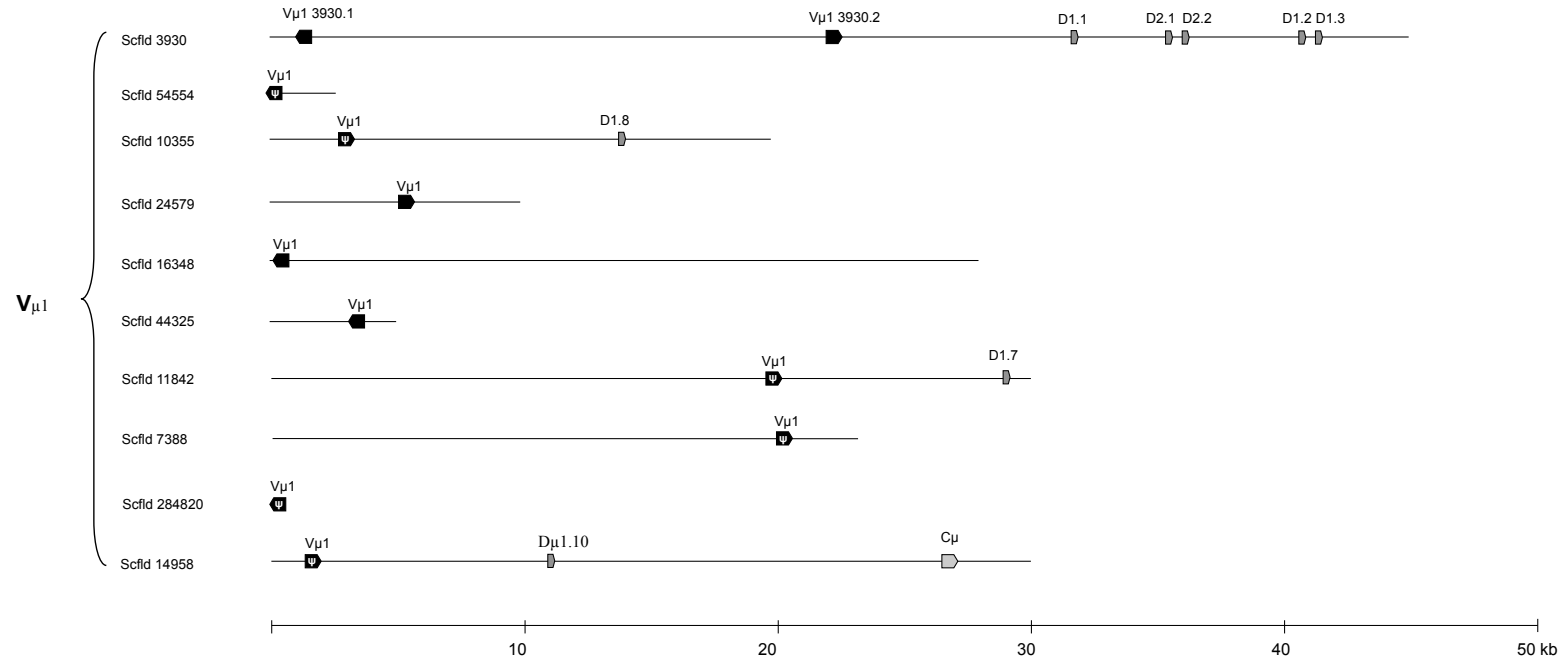
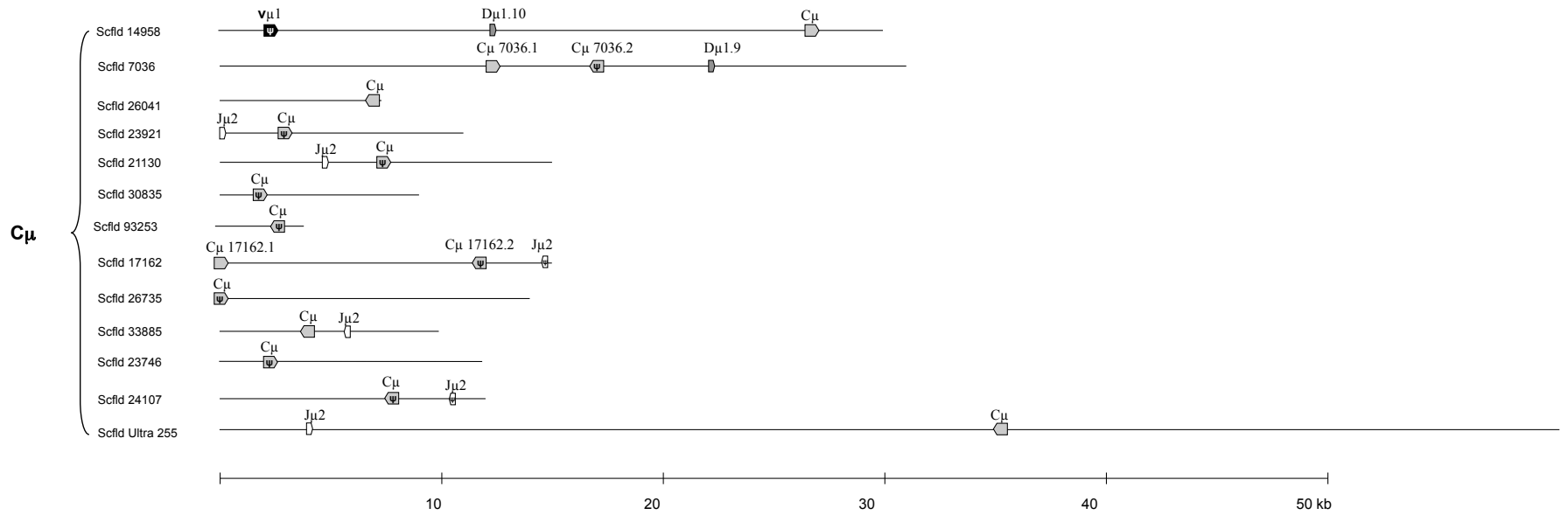


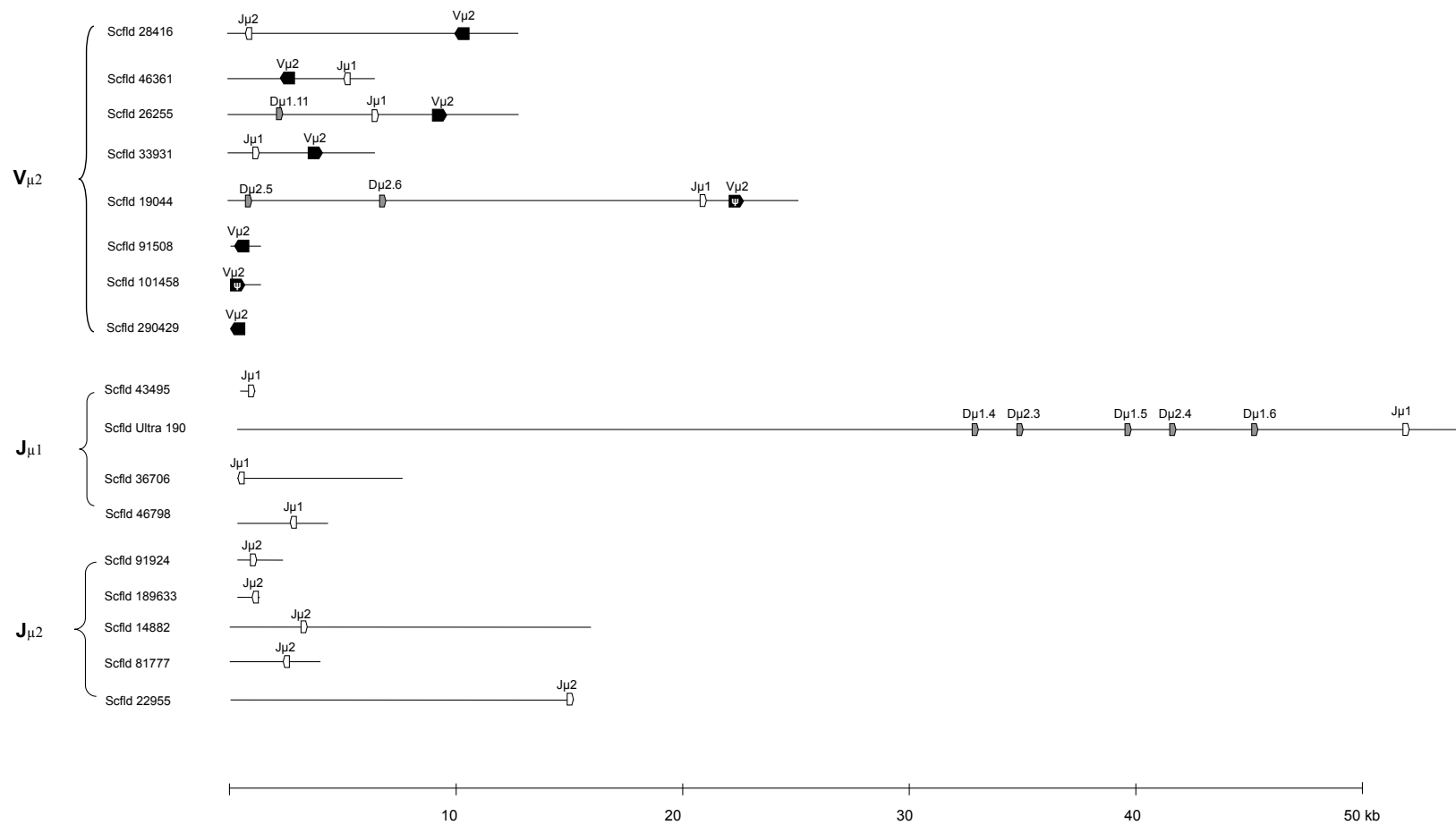
**Supplementary Figure 1:** Diagram of the scaffolds containing TCR $\mu$  genes from the platypus whole genome assembly version 5.0.1. V $\mu$  (black), D $\mu$  (dark grey) J $\mu$  (white) and C $\mu$  (light grey) gene segments are shown in their relative position and transcriptional. Presumptive pseudogenes are indicated with  $\psi$ .

**Supplementary Figure 2:** Alignment of the predicted amino acid sequences of the six functional platypus C $\mu$  identified in the whole genome assembly with their homologues from marsupials and conventional TCRs from a variety of mammalian species. Dashes indicate identity with the first sequence and dots indicate gaps inserted to generate the alignment. Conserved cysteines involved in intra-domain disulfide bonds are indicated in shade.

**Supplementary Figure 3:** Sequences of D $\mu$  including the flanking RSS and three alternative translations. D $\mu$  coding sequences are shown lowercase and RSS are uppercase. Heptamers and nonamers are in bold. Stop codons are indicated by dashes. For those D $\mu$  that were found used in cDNA sequences, the number of clones found is indicated to the right of the reading frame being used.

# Supplementary Fig. 1





# Supplementary Fig. 2

		. . . . .10 . . . . .20 . . . . .30 . . . . .40 . . . . .50 . . . . .60 . . . . .70 . . . . .80 . . . . .90 . . . . .100 . . . . .110.
C <sub>μ</sub>	platypus 62	APAESQPT.VFILRNQSFGGCLVKDFYPKELSLTLSAPRPPLTEPLQATASSS.....QGYTTTIRIG...QFS.....EEDSVTCSVRHGVSQINVTE
	platypus 58	-R-----V---N-AT---Y---K---S---TS-----M-PTA.....-SAV-----R.....-T---AW-----
	platypus 36	-----M--V--K--AS-----EK-----STL--K---M-PTA.....-SAVG-----G.....-GET-I-----M---M--
	platypus 41	-R-----K--V---AA-----K-----SS-----PTA.....-SAVG-----G.....KVET-----Q---M---M--
	platypus 85	-----M--VM---AA-----S-----T-H-R-M---M-PT.....W---SAVG-----G.....-A-M---Q-----
	platypus 55	-----V-----H-----
	opossum 08	PRDPLS-S--VV-S-DAVA---S-----IDAS--SLGASVSAQALTV-PII.....H-V-SA-Q---KAG.....DN-----K-LGKETHESH
	opossum 06	PRDPLS-S--VV-S-DAVA---S-----IDAS--SLGASVSAQALTV-PII.....H-V-SA-Q---KAG.....DN-----K-LGKETHESH
	opossum 04	PRDPLS-S--VV-S-DAVA---S-----IDAS--SLGASVSAQALTV-PTI.....H-V-SA-Q---KAG.....DN-----K-LGKETHESH
	opossum 02	PRDPLS-S--VV-S-DAVA---S-----IDAS--SLGASVSAQAETV-PTI.....H-V-SA-Q---KAG.....DN-----K-LGKETHESH
C <sub>δ</sub>	opossum 01	PR-PLT-S--L--S-DAVA---S-----D-NVS-ASS-ASIPGQVLTV-PTA.....R---SA-Q---RVD.....-N-A-I---Q-LGKE-HMSH
	opossum 07	PR-PLS-S--LV-D-DAVA--IRN-----HVS-TSSGTLISAQNLSTL-PTA.....S---SA-H---RVG.....-N-AII---K-LGKE-HIFH
	opossum 03	PQ-PLS-S--LV-D-NAVA--IRN-----HVS-TSSGTLISAQNLTL-PMA.....T---SA-H---RVG.....-N-AI---K-LEKE-HMSH
	opossum 05	PA-PLS-S--LV-D-DAVA--IRN-----HVS-TSSGTLISAQSLSL-PTA.....S---SA-H---RVG.....-N-AI---K-LGKE-HMSH
	bandicoot93	SQ-PLY-S--LV-S-NAVA--IRN-----HVS-VSS-TLILAKNISLVMP.....N---SAVQ---KIG.....-N-P---L-N-LGKETH-SY
	wallaby 50	LKPPYL-S--MM-D-DSVA--IRN-----NVS-DS-GALISAQPLTLI-MA.....N---SAVQT---KVG.....-N-T---L-K-LGKV-H-SH
	human 24	.QPHTK-S--VMK-GTNVA---E-----DIRIN-V.SSKKI--FDP-IVI-P.....S-K-NAVKL---KYE.....DSN-----Q-DNKTVHS-D
	mouse 94	SQPPAK-S---MK-GTNVA-----VTIS-R.SSKKIV-FDP-IVI-P.....S-K-SAVKL---YG.....DSN-----Q-N-ETVHS-D
	opossum 71	FQET-V-S--VMK-GTNVA-----TVEIQ-H.-DNNPS...GVVATA.....D-KFSAVKL---YKK.....DL-QIN-T-W-NNKVVVASY
	bandicoot95	.QET-L-SKI-VMK-GTNAA-----SIAIN-Y.-ENN.-R...QVVTIV.....N-KFSAVKF---YTQ.....DLENI--T-QYKNQT-MTSY
C <sub>α</sub>	wallaby 47	NQNP-T-S--VMK-GTNVA-----SVDIH-P.-EGKVI...GVVTTA.....N-KFSAVKL---YKQ.....DLEQ-K-T-K-NNNTVEAFY
	platypus 59	NQDYRE-S--L-K-E-TYA-VANN---NAKIHMKL-GKKITITNIEHK-VT.....D-K-SMVQ-T...E-E.....SDGA-N-T-E-EGKYVTPQO
	opossum 04	N-QPALYQ.LRSPKSSNTSV--LT--G.FYNGSIKNET.VTGS-ATVLEMMTME.....SKS-GAVTW-SKSN-TCTDAFR...KDMFDNFQFSGSK-NSS-AEQGFETDR
	human 57	N-DPAVYQ.LRDSKSSDKSV--FT--DSQTNVSQSKDSVYI--DKTVLDMR-MD.....FKSNSAVAVSNKSD-ACANAFNNSIIPEDTFFPSPSS-D-KLVEKSFETDT
	mouse 79	N-EPAVYQ.LKDP-S-DSTL--FT--DSQINVPKTMESGTFI--DKTVLDMKAMD.....SKSNGA-AWSNQT-S-TCQDIFK...ETNATYPSSD-P-DATLTKESFETDM
	echidna 36	N-QPRMYR.LKKPQVNDLSI--FT--GND-VNM-GIRN..IMRA-SVVDVKRLE.....SKSLGIVAWNSLSDWDCQAQAS...EAVYSLSNSSGKV-NAKVNVNENFSSDP
	platypus 99	N-QPRMYH.LKKP-VNDLSV--FT--GNE-VNMMGINN..IKRT-SMVAEKRLA.....SKSLGIVAWNNNLDWKCQAKIS...NITYSLSNSSGKV-NTTAVTENFSSDP
	opossum 07	..QP-EEE..IGEKGKATLV--ATG---DLVE-SWVWNGQETKIGVSTDPPEP--KEHPKEEHS--SLSSRL...RI-APFWRN.....PKNNFR-Q-QFYGIAE-E-W
	human 78	..EP-EAE..ISHTQKATLV--ATG---DHVE-SWVWNGKEVHSGVSTDPQPL.KEQPALNDSR-CLSSRL...RV-ATFWQN.....PRNHFR-Q-QFYGLSE-DEW
	mouse 08	..EP-KAE..IANKQKATLV--ARG-F-DHVE-SWVWNGKEVHSGVSTDPQAY.KES...NYS--CLSSRL...RV-ATFWHN.....PRNHFR-Q-QFHLSEEDKW
C <sub>β</sub>	echidna 35	..DP-EEE..INELGKATLV--ATG-F-DHVE-SWVWNGQETKIGVSTDPQPL.KEQPALNDSR-CLSSRL...RV-AGFWQN.....PSRRFR-Q-FYGLKSDS-W
	platypus 80	..DP-EEE..INELGKATLV--ATG-F-DLVEISWVWNGQETKIGVSTDPQPL.KEQPALNDSR-CLSSRL...RV-AGFWQN.....PWRRFR-Q-HFYGLSDSD-W
	opossum 32	FFLPTSEE..IKQKQSGTYI--LE--F-NVVKTYWKEEDGNSQLDA-FGPITG.....GGNS-SQVSWL...TVKE...DV.....LRKNL-YFYQ-EDLGMPEPKA
C <sub>γ</sub>	human 19	...P-IAE..TK-QKAGTYL--LEK-F-DIIKIHQEKKSNTILGS-EGNNMK.....TND--MKFSWL...TVPE...ES.....LDKEHR-I---ENNKNGIDQ
	mouse 02	...LLLAE..TN-HKAGTYL--LEK-F--VIRVYWEKDKGKILES-EGNTIK.....TNDR-MKFSWL...TVTE...DS.....MAKEHS-I-K-ENNKRG-DQ
	platypus 95	KFFP-SLE..QEQQ-TESYI--F----DVIRMHWKEEGSDKILES-QSDPFR.....VKDK-WQMSWL...TVKK...SS.....PGKIYRLIYK-EKTGR.-EK

### Supplementary Fig. 3

Dμ1.1	<b>GTTTTAGTGT</b> TTGGGTACGCAT <b>CACTGTG</b>	gtaaatatagaac V N I E - I - N K Y R	<b>CACAGCG</b> AAACATCCTCATCAGAGCCCTGT <b>GCAAAAACC</b> 2 1
Dμ1.2	<b>AGTTTAGTGT</b> TTGGCTGTGTAT <b>CACTGTG</b>	atgactggagc M T G - L E D W S	<b>CACAGTG</b> AAACTTCCTCGGCAAGACACCGT <b>ACAAAAACC</b> 4 3
Dμ1.3	<b>AGTTTAGTGT</b> TTGGCTGTGTAT <b>CACTGTG</b>	atgactggagc M T G - L E D W S	<b>CACAGTG</b> AAACTTCCTCGGCAAGGACCGT <b>ACAAAAACC</b> 4 3
Dμ1.4	<b>GGTTTAGTGT</b> TTACTGTGTAT <b>CACTGTG</b>	acgactacgac T T T R L R D Y D	<b>CACAGTG</b> AAACCTCCTTGGCAGGGCCCTGT <b>GCAAAAACC</b> 1
Dμ1.5	<b>GGTTTAGTGT</b> TTGGCTGTGAAT <b>CACTGTG</b>	atacttacacgag I L T R Y L H E T Y T	<b>CACGATG</b> AAACTTCTCGGCAAGGACCCTGT <b>GCAAAAACC</b> 1 1
Dμ1.6	<b>GGTTTAGTGT</b> TTGGATGTGTAT <b>CGCTGTG</b>	atgatggaaa M M E - W K D G	<b>CACAATG</b> AAACTTCCTTGGCAGAGTCTCTGAG <b>CAAAAACC</b> 1
Dμ1.7	<b>AGTTTAGTGT</b> TCATTGTGTAT <b>CACTGTG</b>	ctggctgtagaac L A V E W L - N G C R	<b>CACAGTG</b> AAACTTCCTCTGCAAGGCCGTGT <b>TCAAAAACC</b> 1
Dμ1.8	<b>AATTTAGTGT</b> TTGGTCGTGTAT <b>CACTGTG</b>	gtgggagtggaaac V G V E W E W N G S G	<b>CACAGTG</b> AAATTCCTTTGCTGGGCCCTGG <b>GCAAAAACC</b> 7 4 1
Dμ1.9	<b>GGTTTAGGGT</b> TTGGCCATGC <b>CACTGTG</b>	gtggacataggac V D I G W T - D G H R	<b>CACAGTG</b> AAACATCCTCTGCAAGGCCCTGT <b>GCAAAAATC</b> 2
Dμ1.10	<b>GTTTTAGTGT</b> TTGGCCATACAT <b>CACTGTG</b>	gtagatagaggac V D R G - I E D R - R	<b>CACAATG</b> AAACGTCCTCGGCAAGGCCCTGT <b>GCAAAAACC</b> 1 4
Dμ1.11	<b>GGTTTAGTGT</b> TTGATGGTGTAT <b>CACTGTG</b>	attcctggaac I P G F L E S W N	<b>CACAGTG</b> AATCTCCTCAGCAAGGCTCTGT <b>GCAAAAACC</b> 1 4 1
Dμ1.12	<b>GGTTTAGGGT</b> TTGGCCATGCAT <b>CACTGTG</b>	gtggatataggac V D I G W I - D G Y R	<b>CACAGTG</b> AAACGTCCTCTGCAAGGCCCTGT <b>GCAAAAACC</b> 1 1
Dμ1.13	<b>GGTTTAGTGT</b> TTAGCTGTGTAT <b>CACTGTG</b>	aaggatggagc K D G R M E G W S	<b>CACAGTG</b> AAAGCTCCTCGGCAAGGCCCTGT <b>GTTAAAACC</b> 2
Dμ1.14	<b>AGTTTAGTGT</b> TCATTGTGTAT <b>CACTGTG</b>	atgcatgctgtac M H A V C M L Y A C C	<b>CACAGTG</b> AAACTTCCTCTGCAAGGAAC <b>TGTGCAAAAACC</b> 1
Dμ1.15	<b>AGTTTAGTGT</b> TTGGCTGTGTAT <b>CACTGTG</b>	atgcctggagc M P G C L E A W S	<b>CACAGG</b> AAACTTCCTCGGCAAGGACCAT <b>GCAAAAACC</b> 2
Dμ1.16	<b>GACTTAGTGT</b> TTAGCTGTGTAT <b>CACTGTG</b>	aaatatggagc K Y G N M E I W S	<b>CACGGTG</b> AAACCTCCTTGGCAGGGCCCTGT <b>GTAACAACA</b> 1
Dμ1.17	<b>AGTTTAGTGT</b> TCATTGTGTAT <b>CACTGTG</b>	ctggctgtagtac L A V V W L - Y G C S	<b>CACAGTG</b> AAACTTCCTCTGCAAGGCCGTGT <b>TCAAAAACC</b> 1
Dμ1.18	<b>AGTTTAGTGT</b> TCATTGTGTAT <b>CACTGTG</b>	atgcatggagtac M H G V C M E Y A W S	<b>CACAATG</b> AAACTTCCTCTGCAAGG <b>CACTGTGCAAAAACC</b> 1
Dμ1.19	<b>AGTTTAGTGT</b> TCATTGTGTAT <b>CACTGTG</b>	atgcatgcagtac M H A V C M Q Y A C S	<b>CACAATG</b> AAACTTCCTCTGCAAGG <b>CACTGTGCAAAAACC</b> 1
Dμ1.20	<b>GGTTTAGTGT</b> TTGGCTATGTAT <b>CACTGTG</b> nonamer 12bp spacer heptamer	cagactggtat Q T G R L V D W Y	<b>CACAGTG</b> AAATTCCTCTGCAAGGCC <b>CCATGCAAAAACC</b> heptamer 23bp spacer nonamer 1 1

Dμ2.1	<b>AAAAGATAGT</b> GTTTGTGTGCACAGTG	tgggcaggacagggagaca	<b>CACACTGGACT</b> TTTCAGCCACATAAGCACT <b>CATAAGTGA</b>
		W A G Q G D 3	
		G Q D R E T 4	
		G R T G R	
Dμ2.2	<b>AAAAGATAGT</b> GTTTGTGTGCACAGTG	tgggcaggacagggagaca	<b>CACACTGGACT</b> TTTCAGCCACACAAGCACT <b>CATAAGTGA</b>
		W A G Q G D 3	
		G Q D R E T 4	
		G R T G R	
Dμ2.3	<b>AAAGTATGGC</b> TTTTGTGTGCACAGTG	tggtcataacagggagata	<b>CATTTTGGCCT</b> TTTCAGCCACACAAGCACT <b>CCCTTCAAA</b>
		W S - Q E D 7	
		G H N R R I	
		V I T G G	
Dμ2.4	<b>AAAAATATGGC</b> TTTTGTGTGCACAGTG	tggtcaggacagggagaca	<b>CACATTGGCCT</b> TTTCAGCCACACAAGCACT <b>CACAAGTGA</b>
		W S G Q E T 3	
		G Q D R R	
		V R T G D 1	
Dμ2.5	<b>AAAAGATGGC</b> TTTTGTGTGCACAGTG	tggttaggttaggggagaca	<b>CACATTGGACT</b> TTTCAGCCACACGAGCACT <b>CATAAGGGA</b>
		W L G - G D 1	
		G - V R G T 1	
		V R L G G	
Dμ2.6	<b>AAAAGATGGC</b> TTTTGTGTATGCACAGTG	tggtcaggtaggggagaca	<b>CACACGT</b> TGCACTTTTCAGCCACACAAGCACT <b>CATAAGGGA</b>
		W S G - G 1	
		G Q V R G	
		V R L G 1	
Dμ2.7	<b>AAAAGATGGC</b> TTTTGTGTGCACAGTG	tggtcaggacaggggagaca	<b>CACATTGGCCT</b> TTTCAGCCACACAAGCAAT <b>CAACAGTGA</b>
		W S G Q G D 1	
		G Q D R G T	
		V R T G G	
Dμ2.8	<b>AAAAGATGGC</b> TTTTGTGTGCACAGTG	tggtcaggtaggggagaca	<b>CACACGT</b> TGGATTTTCAGCCACACAAGCACT <b>CATAAGGGA</b>
		W S D - G 1	
		G Q I R G 2	
		V R L G	
Dμ2.9	<b>AAAAGATGAC</b> TTTTGTGTGCACAGTG	tggtcaggacaggggagaca	<b>CATATTGGCCT</b> TTTCAGCCACATAAGCAAT <b>CAACAGTGA</b>
		W S G Q G D 1	
		G Q D R G T	
		V R T G G	
Dμ2.10	<b>AAAAATATGGC</b> TTTTGTGTGCACAGTG	tggtcaggacaggggagaca	<b>TACATTGGCCT</b> TTTCAGCCACACAAGCACT <b>CACAAGTGA</b>
		W S G Q G T	
		G Q D K G 1	
		V R T R D	
Dμ2.11	<b>AAAAGATGGC</b> TTTTGTGAGCGCACAGTG	tggtcaggtaggggagaca	<b>CACATTGGACT</b> TTTCAGCCACACGAGCACT <b>CATAAGGGA</b>
		W P G - R D	
		G Q V R G T	
		A R L E G 1	
Dμ2.12	<b>AAAAGATGGC</b> TTTTGTGTGCACAGTG	tgatcaggtaggggagaca	<b>CACACGT</b> TGGACTTTTCAGCCACACAAGCACT <b>CAGAAGGGA</b>
		- S G - G 1	
		D Q V R G	
		I R L G	
Dμ2.13	<b>AAAAGATGGC</b> TTTTGTGAGTGCACAGTG	tggtcaggtaggggagaca	<b>CACAATGGACT</b> TTTCAGCCACCCGAGCACT <b>CATAAGGGA</b>
		W P G K R D	
		G Q V R G T	
		A R - E G 1	
Dμ2.14	<b>AAAAGATGGC</b> TTTTCTGTGTACACAGTG	tggtcaggtaggggagaca	<b>CACATTGGACT</b> TGTCATCCACACAAGCACT <b>CATAAGGAG</b>
		W S G - G D	
		G Q V R G T 2	
		V R L G G	
Dμ 2.15	<b>AAAAGATGGC</b> TTTTGTGTGCACAGTG	tggtcaggtaggggagaca	<b>CACATTGGCCT</b> TTTCAGCCACACAAGCACT <b>CCCTAGATG</b>
	<b>nonamer 12bp spacer heptamer</b>	W T G Q G D 1	<b>heptamer 23bp spacer nonamer</b>
		G Q D R G T 2	
		D R T G G	

Supplementary Table 1: *The V, D, J and C usage in platypus TCR $\mu$  cDNA sequences*

Clone name	V $\mu$ 1 <sup>#</sup>	D $\mu$	D $\mu$	D $\mu$	D $\mu$	J $\mu$ 1	V $\mu$ 2	D $\mu$ ?	J $\mu$ 2	C $\mu$
21F	16348	2.3	1.2/1.3	1.9/1.12	1.20	46361	46361		91924	17162.1
26R	16348	1.4	1.8/1.11	1.8/2.1/2.2	1.8/1.11	46361	46361	2.8	91924	17162.1
2.22	16348	1.13	1.8/2.1/2.2	1.8/1.11	1.15	46361	46361		91924	17162.1
3815	16348	1.20	1.9	1.1/1.7/1.8/1.11		46361	46361	2.8	91924	17162.1
1915	16348	1.2/1.3	1.8/1.11	1.8/2.1/2.2		46361	46361		91924	17162.1
1953	16348	1.6/1.8/1.11	1.20	1.8/2.1/2.2	1.10/2.3	46361	46361		91924	17162.1
1954	16348	1.2/1.3	1.5	1.10/2.3		46361	46361		91924	17162.1
1955	16348	1.10	1.2/1.3	2.5	1.8/2.1/2.2	46361	46361		91924	17162.1
4951	16348	1.1	1.2	2.1/2.2	1.12	46361	46361		91924	21130
4942	16348	2.11/2.13	1.2/1.3			46361	46361		91924	17162.1
786*	16348	1.12	1.1			46361				
6	16348	2.12	2.2/2.3			46361	46361		91924	17162.1
17	16348	1.8/2.1/2.2	2.6/2.14	1.8/2.1/2.2		46361	46361		91924	17162.1
2.34	24579	1.8/2.1/2.2	1.8/2.1/2.2	1.8/1.11		46361	46361		91924	17162.1
10	24579	1.2/1.3	2.1/2.2	1.13		36706	28416		28416	26041
36F*	3930.2	1.8/2.1/2.2	1.1/1.7/1.8/1.11	2.3/2.4		46798				
4966	3930	1.2/1.3	1.5			46798	33931		21130	21130
1.22	3930	2.4/2.6	1.15			46798	33931		21130	21130

\* 3' partial sequence lacking V2 domain.

# The number in the column designates the scaffold on which germ-line gene segment is identified.