGCATATGGGGGCGGG 420
P.antarcticum         TGGGAGATAGCTGTAATACAGGGGGAGGCCGCAGGGGTCTCAGAAGCCTACGGGGGCGGG 420
P.cerebropogon        TCGGAGATAAGTGTGGTACAAGGAGAAAGTGATGGGGTGTCAGAAGCATATGGGGGCGGG 420
P.scotti              TCGGAGATAAGTGTGGTACAAGGAGAAAGTGATGGGGTGTCAGAAGCATATGGGGGCGGG 420
H.velifer             TCGGAGATAAGTGTGGTACAAGGAGAAAGTGATGGGGTGTCAGAAGCATATGGGGGCGGG 420
H.antarcticus         TCGGGGATGAGTGTAATACAAGGAGAGAGTGAAGGGGTGTCAGAAGCATACGGGGGCGGG 420
C.myersi              GCAGAGATAAATGTAGTGCAAGGAGGGACTGAGGGGGTGTCAGAAGCGTACGGGGGCGGG 420
C.rastrospinosus      GCAGAGATAAATGTAGTGCAAGGAGAGACTGAGGGAGTGTCAGAAGCATATGGGAGCGGG 420
C.aceratus            GCAGAGATAAATGTAGTGCAAGGAGAGACTGAGGGAGTGTCAGAAGCGTATGGGAGCGGG 420
C.esox                GCAGAGATAAATGTAGTGCAAGGGGAGACTGAGGGAGTGTCAGAAGCATATGGGAGTGGG 420
R.glacialis           TCAGAGTTAAGTGTAGTGCAAGGAGAGACTGATGGAGTGTCAGAAGCATATGGGAGTGGG 420
B.variegatus          TGTGAATTTTCACTCTTTCGGGGGGATACAGGAGGGGTTGCTATAATGTACTCTTCGGGG 420
P.urvillii            AATGAGTTTTCTATATGTTGTGAGGAGGCTCAGGGGATGTCAGAGGCTTATGGGTTTGGG 420
E.maclovinus          ATAGGGATGTCCTCTTGTGTAACGGAATATGATGGAATGTCACAGATATATGGGCCCGGG 420
                         *                             **  *  *       **       **

N.angustata           GGTATTCTGTTAATTACTTGTGCGTGGGCACTTCTGGTTGCTCTTTATGTTGCTTTAGAG 480
N.microlepidota       GGGTTTTTGTTAATTATTTGTGCGTGGGCACTTTTGGTTGCTCTTTACGTTGCTTTAGAG 480
N.coriiceps           GGCATCTTGTTGCTTACTTGCGGGTGGGCTCTTCTGGTAGCTCTTTATGTTGCTTTGGAG 480
N.rossii              GGCATTTTGTTGGTCACCTGTGGGTGGGCTCTGCTGGTAGCCCTTTATGTTGCTCTAGAG 480
T.bernacchii          GGCATTCTTCTGCTCACTTGCGCATGGGCCCTGCTGGTGGCCCTCTATGTTGCCCTAGAA 474
T.eulepidotus         GGCATTCTTCTGATCACTTGTGCATGGGCCCTGCTGGTAGCCCTCTATGTTGCTCTAGAA 474
P.borchgrevinki       GGCATTATCCTGATCTCTTGTGCATGGGCCTTGCTGGTAGCCCTCTACGTTGCTCTAGAA 474
T.newnesi             GGCATTCTTCTAATCACTTGTGCATGAGCCCTGCTGGTAGCCCTCTATGTTGCCCTGGAA 474
L.squamifrons         GGTATTCTTCTGATTACCTGTGCATGAGCCCTACTGGTGGCCCTTTATGTCGCTCTAGAA 480
P.antarcticum         GGGTTCCTAGTAGTGACGTGTGGATGGGCCCTGTTGATTGCCCTTTATGTGGCGCTGGAG 480
P.cerebropogon        GGAATCATATTGATCACTTGTGGGTGGGTTCTGCTGGTGGCTCTTTATGTTGCTTTGGAG 480
P.scotti              GGAATCATATTGATCACTTGTGGGTGGGTTCTGCTGGTGGCTCTTTATGTTGCTTTGGAG 480
H.velifer             GGAATCCTATTGATCACTTGTGGGTGGGTTCTGCTGGTGGCTCTTTATGTTGCTTTGGAG 480
H.antarcticus         GGAATCTTGCTGATCGTCTGTGCGTGGGTGTTGGTGGTGGCTCTCTTTGTTGCTTTGGAA 480
C.myersi              GGCATGTGTTTAGTTATTTGTGCGTGGGTGTTGCTGGTGGCCCTTTATGTTGCTTTGGAG 480
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C.rastrospinosus       GGTATGTGTTTAGTTATTTGTGCGGGGGTGTTGCTGGTGGCTCTTTATGTTGCTTTGGAG 480
C.aceratus             GGCATGTGCTTAGTTATTTGTGCGTGGGTGTTGCTGGTGGCTCTTTATGTTGCTTTGGAA 480
C.esox                 GGCATATGTTTAGTTATTTGTGCGTGGGTGTTGCTGGTGGCCCTTTATGTTGCTTTGGAG 480
R.glacialis            GGTATTTGCTTAGTTGTCTGTGCGTGGGTGTTGTTGGTGGCCCTTTATGTTGCTTTGGAA 480
B.variegatus           GGCGGGATATTGGTTTTAAGCGCATGGGTGTTGTTTCTTACTTTATTTGTGGTGTTGGAG 480
P.urvillii             GGGTTTTTGTTAATTATAAGTGCTTGGGTGTTGTTACTTGCCTTATTTGTTGTTTTGGAA 480
E.maclovinus           GGCTTTCTGTTAGTTCTTTGTGCCTGGGTTCTATTGTTAACTTTATTTGTTGTGCTAGAA 480
                       **        *  *     * *   * *   *  *  *  *  * *  ** *   * **

N.angustata            GTGTCGCGGGGTTGCAATCGGGGGACAGTGCGGCCAATTGAGTAG 525
N.microlepidota        GTGTCGCGGGGCTGTAGCCGAGGGACAGTGCGGTCAATCAAATAG 525
N.coriiceps            GTGTCACGGGGTTGCAGCCGGGGGCCAGTGCGGCCAATCAAGTAG 525
N.rossii               GTGTCCCGGGGTTGCAGCCGGGGGCCAGTGCGACCAATCAAGTAG 525
T.bernacchii           GTATCGCGGGGTTGTAGTCGGGGGCCGGTCCGCCCTATCAAAAGG 519
T.eulepidotus          GTATCGCGGGGTTGTAGTCGGGGGCCGGTTCGCCCTATCAAAAGG 519
P.borchgrevinki        GTATCACGGGGTTCTAGTCGGGGGCCGGTCCGCCCTATCAAAAGG 519
T.newnesi              GTGTCGCGGGGCAGTAGCCGGGGGCCGGTCCGGCCTATCAAAAGG 519
L.squamifrons          GTATCACGGGGCTCTAACCGGGGGCCGGTTCGCCCTGTT---AGG 522
P.antarcticum          GTGTCTCGGGGTCCTAGCCGGGGGCCGGTACGCCCATTCAAAAGG 525
P.cerebropogon         GTGTCGCGGGGTTGCAGCCGGGGGCCTGTACGGCCAATTAAAAGG 525
P.scotti               GTGTCGCGGGGTTGCAGCCGGGGGCCTGTACGGCCAATTAAAAGG 525
H.velifer              GTGTCGCGGGGTTGCAGCCGGGGGCCTGTACGGCCAATTAAAAGG 525
H.antarcticus          GTGTCGCGGGGTTGCAGCCGTGGGCCGGTACGGTCAATTAAAAGG 525
C.myersi               GTGTCTCGGGGTAGTAGCCGGGGGCCGGTGCGGCCCATTAAAAGG 525
C.rastrospinosus       GTGTCTCGGGGTAGTAGCCGGGGGCCGGTGCGGCCCATTAAAAGG 525
C.aceratus             GTGTCCCGGGGCAGCAGCCGGGGGCCGGTGCGGCCAATTAAAAGG 525
C.esox                 GTATCCCGGGGTAGTAGCCGGGGGACGGTGCGGTCAATTAAAAGG 525
R.glacialis            GTGTCGCGGGGTAGCAGCCGGGGCCCGGTGCGGCCAATTAAAAGG 525
B.variegatus           CTTACACGAGGTTTGAGTCGAGGGGCTTTGCGGGCTGTT---TAG 522
P.urvillii             ATAACGCGGGGGGCAAGTCGGGGGCCGCTTCGGTCGGTTAAAAGG 525
E.maclovinus           ATGACGCGAGGATCCAGCCGAGGGCCTCTTCGGTCAGTTAAAAGA 525
                        *  * ** **    *  ** **  *  * **  *  *  Stop codon
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(B)

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N.coriiceps            MLYCGYLLMFGVVVGMAAVAANPSPFYAALGLVMVAAMGCGFIMCLGGTFSCLVLFLIYL 60
N.rossii               MLYCGYLLMFGVVVGMAAVAANPSPFYAALGLVMVAAMGCGFIMCLGGTFSCLVLFLIYL 60
N.angustata            MFYYGYLLMLGVVVGMAAVAANPSPFYAALGLVMVAAMGCGFIMCLGGTFSCLVLFLIYL 60
N.microlepidota        LLYYGYLLMFGVVVGMAAVAANPSPLYAALGLVLVAAMGCGFIMCLGGTFSCLVLFLIYL 60
T.bernacchii           MMYCGNLLMLGVVVGTAAVAANPSPFYAALGLVVVAAMGCGFIMCSGGTFLCLILFLIYL 60
T.eulepidotus          MMYCGNLLMLGVVVGTAAVAANPSPFYAALGLVMVAAMGCGFIMCSGGTFLCLILFLIYL 60
T.newnesi              MSYCGDLLMLGVVVGTAAVAANPSPFYAALGLVMVAAMGCGFIMCSGGTFLCLILFLIYL 60
P.borchgrevinki        MMYCGNLLLLGVVVGTAAVAANPSPFYAALGLVTVAAMGCGFIMCSGGTFLCLILFLIYL 60
L.squamifrons          MLYYGYSLMLGVVVGMAAVAANPSPLYAALGLVMVAAMGCGSIICSGGTFLCLVLFLIYL 60
P.cerebropogon         MLYCGYLLMLGVVVGMAAVAANPSPFYAALGVVVVAAAGCGLIMCFGGTFLCLVLFLIYL 60
P.scotti               MLYCGYLLMLGVVVGMAAVAANPSPFYAALGVVVVAAAGCGLIMCLGGTFLCLVLFLIYL 60
H.velifer              LLYCGYLLMLGVVVGMAAVAANPSPFYAALGVVVVAAAGCGLIMCLGGTFLCLVLFLIYL 60
H.antarcticus          MLYCGYLLMLGVVVGVVAVAASPAPFYAGLGVVGAAAAGCGLIMCLGGTFLCLVLFLIYL 60
C.myersi               MLYCGYLLMLGVVVGMVVVAANPSPFYAALGVVMVAAAGCGFIMCVGGTFLCLVLFLIYL 60
C.rastrospinosus       MLYCGYLLMLGVVVGMVLVAANPSPFYAALGVVMVAAAGCGFIMCVGGTFLCLVLFLIYL 60
C.aceratus             MLYCGYLLMLGVVVGMVVVAANPSPFYAALGVVMVAAAGCGFIMCVGGTFLCLVLFLIYL 60
C.esox                 MMYCGYLLMLGVVVGMVVVAANPSPFYAALGVVMVAAAGCGFIMCVGGTFLCLVLFLIYL 60
R.glacialis            MLYFGYLVMLGVVVGMVVVAANPSPFYAALGVVMVAAAGCGFIMCVGGTFLCLVLFLIYL 60
P.antarcticum          VLYWGYLFMLGMVVGMAAVASNPPPFYAALGLVVVAAMGCGLILSLGGTFSCLVLFLIYL 60
B.variegatus           MTYFVSFSLLGLVLGLVAVASNPSPYFAALGLVVVAGMGCGVIMGHGGSFLSLVLLLIYL 60
E.maclovinus           MAYFVYLFMVGLVLGMAVVAVNLSPYFAAFGLVVVAGMGCGVIISFGGSFLSLVLFLIYL 60
P.urvillii             MGYLMSLFMLGLVLGMVAVAANPSPYFAAFGLVMVAGMGCGVVMGYGGAFLSLVLFLIYL 60
                       : *     :.*:*:* . ** . .* :*.:*:* .*. *** ::  **:* .*:*:****

N.coriiceps            GGMLVVFAYSVALCSDALPTGLAEGSVLKSMVGYLGLTGVVALFQQSSEAPLFWWFVGQE 120
N.rossii               GGMLVVFAYSVALCADALPTGLAEGSVLKSMVGYLGLTGVMALSQQSSEAPLFWWFVGQE 120
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                                   74C   80T      89K             105Q  111P*
N.angustata           GGMLVVFAYSVALCADALPTGLAEGWVLKSMVGYLGLTGGMAFSQWSLEAPLFWWFMGQE 120
N.microlepidota       GGMLVVFAYSVALCAEDLPTGLAEGLVFKAMVGYLGVTGGVALFQWSPEAPLFWWSVGEE 120
T.bernacchii          GGMLVVFAYSVALCADPFPTGLAGGSVAKYMTGYLVLTGGVA--QCTTLGPPFWWSVGQE 118
T.eulepidotus         GGMLVVFAYSVALCADPFPTGLAGGSVAKYMVGYLVLTGGVA--QCTTLGPPFWWSVGQE 118
T.newnesi             GGMLVVFAYSVALCADPFPTGLAGGSVAKYMVGYLALTGVVA--QCTTLGPPFWWSVGQE 118
P.borchgrevinki       GGMLVVFAYSVALCADPFPTGLAEGSVAKYMAGYLLLTGGAA--QCTTLAPPFWWSVGQE 118
L.squamifrons         GGVLVVFAYSVALCADPFPTGLAGESVAKCMLGYLVLTGGMALSQWNMDSPPFWWSVGQE 120
P.cerebropogon        GGMLVVFAYSAALCAETHPTGLAEGSVLKCMGGYLAGVAGAALFQGGSEAPLFWWLAGEE 120
P.scotti              GGMLVVFAYSAALCAETHPTGLAEGSVLKCMGGYLAGVAGAALFQGSSEAPLFWWLAGEE 120
H.velifer             GGMLVVFAYSAALCAETHPTGLAEGSVLKCMGGYLAGVAGAALFQGSSEAPLFWWLAGEE 120
H.antarcticus         GGMMVVFAYSAALCSETRPTGLAEGSVLKCMGGYFVVAAGVALYQGGMEGPPFWWFAGEE 120
C.myersi              GEMLVVFAYSAALCAEVHPTGLSEGSVLKCMVGCFGVVSGVALLQGSPEAPLYWWFVGEE 120
C.rastrospinosus      GGMLVVFAYSAALCAEVHPTGLSEGSVLKCMVGYFGVVSGVALLQGSPEAPLYWWFVGEE 120
C.aceratus            GGMLVVFAYSAALCAEVHPTGLSEGSVLKCMMGYFGVVLGVGLLQGNPEAPLYWWFVGEE 120
C.esox                GGMLVVFAYSAALCAESHPTGLTEGSVLKAMVGYLGVVFGAAVFQGTAEAPLFWWFVGEE 120
R.glacialis           GGMLVVFAYSAALCAEVHPTGLAEGSVLKCMVGYFAVVAGVAVFQGSSEAPVYWWFMGEE 120
P.antarcticum         GGMLVVFAYSVALCAGAPSTGLGEESVLKCMAGYLGVTGVVGFLQGSSEGPAFWWFAGGE 120
B.variegatus          GGMLVVFAYSAALAAEPFPESWGSGSVMLYMFLYLMATGVVGSMLFEGWYEFSWGVVDEF 120
E.maclovinus          GGILVVFAYSAALAAEPFPESFGTTGAVLYFMGLVGVVGVFAILYIEEGSGLGGCSSDEV 120
P.urvillii            GGMLVVFAYSAALVAEPYPEGLGSAPVLVYVVGFLGMIGGVGIVCWEGWDEAVWFSTECT 120
                      * ::******.** :   . .     .   .   .
                         124S
                           126M**
N.coriiceps           SEMSVMQGETEGVSEAYGSGGILLLTCGWALLVALYVALEVSRGCSRGPVRPIK 174
N.rossii              SEMSVMQGETDGVSEAYGSGGILLVTCGWALLVALYVALEVSRGCSRGPVRPIK 174
N.angustata           LEMSAMQGETEGVSEVYGSGGILLITCAWALLVALYVALEVSRGCNRGTVRPIE 174
N.microlepidota       SEMSVMQGEIEGVSEAYGSGGFLLIICAWALLVALYVALEVSRGCSRGTVRSIK 174
T.bernacchii          ADMSAMQGETEGVSEAYGSGGILLLTCAWALLVALYVALEVSRGCSRGPVRPIK 172
T.eulepidotus         ADMSAMQGEAEGVSEAYGGGGILLITCAWALLVALYVALEVSRGCSRGPVRPIK 172
T.newnesi             ADMSAMQGETEGVSEAYGGGGILLITCAWALLVALYVALEVSRGSSRGPVRPIK 172
P.borchgrevinki       ADMSAMQGEAEGVSEAYGGGGIILISCAWALLVALYVALEVSRGSSRGPVRPIK 172
L.squamifrons         ADMSAMQGETEGVSEAYGGGGILLITCAWALLVALYVALEVSRGSNRGPVRPV- 173
P.cerebropogon        SEMSVVQGESDGVSEAYGGGGIMLITCGWVLLVALYVALEVSRGCSRGPVRPIK 174
P.scotti              SEMSVVQGESDGVSEAYGGGGIMLITCGWVLLVALYVALEVSRGCSRGPVRPIK 174
H.velifer             SEMSVVQGESDGVSEAYGGGGILLITCGWVLLVALYVALEVSRGCSRGPVRPIK 174
H.antarcticus         SGMSVMQGESEGVSEAYGGGGILLIVCAWVLVVALFVALEVSRGCSRGPVRSIK 174
C.myersi              AEMNVVQGGTEGVSEAYGGGGMCLVICAWVLLVALYVALEVSRGSSRGPVRPIK 174
C.rastrospinosus      AEMNVVQGETEGVSEAYGSGGMCLVICAGVLLVALYVALEVSRGSSRGPVRPIK 174
C.aceratus            AEMNVVQGETEGVSEAYGSGGMCLVICAWVLLVALYVALEVSRGSSRGPVRPIK 174
C.esox                AEMNVVQGETEGVSEAYGSGGMCLVICAWVLLVALYVALEVSRGSSRGTVRSIK 174
R.glacialis           SELSVVQGETDGVSEAYGSGGICLVVCAWVLLVALYVALEVSRGSSRGPVRPIK 174
P.antarcticum         WEMAVMQGEAAGVSEAYGGGGFLVVTCGWALLIALYVALEVSRGPSRGPVRPFK 174
B.variegatus          CEFSLFRGDTGGVAMMYSSGGGMLVLSAWVLFLTLFVVLELTRGLSRGALRAV- 173
E.maclovinus          IGMSSCVTEYDGMSQIYGPGGFLLVLCAWVLLLTLFVVLEMTRGSSRGPLRSVK 174
P.urvillii            NEFSMCCEEAQGMSEAYGFGGFLLIMSAWVLLLALFVVLEMTRGASRGPLRSVK 174
                        :        *::  *. **  :: .. .*.::*:*.**::** .**.:*..
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Fig. S1.--- Sequence alignments of mitochondrial NADH dehydrogenase subunit 6 (ND6) gene nucleotides and deduced amino acid residues of 22 notothenioid spp. Abbreviations of the species names refer to the main manuscript. Dashed lines indicate introduced gaps by alignment. **(A)** H-strand (sense strand) nucleotide sequence alignment of ND6 gene. Start and stop codons are boxed. Asterisks indicate nucleotide identity in the column. **(B)** Amino acids sequences alignment of deduced ND6 residues. Asterisks stands for identical residues, ':' for conservative substitutions and '.' for semiconservative substitutions. The seven amino acids identified by branch-site model A test to be under positive selection (Table 1) are indicated and highlighted in yellow.

Supplementary Material

(A) Possible Causes for Misdiagnosis of a 'Missing' ND6 – detailed description

In the study by Papetti et al. (2007), loss of ND6 gene in Antarctic notothenioids was inferred based on: (1) its absence at the canonical position in the mtDNA of all the species they examined; (2) no ND6 could be found in the complete mt genome of the icefish *Chionodraco rastrospinosus*; (3) in DNA dot blot, and (4) in RT-PCR for ND6 transcript, they found no evidence of mt ND6 gene copies in heteroplasmy, or of a functional ND6 gene copy in the nuclear genome respectively. Our results provide clear evidence for alternate explanations to these four observations, detailed below.

(1) We confirmed the PCR-based result of Papetti et al (2007) of the absence of ND6 at the canonical position between ND5 and Cytb in the mtDNA of Antarctic notothenioids, replaced by a short non-coding sequence, in contrast to their basal non-Antarctic cousin, *B. variegatus, P. urvillii*, and *E. maclovinus*. However, it is not a result of ND6 loss, but the degeneration of the ND6 copy at the canonical position after the regional duplication (ND6-through-CR) in the common ancestor to the Antarctic clade. The $ND6_{CR}$ copy embedded in the rearranged CR became the functional gene, and likewise, for the $tRNA^{Glu}$, which was reportedly missing.

(2) The apparent absence of a ND6 gene in the “complete mtDNA sequence” of their test species *C. rastrospinosus* supported their inference of ND6 loss. However, the missing ND6 gene in *C. rastrospinosus* is not due to gene loss, but to the fact that the reported mt genome sequence was incomplete, specifically in the protion of the CR where we discovered the embedded $ND6_{CR}$ and $tRNA^{Glu}$ genes. The annotations for *C. rastrospinosus* mtDNA in GenBank (DQ526431) (Papetti et al. 2007) specify that it is a partial genome, and that there are

two gaps in the mid-section of the D-Loop (Control Region), one of unknown length. The first gap in the mid-section of the CR is where we found the translocated ND6 gene in Antarctic notothenioids including *C. rastrospinosus* (figs. 1, 2). The reported sequence downstream from the first CR gap in *C. rastrospinosus* (DQ526431) is ~96% identical to the sequence downstream from $ND6_{CR}$/$tRNA^{Glu}$ genes that we obtained for this species, indicating the first gap in DQ526431 is the location of $ND6_{CR}$/$tRNA^{Glu}$ genes, rather than wholly non-coding CR sequence.

(3) In Southern dot blot analyses, Papetti et al. (2007) used *E. maclovinus* ND6 as probe to detect ND6 presence in the DNA of other species, and found positive hybridization in the related basal non-Antarctic notothenioid *B. variegatus*, as well as in the unrelated teleosts *Danio rerio*, *Sparus aurata*, and *Seriola dumerilii*, but not in Antarctic notothenioids *C. rastrospinosus* and *H. antarcticus*. They inferred, quite logically, that the efficacy of the *E. maclovinus* ND6 probe was proven, thus the dot blot result was taken as support for the homoplasmic lack of mt ND6 in the Antarctic notothenioids. We deduced however that the absence of hybridization very likely resulted from the absence of $ND6_{CR}$ in their preparation of the mt DNA. To generate mtDNA of the two Antarctic test species for the dot blot, they used six pairs of degenerate primers to PCR amplify the mt genome in six presumably overlapping fragments. The amplified regions can be readily deduced from the mnemonic primer names (supplementary table S2 in Papetti et al. 2007), with which we found the fragment from Cytb to D-loop (amplified with primer pair cytbfornot/d-looprevnot) would not overlap with the succeeding fragment from 12S rRNA to ND1 (amplified with primer pair 12Sfornot/ND1revnot), such that there would be a sizable gap spanning much of the rearranged CR or non-coding region (that we now know to exist) through part of 12S rRNA. This gap is where we found the $ND6_{CR}$ gene in *H. antarcticus*, *C.*

rastrospinosus and other Antarctic notothenioids (fig. 2). Thus, the negative dot blot hybridization to *E. maclovinus* ND6 probe for *H. antarcticus* and *C. rastrospinosus* resulted from the exclusion of the rearranged CR portion containing the $ND6_{CR}$ gene in the PCR generated test mt DNA, rather than ND6 gene loss from Antarctic notothenioids mt genomes.

(4) To detect ND6 gene transcripts, Papetti et al. (2007) used degenerate primers designed to match conserved regions of teleost ND6 sequences, and successfully RT-PCR amplified ND6 cDNA from *D. rerio* RNA, but not from the Antarctic notothenioids *C. rastrospinosus* and *T. eulepidotus* RNA. They inferred, again logically, that this result supported the complete absence of ND6 transcripts in the transcriptome, both mitochondrial and nuclear, of the two Antarctic notothenioid species. An alternate explanation is the lack of primer specificity for the target sequence in Antarctic notothenioid species to permit successful amplification. Their degenerate primers have only 55-61% nucleotide match with the target sites in the $ND6_{CR}$ sequence of *C. rastrospinosus* and *T. eulepidotus* we obtained in this study and additionally they lack 3' complementarity, as opposed to a 73-74% nucleotide match and 3' complementarity to the corresponding sites in their positive control *D. rerio* (figure in section B). Indeed, we first tried the same degenerate primers and PCR cycling conditions, and failed to amplify ND6 cDNA from Antarctic notothenioids. Only when we designed species-specific primers to prime the first strand cDNA synthesis from $ND6_{CR}$ mRNA of each species, and paired it with an appropriate forward primer (supplementary table S1) for the subsequent PCR amplification, that $ND6_{CR}$ transcripts were successfully amplified (fig. 4).

(B) Comparisons of the sequences of degenerate primers (in blue) used by Papetti et al. (2007) to amplify ND6 gene transcripts from *C. rastrospinosus* and *T. eulepidotus*, with the actual sequences of the primer sites in $ND6_{CR}$ we obtained for these two species, and those of ND6 (accession numbers AC024175) from their teleost control *D. rerio*. The nucleotide positions of the primer sites in $ND6_{CR}$ are indicated. The degenerate primers share greater nucleotide identity with the primer site sequences in *D. rerio* ND6 than with notothenioid $ND6_{CR}$ and are deficient in 3' complementarity to $ND6_{CR}$, which are likely the reasons for successful amplification of *D. rerio* ND6 transcript, but not the notothenioid $ND6_{CR}$ transcripts.

C.rastrospinosus — Nucleotide Identity

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                     73 CCCTTTTATGCGGCACTGGGGGT 95
                        ** * ** .**.**. * **. *         56.5%
Nd6int2FORuniv       5'-CCKTATTTYGCKGCHTTWGGKTT-3'

                    482 CGAGACACCTCCAAAGCAACAT 461
                        **.* .*..** *.. *****           54.5%
Nd6int2REVuniv       5'-CGYGTHAHYTCTARHACAACAA-3'
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T.eulepidotus

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                     73 CCCTTTTATGCGGCCCTCGGGTT 95
                        ** * **.* *.**.* ** .**         60.9%
Nd6int2FORuniv       5'-CCKTATTTYGCKGCHTTWGGKTT-3'

                    482 CGCGATACTTCTAGAGCAACAT 461
                        **.* .*..****.. *****           59.1%
Nd6int2REVuniv       5'-CGYGTHAHYTCTARHACAACAA-3'
```

D. rerio

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                     73 CCTTATTTTGCTGCATTTGGTTT 95
                        **.*****.**.**.**.**.**         74.0%
Nd6int2FORuniv       5'-CCKTATTTYGCKGCHTTWGGKTT-3'

                    485 CGAGTAACCTCTAGTACAACAA 464
                        ** **.*..****..*******          73.0%
Nd6int2REVuniv       5'-CGYGTHAHYTCTARHACAACAA-3'
```

R = A,g; Y = C,T; M = A,C; K = g,T; S = g,C; W = A,T; H = A,C,T; B = g,T,C; V = g,C,A; D = g,A,T; N = A,C,g,T