Dottorato di ricerca industriale (2018)

Cronoprogramma

		Approcci sperimentali utilizzati	I Anno												II Anno												III Anno												Deliverables
			1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
WP1. Analisi molecolare e morfo-funzionale del sistema digerente di zebrafish	A1. Analisi trascrittomica, lungo l'asse rostro-caudale, del sistema digerente di zebrafish	1. Consultazione Banche Dati* 2. Genomica e trascrittomica funzionale comparata* 3. Bioinformatica*				§	§	§																															D1. Analisi completa del trascrittoma del sistema digerente di zebrafish confrontato con dati provenienti da altri pesci e/o vertebrati superiori (mammiferi e uomo compreso) (mesi 1-6)
	A2. Identificazione, selezione e verifica per espressione dei geni principalmente coinvolti nelle funzioni alimentari (digestione, assorbimento, secrezione, motilità, immunità) nel sistema digerente di zebrafish adulto	1. Isolamento di acidi nucleici (DNA e RNA) e proteine* 2. Real-time PCR semi-quantitativa e quantitativa* 3. *In situ* hybridization 4. Analisi per Western blot* 5. Analisi ed elaborazione dei dati				§	§	§	§	§	§	§	§	§																									D2. Identificazione molecolare e localizzazione dei prodotti di espressione genica di almeno 1/3 dei geni differenzialmente espressi lungo il sistema digerente di zebrafish adulto**** (mesi 4-15)
	A3. Identificazione, selezione e verifica per espressione dei geni principalmente coinvolti nelle funzioni alimentari (digestione, assorbimento, secrezione, motilità, immunità) nel sistema digerente di larve e giovanili di zebrafish	1. Isolamento di acidi nucleici (DNA e RNA) e proteine* 2. Real-time PCR semi-quantitativa e quantitativa* 3. *In situ* hybridization* 4. Whole-mount *In situ* hybridization* 5. Analisi per Western blot* 6. Analisi ed elaborazione dei dati							§	§	§	§	§	§																									D3. Identificazione molecolare e localizzazione dei prodotti di espressione genica durante lo sviluppo larvale e giovanile di zebrafish, con particolare riferimento ai momenti critici dello sviluppo (ad es. giorni 7-12 e giorni 28-35 dopo la fecondazione) (mesi 7-15)
WP2. Messa a punto di diete speciali contenenti molecole con particolare valore alimentare e/o nutraceutico	A1. Identificazione e selezione di molecole con particolare valore alimentare e/o nutraceutico^ in zebrafish	1. Consultazione Banche Dati 2. Consultazione di collezioni chimiche (*chemical libraries*)																																					D1. Identificazione di molecole con valore alimentare e/o nutraceutico utili per favorire almeno una funzione alimentare (mesi 1-3)
	A2. Verifica del potenziale delle molecole sulle funzioni alimentari	1. Screening di molecole mediante incubazione con embrioni e larve di zebrafish*				§	§	§	§	§	§	§	§	§																									D2. Selezione di almeno 3 molecole con valore alimentare e/o nutraceutico (mesi 4-12)
	A3. Preparazione di diete *ad hoc* contenenti le molecole selezionate (da sole o in combinazione)	1. Preparazione delle diete e dei mangimi contenenti le molecole selezionate*** 2. Analisi ed elaborazione dei dati																																					D3. Preparazione di almeno 3 diete e 3 mangimi contenenti le molecole selezionate (mesi 13-15)
WP3. Analisi degli effetti delle diete speciali sulla capacità di crescita, e più in generale sul benessere, di zebrafish	A1. Ricerca e sviluppo di una tecnologia per l'ottimizzazione della misura degli effetti delle diete in zebrafish	1. Ricerca e sviluppo sul sistema Tritone (Tecniplast S.p.A.)** 2. Implementazione di nuove tecnologie sul sistema Tritone (Tecniplast S.p.A.)**																$	$	$	$	$	$	$	$	$													D1. Integrazione di almeno un nuovo elemento tecnologico nel sistema di distribuzione del cibo detto Tritone (mesi 16-24)
	A2. Messa a punto di protocolli *ad hoc* per l'analisi degli effetti delle diete su crescita, morfologia e, più in generale, benessere degli zebrafish in allevamento	1. Standardizzazione di protocolli sperimentali che tengano conto anche delle normative vigenti**																					$	$	$	$													D2. Definizione di almeno un protocollo operativo per la somministrazione controllata ed efficace dei mangimi nel sistema di distribuzione del cibo detto Tritone (mesi 21-24)
	A3. Analisi morfometrica, funzionale, e dell'accrescimento in zebrafish trattati con le diete speciali rispetto a zebrafish trattati con diete di controllo	1. Analisi biometriche, fisiologiche e comportamentali che tengano conto anche delle normative vigenti 2. Analisi ed elaborazione dei dati																																					D3. Definizione in condizioni standardizzate degli effetti benefici delle diete/mangimi speciali rispetto alle diete/mangimi di controllo (mesi 25-36)

*Parte dell'attività di ricerca di base sarà sviluppata presso il Dipartimento di Biologia dell'Università di Bergen (Bergen, Norvegia). (§) Periodo previsto per le attività nell'Università straniera: 9 mesi

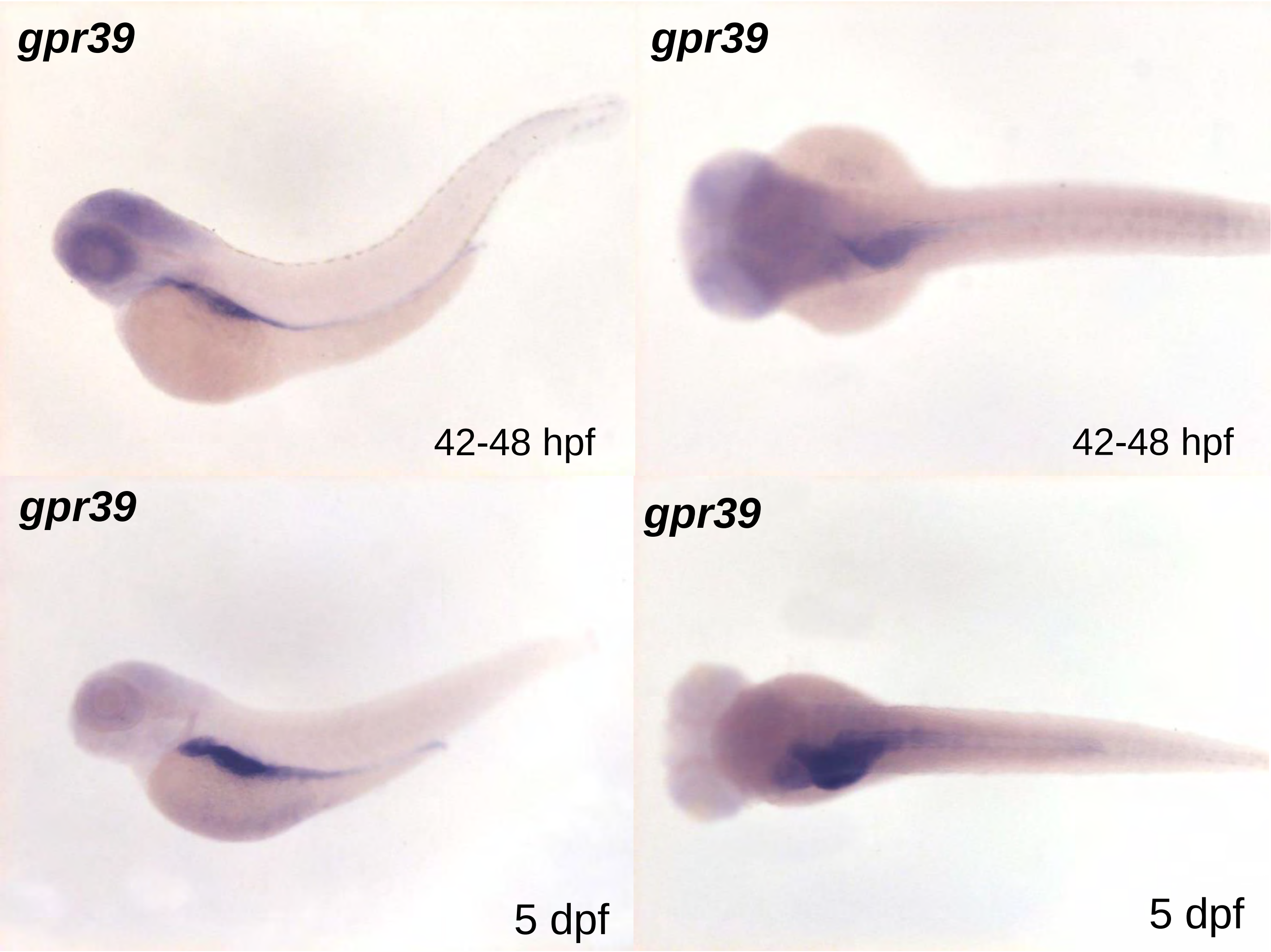
**Gli studi di ricerca e sviluppo e la tecnologia che ne deriverà sarà sviluppata nell'azienda partner di questo progetto, Tecniplast S.p.A. (Buguggiate, VA), durante lo stage in azienda del dottorando. ($) Periodo previsto per le attività in azienda: mesi 9

***Diete e mangimi verranno sviluppati e preparati con l'ausilio di SPAROS LDA [impresa spin-off del Centro per le Scienze Marine di Algarve (CCMAR) dell'Università dell'Algarve, Portogallo].

****Si stima che il trascrittoma del sistema digerente dello zebrafish adulto esprima almeno 22000 geni.

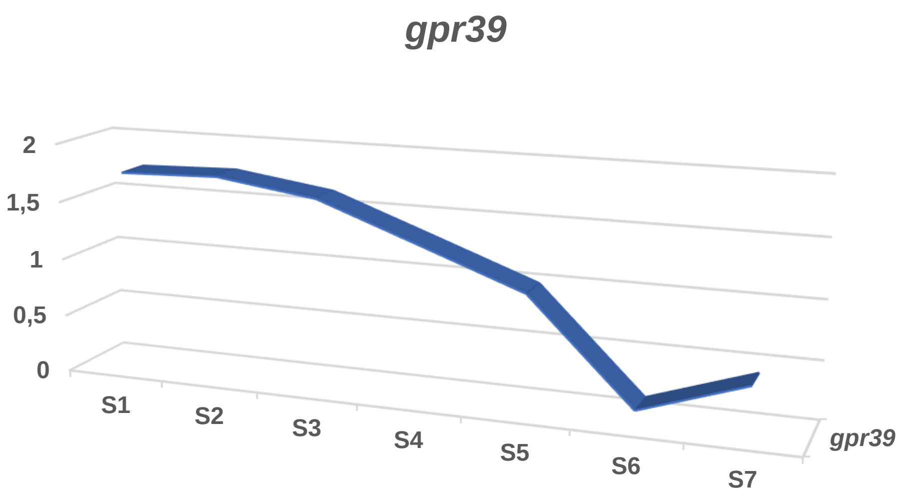
^Le molecole saranno selezionate tra piccoli peptidi, sostanze peptido-mimetiche e/o peptido-simili sulla base delle informazioni di letteratura e sulla base delle esperienze pregresse maturate nei laboratori di ricerca, italiano e norvegese, partecipanti al progetto; particolare attenzione sarà data alle molecole peptidiche di origine dietetica, derivanti dai processi digestivi delle proteine di origine alimentare.

gpr39



Expressed in intestinal bulb and in brain

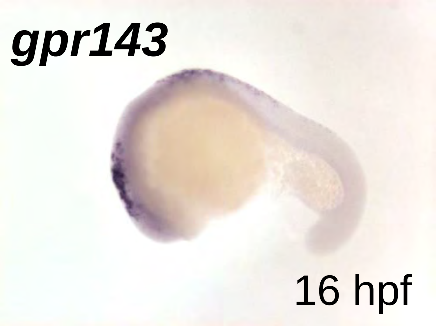
Adult gut

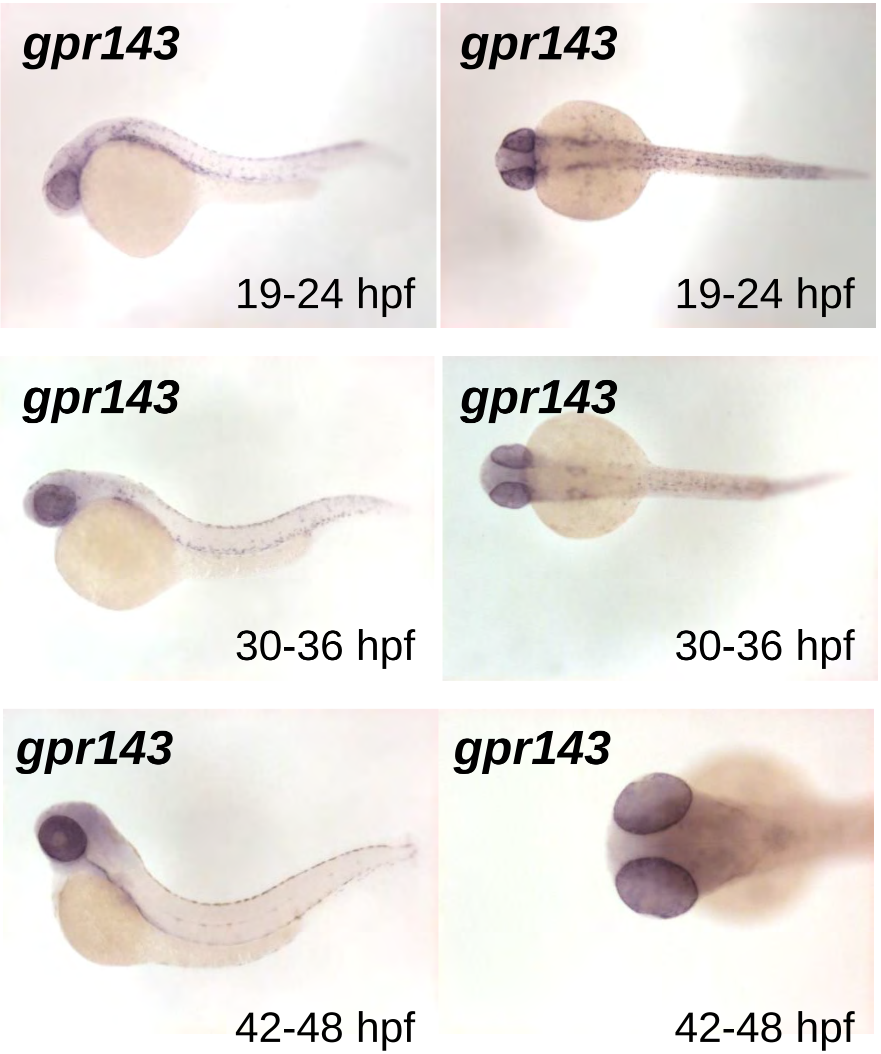


GPR39 - This gene is a member of the ghrelin receptor family and encodes a rhodopsin-type G-protein-coupled receptor (GPCR). The encoded protein is involved in zinc-dependent signaling in epithelial tissue in intestines, prostate and salivary glands. The protein may also be involved in the **pathophysiology of depression**.

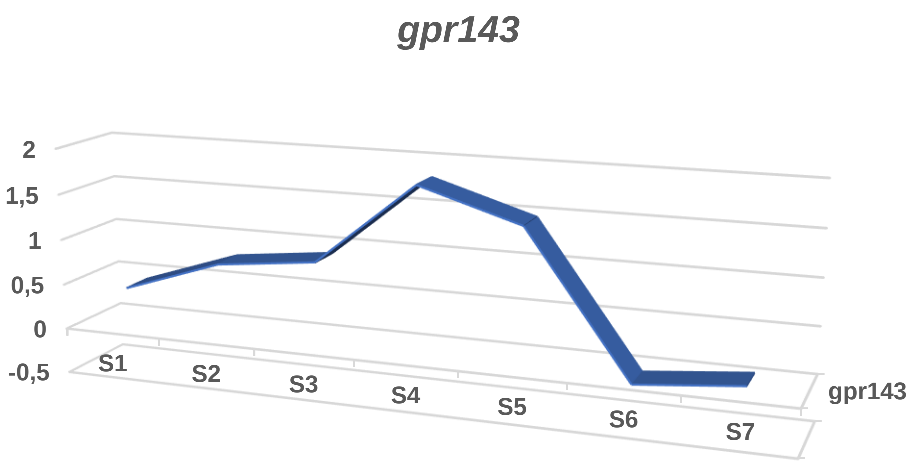
gpr39

Expressed in cephalic neural crest cells. Expressed in pigment cells and in retina pigmented epithelium





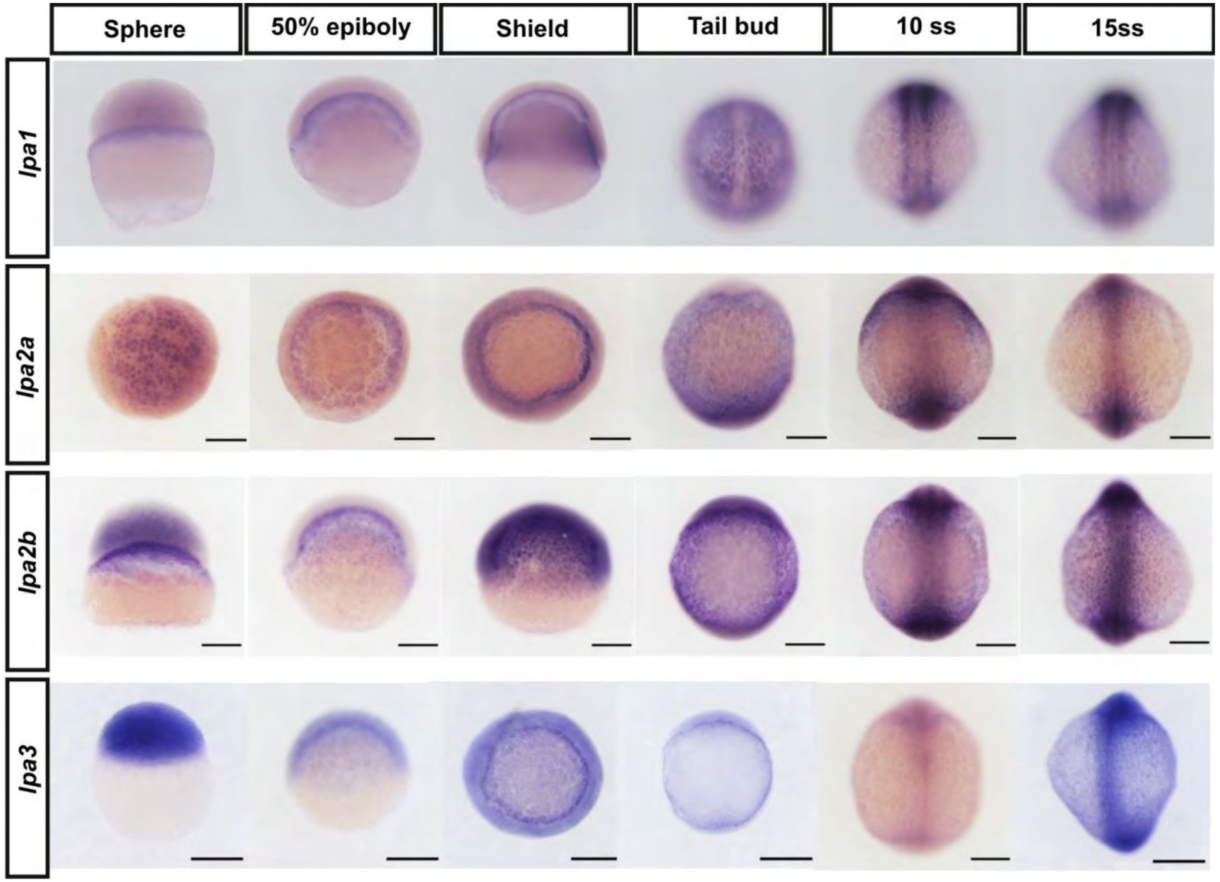
Adult gut

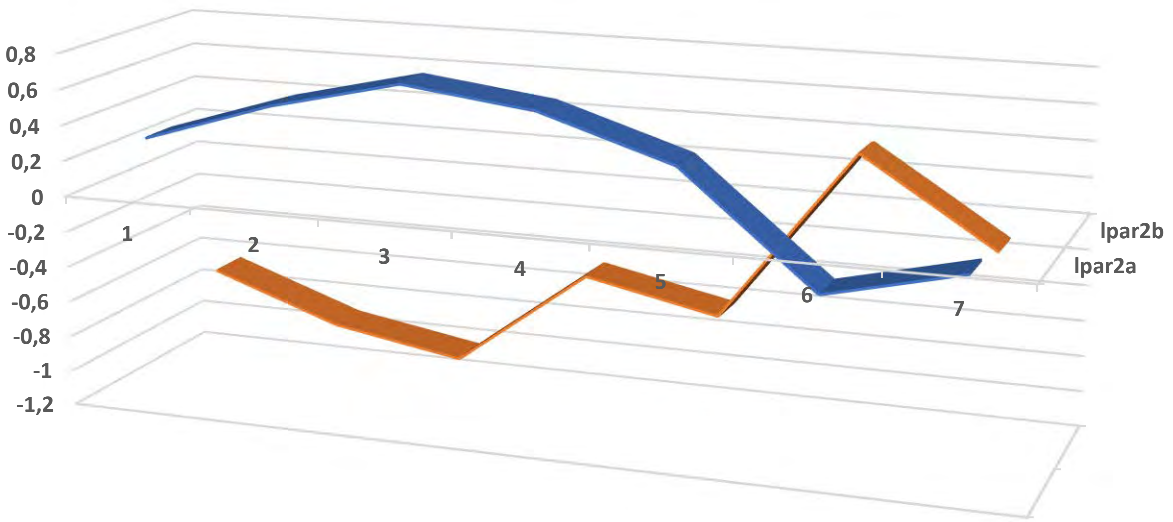


GPR143 - This gene encodes a protein that binds to heterotrimeric G proteins and is targeted to melanosomes in pigment cells. This protein is thought to be involved in intracellular signal transduction mechanisms. Mutations in this gene cause **ocular albinism type 1**, also referred to as **Nettleship-Falls type ocular albinism**, a severe visual disorder. A related pseudogene has been identified on chromosome Y.

LPA2

Adult gut





LPA2 - This gene encodes a member of family I of the G protein-coupled receptors, as well as the EDG family of proteins. This protein functions as a lysophosphatidic acid (LPA) receptor and contributes to Ca2+ mobilization, a critical cellular response to LPA in cells, through association with Gi and Gq proteins. An alternative splice variant has been described but its full length sequence has not been determined.

Conclusions

Integrating…

- Digestion (digestive enzymes…)
- Terminal digestion (terminal digestive enzymes…)
- Absorption (transporters…)
- Sensing (receptors…)
- …

- Defining phenotypes (biometry, anatomy, physiology, behaviour…)

Conclusions

Zebrafish

Commercial fish species

The group in Lecce

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Grazie per l’attenzione

Sea bass PEPT1 kinetic analysis by TEVC

Fig. 1 a, b Records of membrane currents from an oocyte transfected with sbPepT1 kept at −60-mV holding potential and exposed to three different dipeptides and histidine (all at 1 mM) during the periods indicated by the *gray bars*. *Top trace* pH7.5, *bottom trace* pH 6.5. c A noninjected oocyte exposed to the same peptides does not generate currents. Note the different time scales

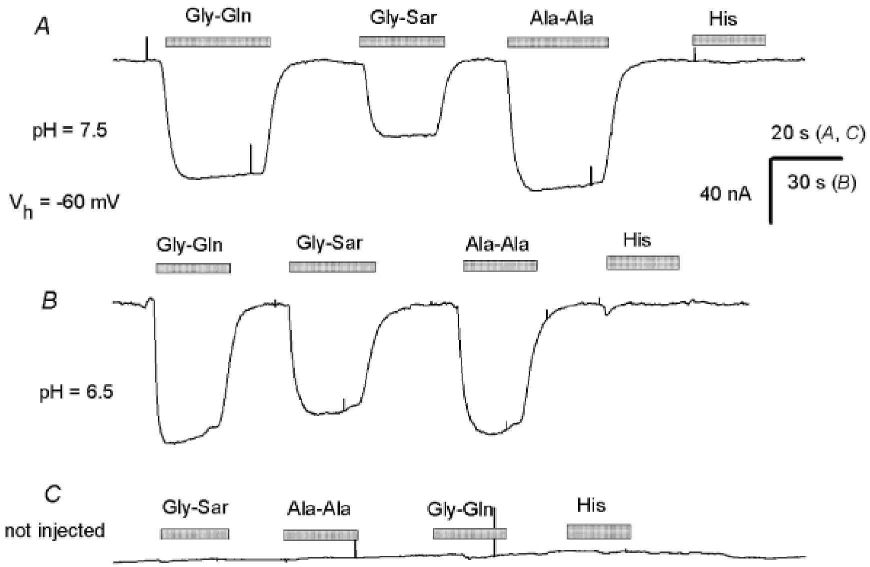
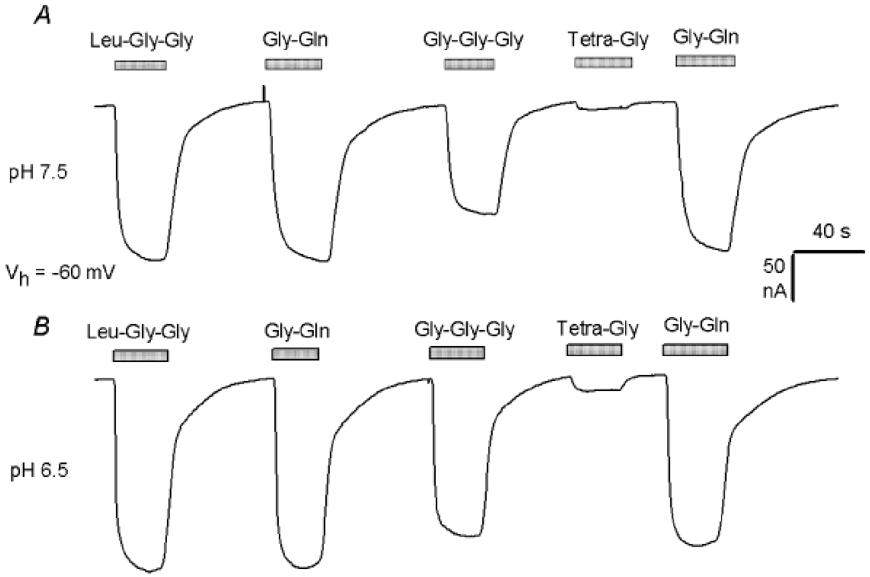


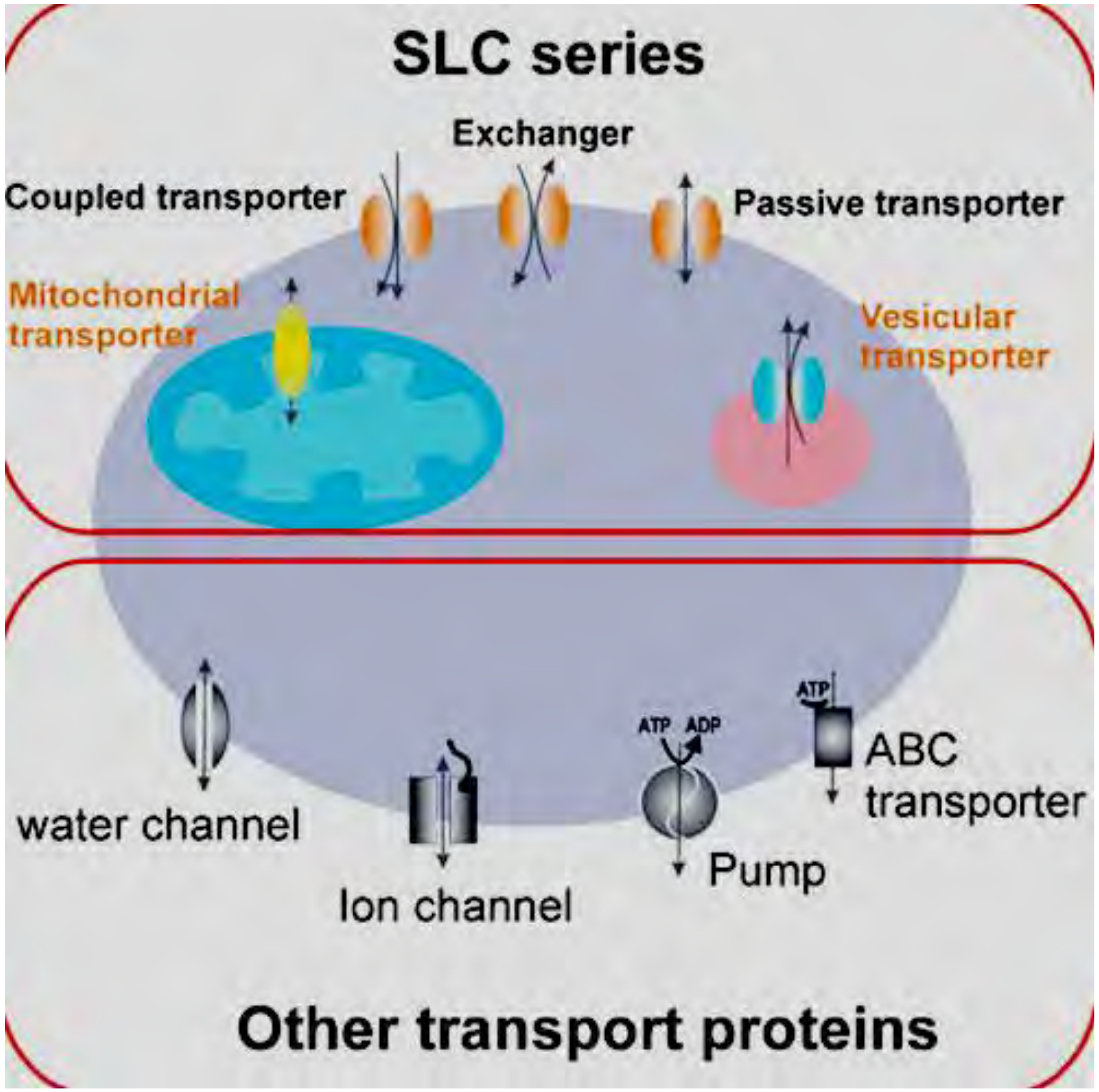
Fig. 2 a, b Records of membrane currents from an oocyte transfected with sbPepT1 kept at −60-mV holding potential and exposed to two different tripeptides and one tetrapeptide (all at 1 mM) during the periods indicated by the *gray bars*. *Top trace* pH7.5, *bottom trace* pH 6.5. Exposures to 1 mM Gly-Gln were also used as reference responses



Data from:
Sangaletti et al., Pflügers Arch. (2009)

The HUGO Solute Carrier (SLC) family series

Ion-



The SLC series includes passive transporters, ion-coupled transporters and exchangers

Passive transport

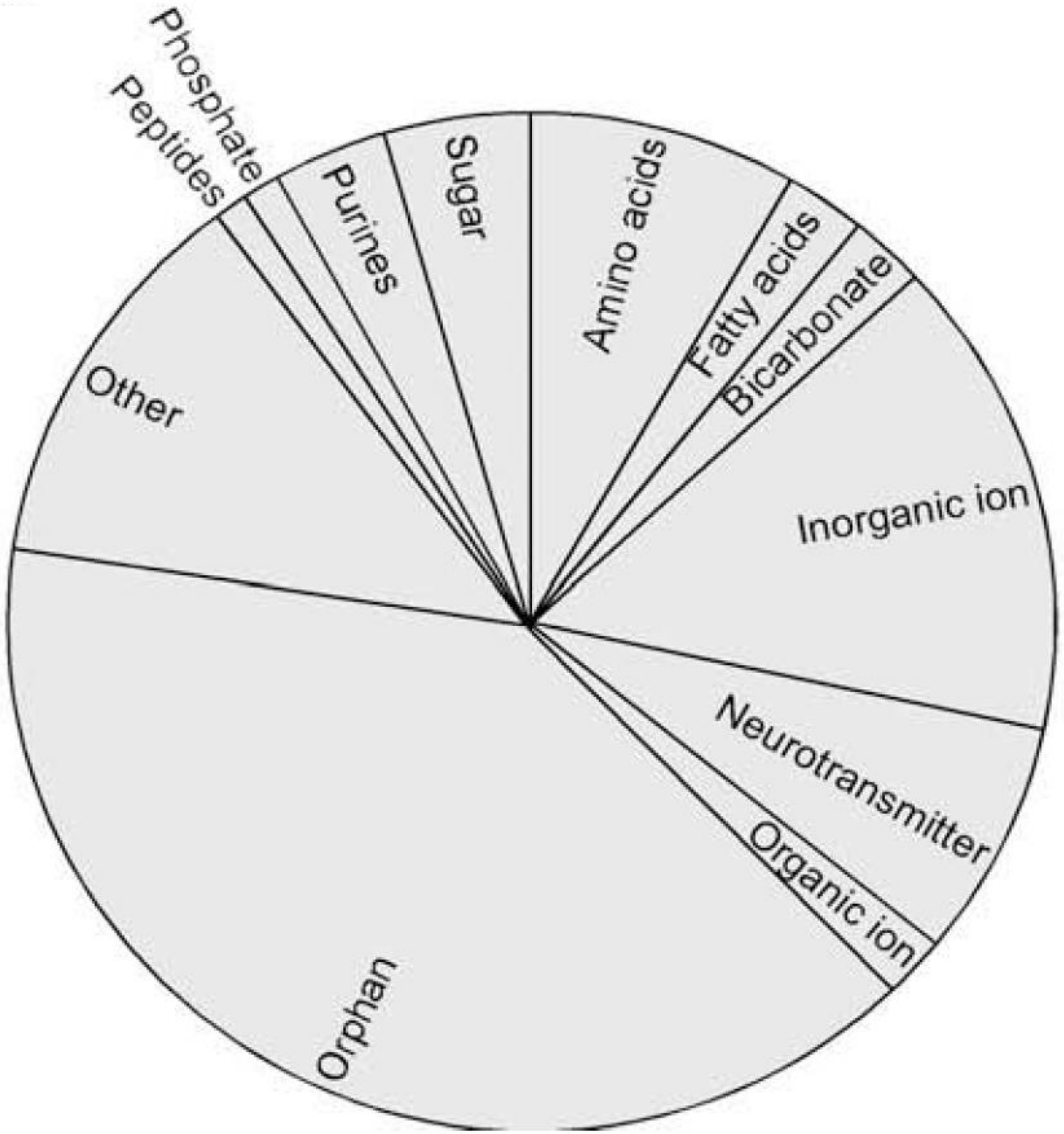
A kind of transport by which ions or molecules move along a concentration gradient, which means movement from an area of higher concentration to an area of lower concentration.

Coupled transport

The linked, simultaneous transport of two substances across a cell membrane (or another intracellular membrane). If the two substances are moving in the same direction (both into the cell or both out of the cell) it is called symport. If the two substances are moving in opposite directions (one moves into the cell while the other moves out) it is called antiport.

Adapted from:
Hediger et al., Pflügers Arch. 447:485 (2004)

The HUGO Solute Carrier (SLC) family series

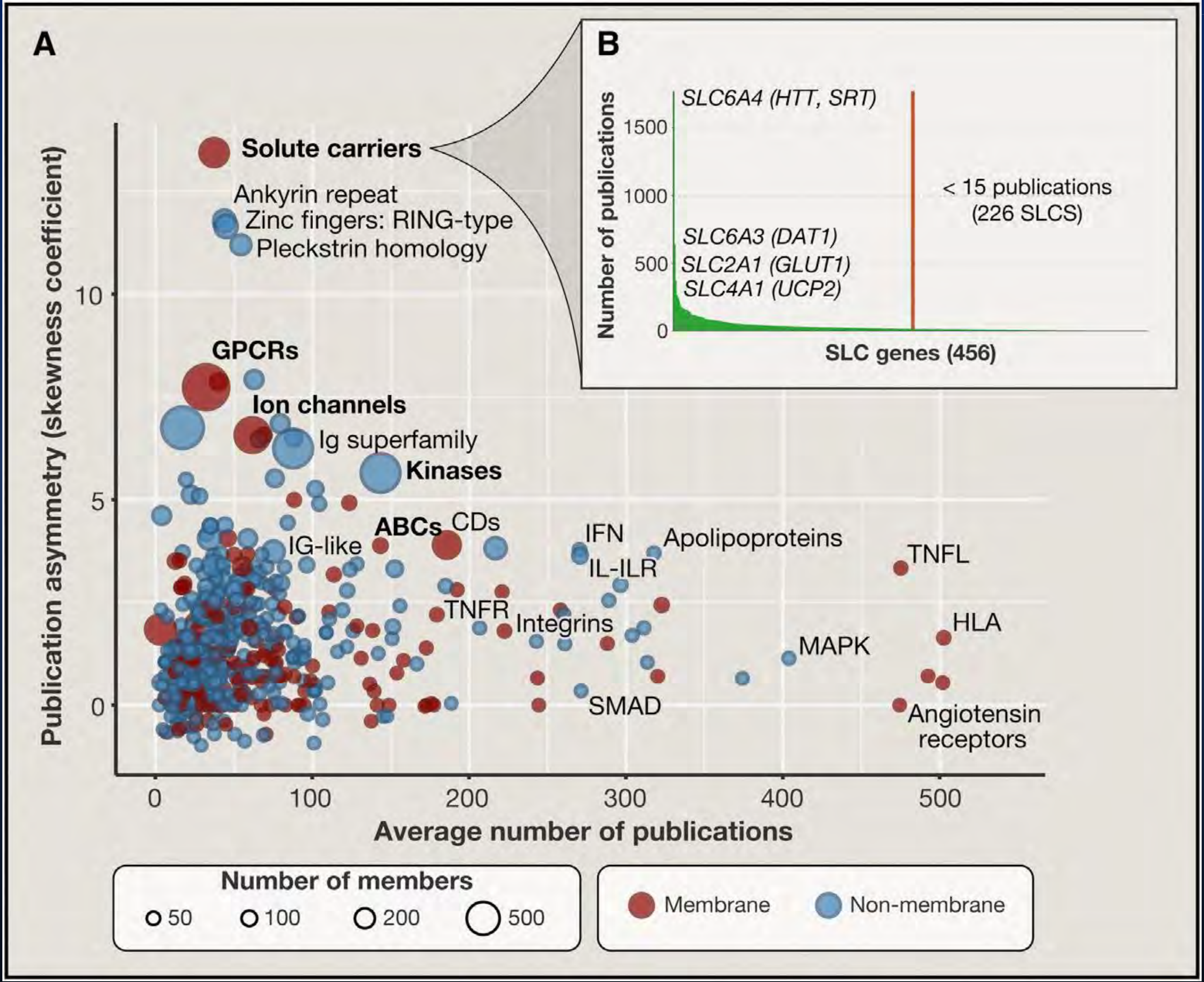


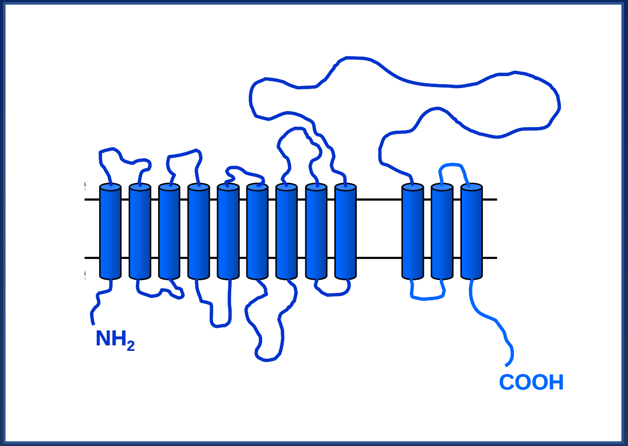
SLCs classification based on the type of substrate they are transporting.

The transporters were classified into ten major groups based on the substrate they are transporting according to the literature. Orphan transporter (substrate unknown) and other (substrate does not fit into any of the ten major categories) are also included as groups in the graph.

Adapted from:
Fredriksson et al., FEBS Lett. 582:3811 (2008)

The HUGO Solute Carrier (SLC) family series



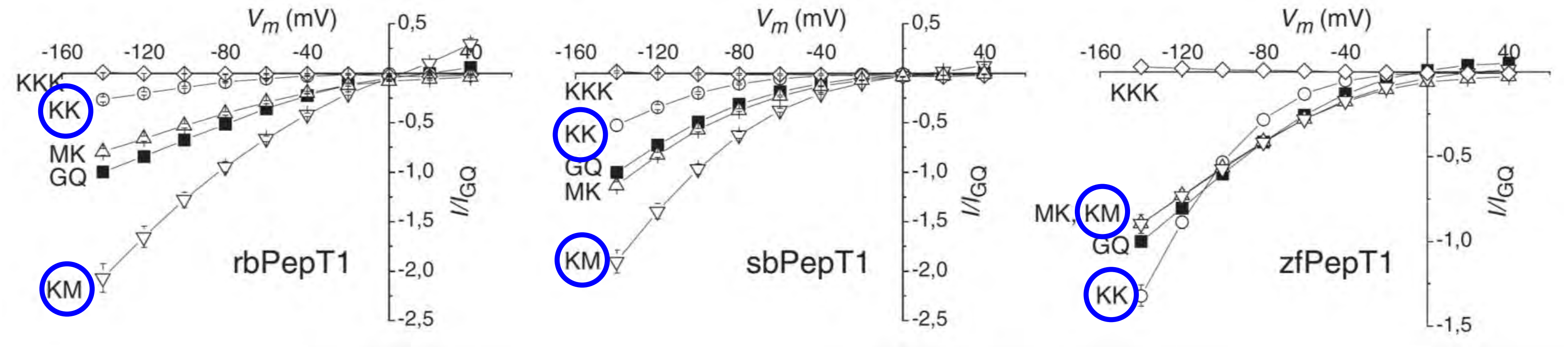


After G-protein-coupled receptors (GPCRs), SLCs are the second-largest family of membrane proteins in the human genome. Also, SLCs are the most neglected group of genes in the human genome

Adapted from:
Cesar-Razquin et al., Cell 162:478 (2015)

Sea bass PepT1

Sea bass PEPT1 kinetic analysis by TEVC



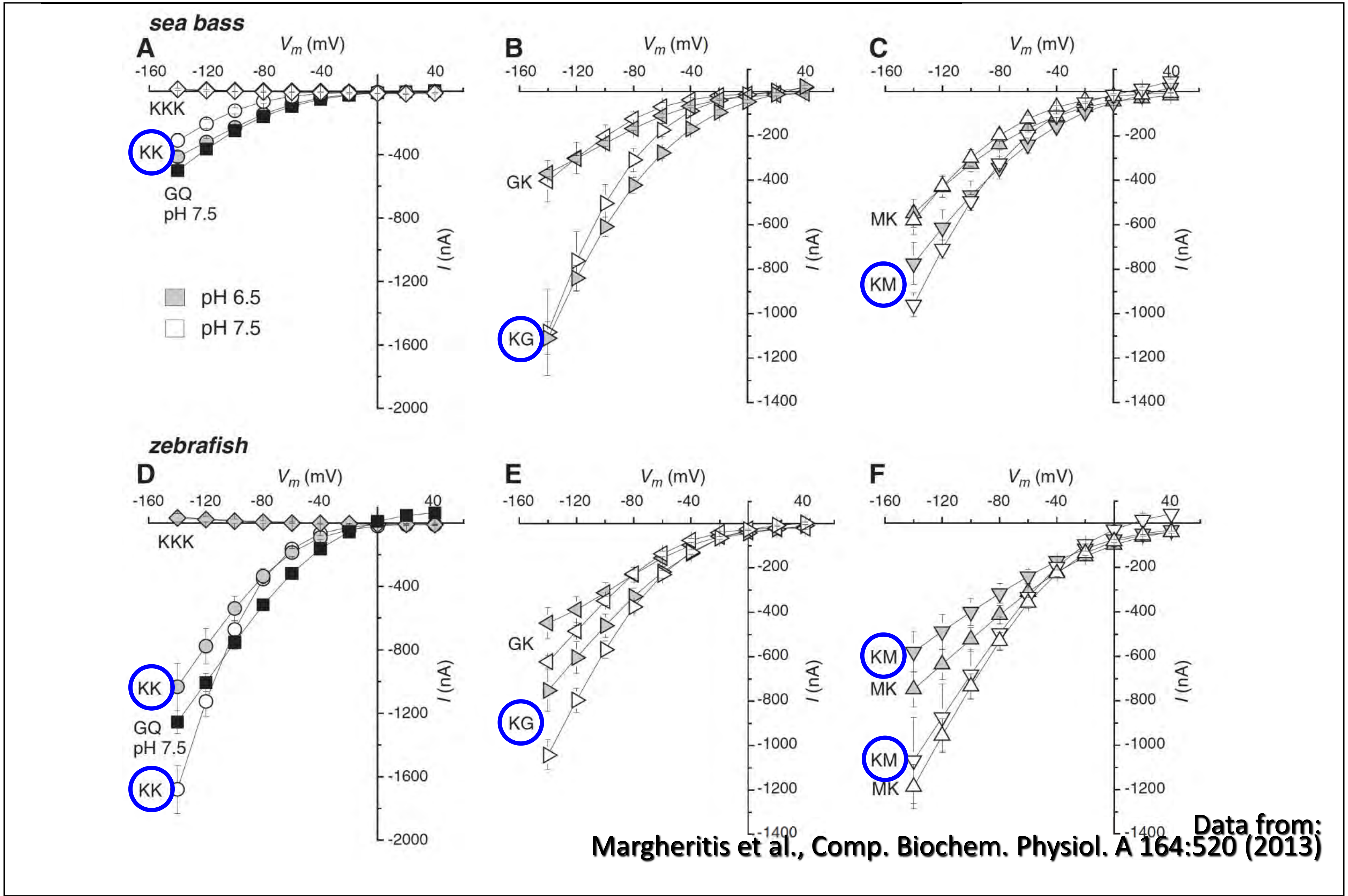
rabbit

sea bass

zebrafish

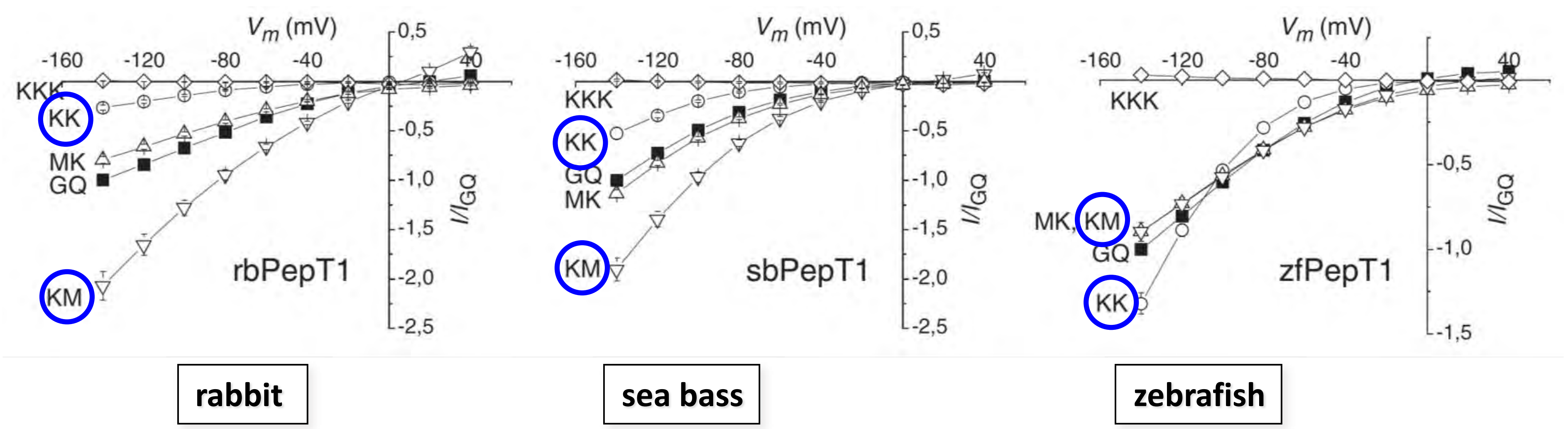
Data from:
Margheritis et al., Comp. Biochem. Physiol. A 164:520 (2013)

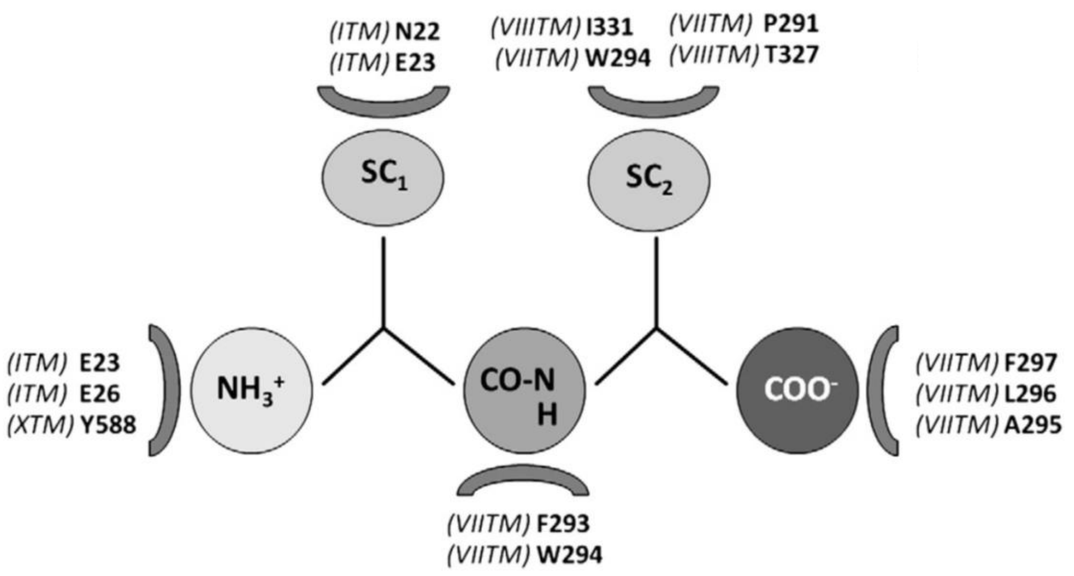
Sea bass PEPT1 kinetic analysis by TEVC



Data from:
Margheritis et al., Comp. Biochem. Physiol. A 164:520 (2013)

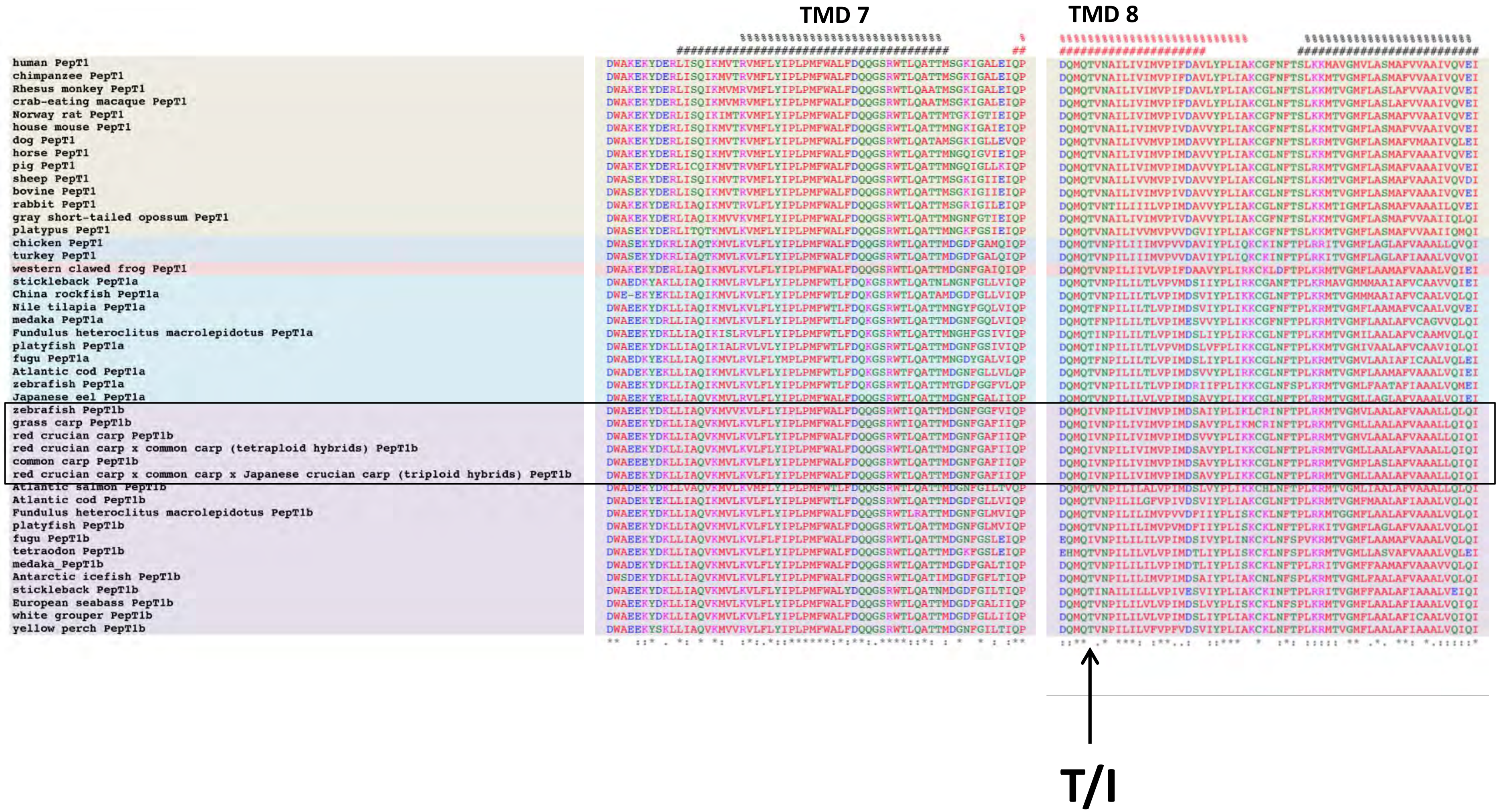
Sea bass PEPT1 kinetic analysis by TEVC



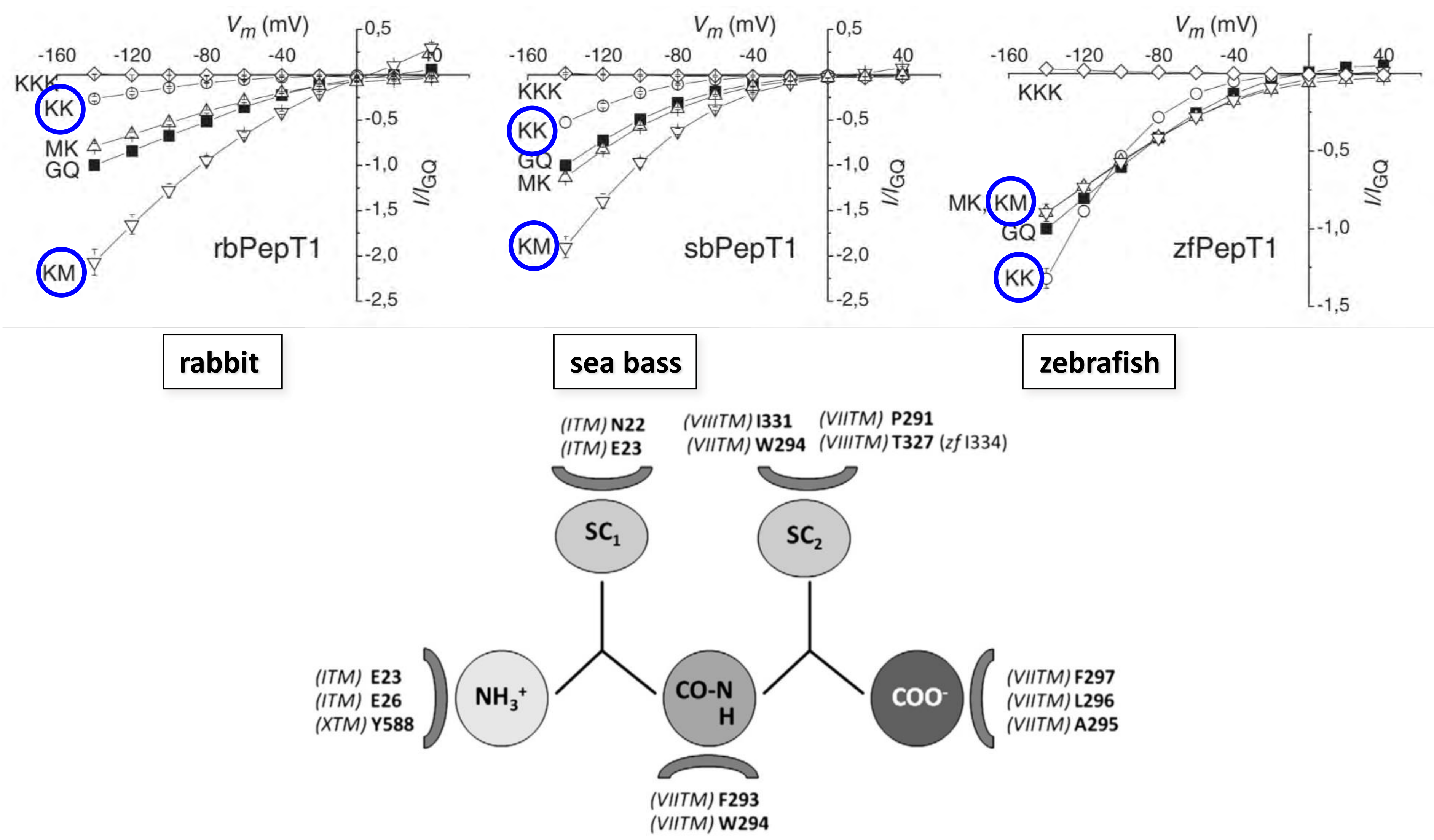


Data from:
Margheritis et al., Comp. Biochem. Physiol. A 164:520 (2013)

Sea bass PEPT1 kinetic analysis by TEVC



Sea bass PEPT1 kinetic analysis by TEVC



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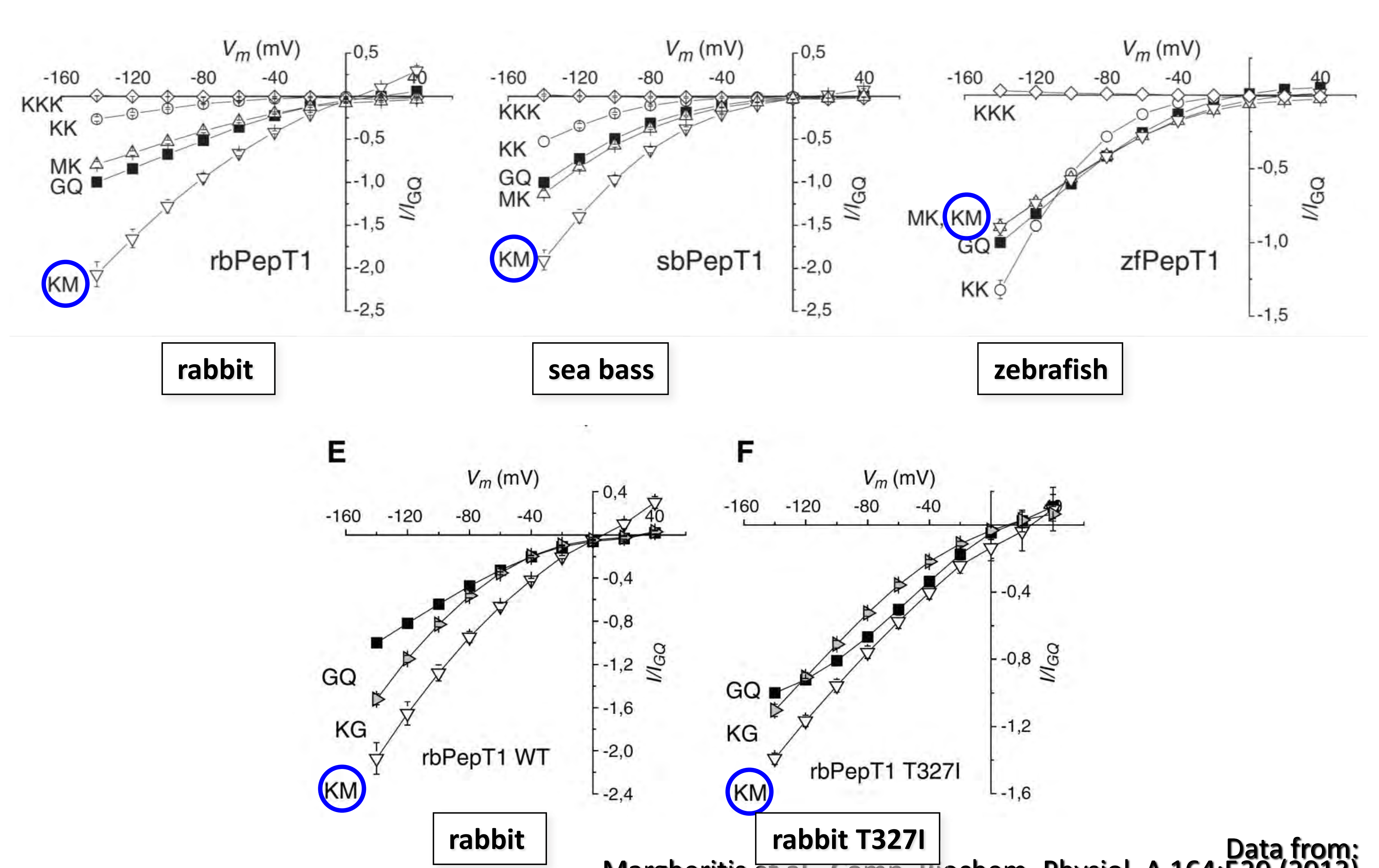
Margheritis et al., Comp. Biochem. Physiol. A 164:520 (2013)

Sea bass PEPT1 kinetic analysis by TEVC



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Margheritis et al., Comp. Biochem. Physiol. A 164:520 (2013)

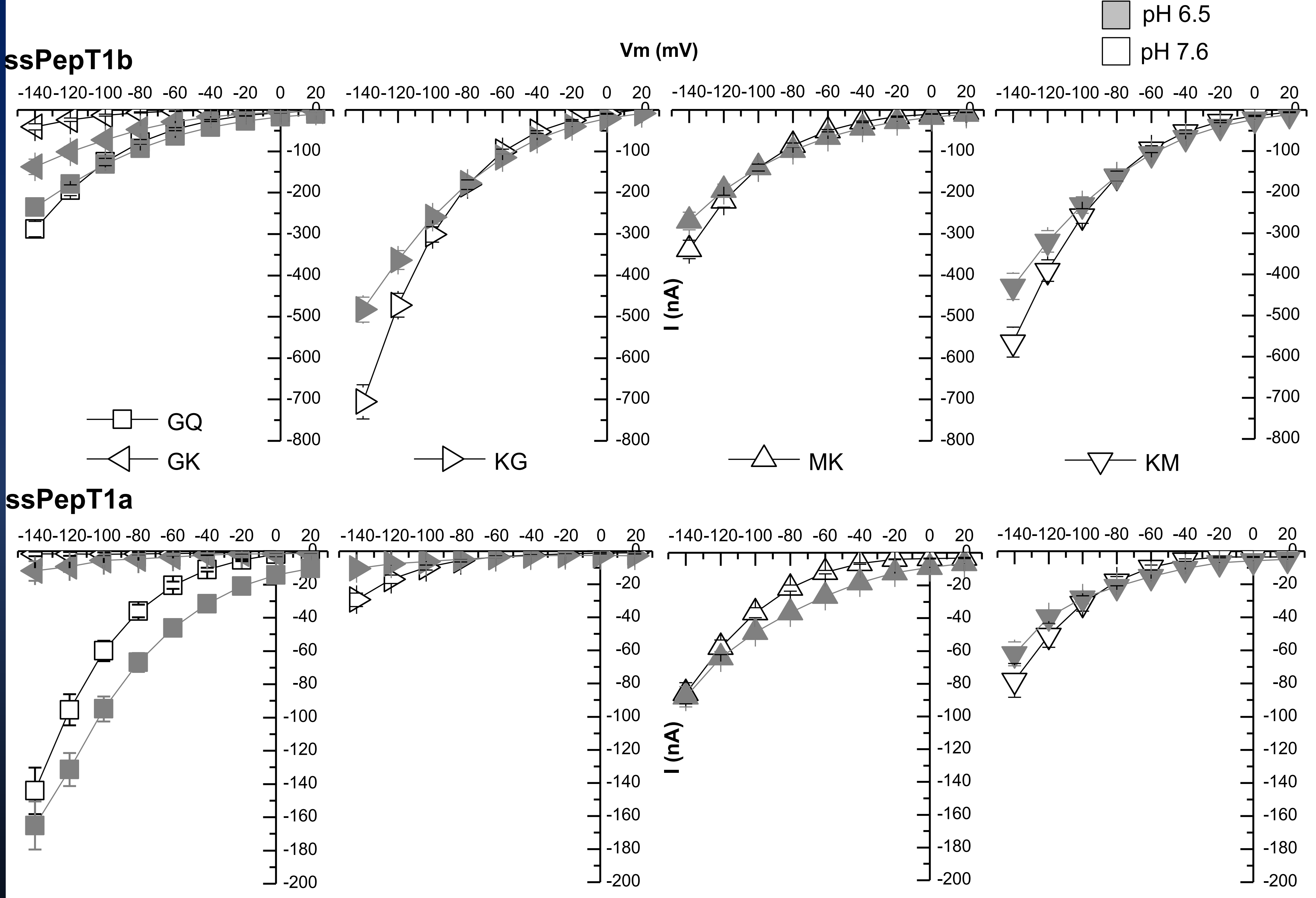
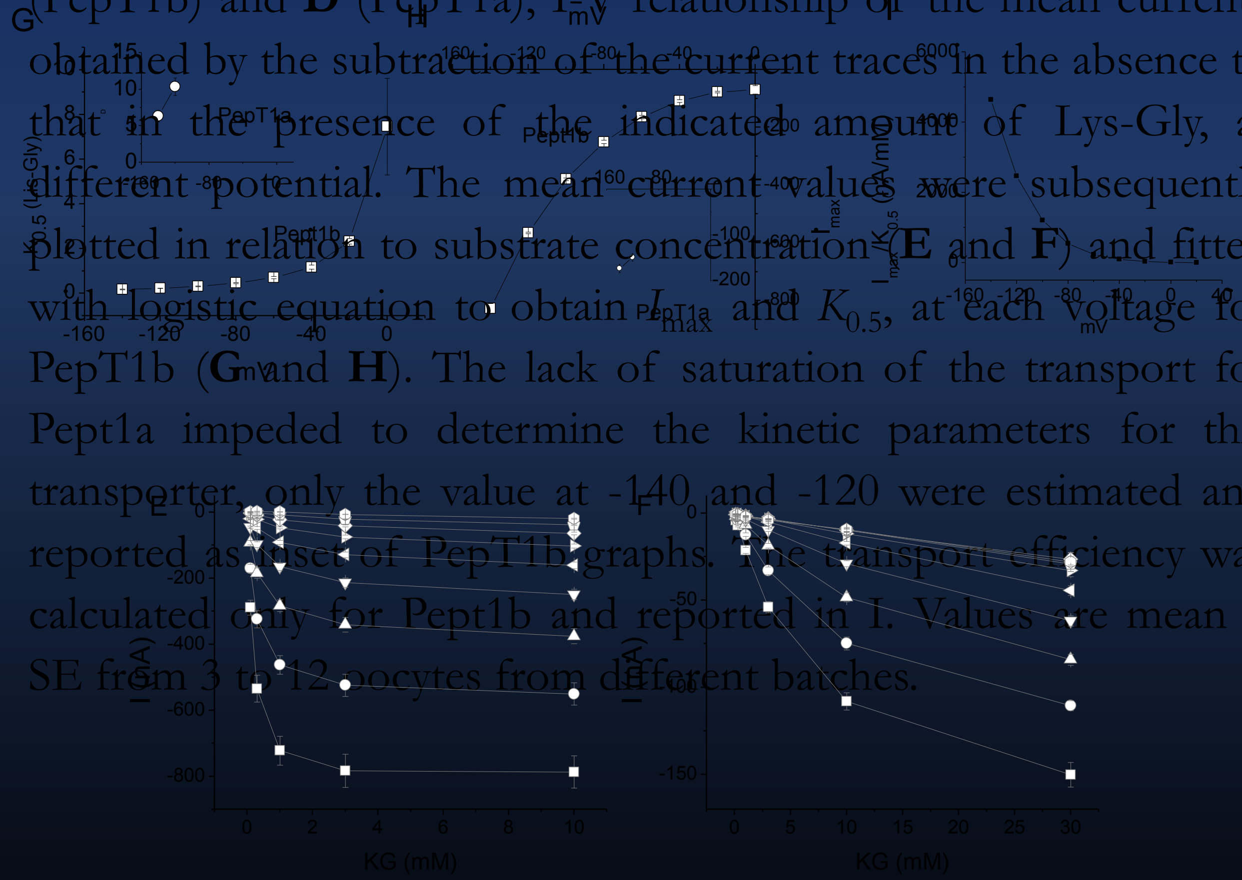
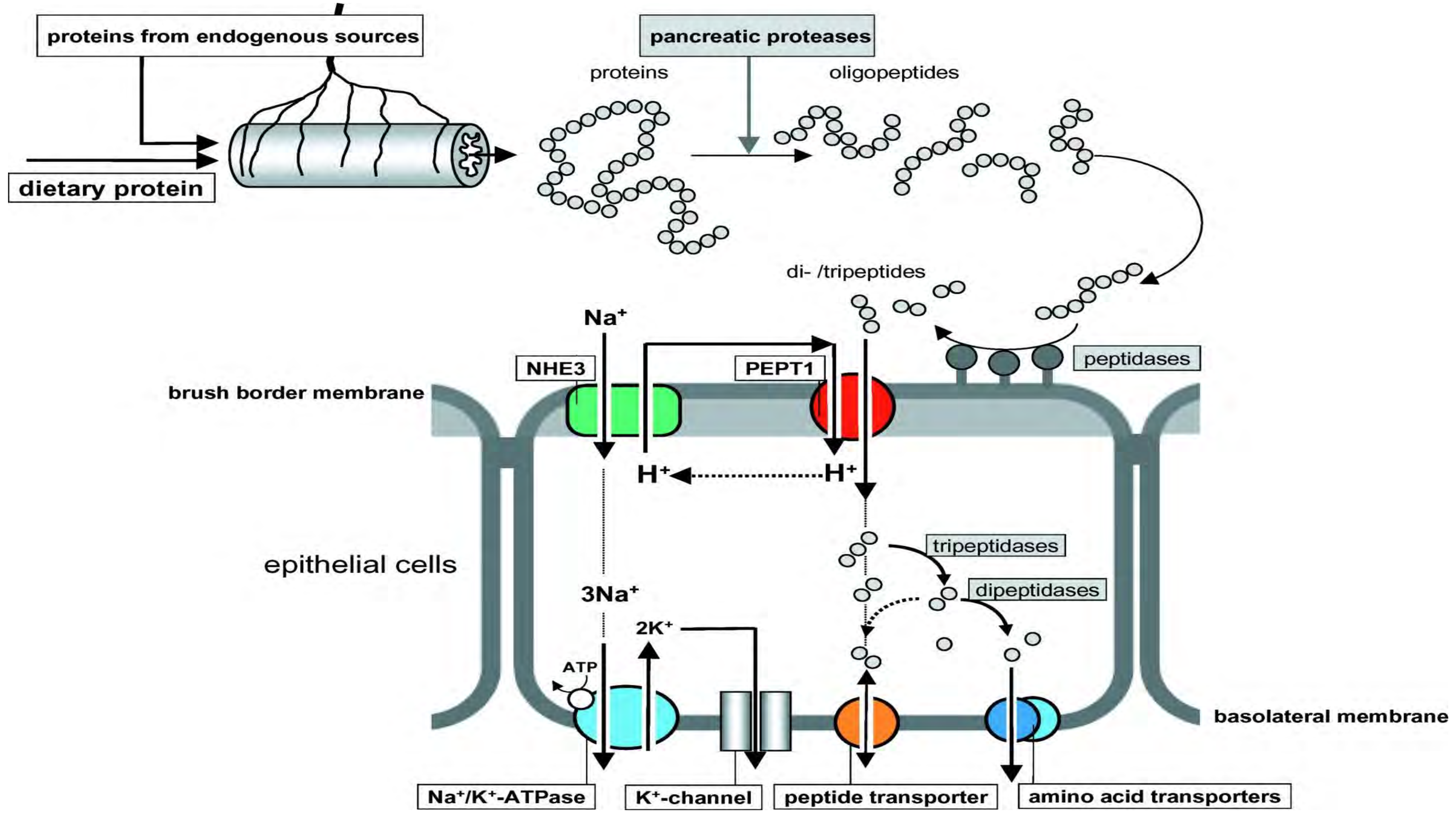

pH 6.5
pH 7.6
ssPepT1b
Vm (mV)
-140 -120 -100 -80 -60 -40 -20 0 20
-100
-200
-300
-400
-500
-600
-700
-800
I (nA)
GQ
GK
KG
MK
KM
ssPepT1a
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

Fig. 5. Dose-response Lys-Gly at pH 7.6 in sodium buffer. A (slc15a1b, pept1b) and **B** (slc15a1a, pept1b), Representative recordings at -60 mV in the presence of the reference di peptide Gly-Gln increasing concentration of Lys-Gly from 0.1 mM to 10 mM for slc15a1b (pept1b) and to 30 mM for scl15a1a (pept1a). **C** (PepT1b) and **D** (PepT1a), I-V relationship of the mean currents obtained by the subtraction of the current traces in the absence to that in the presence of the indicated amount of Lys-Gly, at different potential. The mean current values were subsequently plotted in relation to substrate concentration (**E** and **F**) and fitted with logistic equation to obtain I_{max} and $K_{0.5}$, at each voltage for PepT1b (**G** and **H**). The lack of saturation of the transport for Pept1a impeded to determine the kinetic parameters for this transporter, only the value at -140 and -120 were estimated and reported as inset of PepT1b graphs. The transport efficiency was calculated only for Pept1b and reported in I. Values are mean ± SE from 3 to 12 oocytes from different batches.




proteins from endogenous sources
pancreatic proteases
proteins
oligopeptides
dietary protein
di- /tripeptides
Na+
NHE3
PEPT1
peptidases
brush border membrane
H+
H+
epithelial cells
tripeptidases
dipeptidases
3Na+
2K+
ATP
basolateral membrane
Na+/K+-ATPase
K+-channel
peptide transporter
amino acid transporters

The HUGO Solute Carrier (SLC) family series

- [+] SLC1 High-affinity glutamate and neutral amino acid transporter family
- [+] SLC2 Facilitative GLUT transporter family
- [+] SLC3 Heavy subunits of the heteromeric amin
- [+] SLC4 Bicarbonate transporter family
- [+] SLC5 Sodium glucose cotransporter family
- [+] SLC6 Sodium- and chloride-dependent neurot
- [+] SLC7 Cationic amino acid transporter/glycopr
- [+] SLC8 Na^+/Ca^{2+} exchanger family
- [+] SLC9 Na^+/H^+ exchanger family
- [+] SLC10 Sodium bile salt cotransport family
- [+] SLC11 Proton-coupled metal ion transporter f
- [+] SLC12 Electroneutral cation-coupled Cl cotra
- [+] SLC13 Human Na^+-sulfate/carboxylate cotrans
- [+] SLC14 Urea transporter family
- [+] SLC15 Proton oligopeptide cotransporter fam
- [+] SLC16 Monocarboxylate transporter family
- [+] SLC17 Vesicular glutamate transporter family
- [+] SLC18 Vesicular amine transporter family
- [+] SLC19 Folate/thiamine transporter family
- [+] SLC20 Type III Na^+-phosphate cotransporter family
- [+] SLC21 Organic anion transporter family
- [+] SLC22 Organic cation/anion/zwitterion transporter family
- [+] SLC23 Na^+-dependent ascorbic acid transporter family
- [+] SLC24 $Na^+/(Ca^{2+}\text{-}K^+)$ exchanger family
- [+] SLC25 Mitochondrial carrier family
- [+] SLC26 Multifunctional anion exchanger family
- [+] SLC27 Fatty acid transporter family
- [+] SLC28 Na^+-coupled nucleoside transport family
- [+] SLC29 Facilitative nucleoside transporter family
- [+] SLC30 Zinc efflux family
- [+] SLC31 Copper transporter family
- [+] SLC32 Vesicular inhibitory amino acid transporter family
- [+] SLC33 Acetyl-CoA transporter family

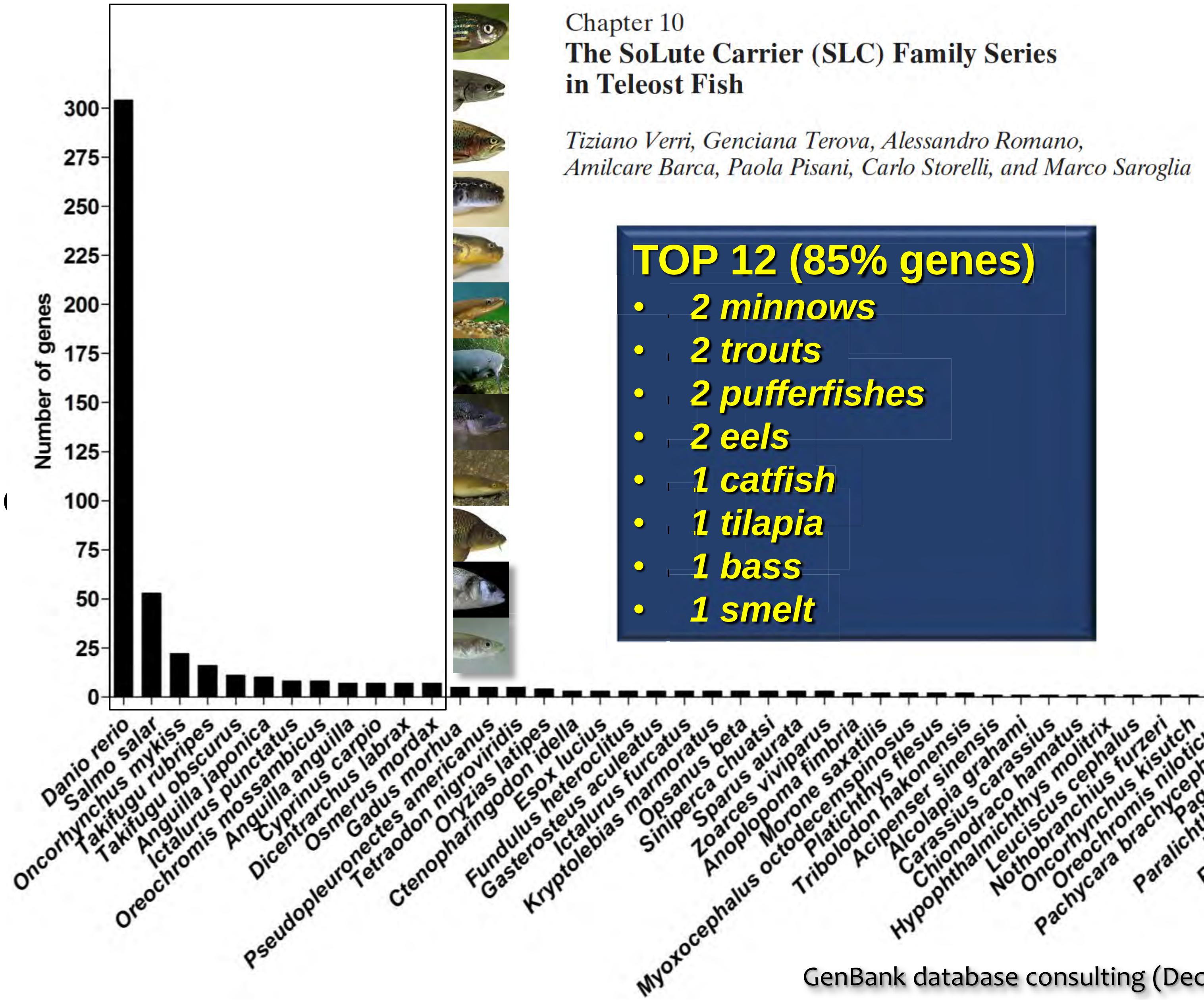
- [+] SLC34 Type II Na^+-phosphate cotransporter family
- [+] SLC35 Nucleoside-sugar transporter family
- ed amino acid transporter family
- hate/phosphate exchanger family
- System N sodium-coupled neutral amino acid
- sporter family
- on transporter family
- gnesium transporter family
- m transporter family
- lent, system-L-like amino acid transporter family
- ransporter family
- ansporter family
- orter family
- Toxin Extrusion (MATE) family
- rter family
- d transporter family
- [+] SLC50 Sugar efflux transporters
- [+] SLC51 Transporters of steroid-derived molecules
- [+] SLC52 Riboflavin transporter family
- [+] SLC53 Phosphate carriers
- [+] SLC54 Mitochondrial pyruvate carriers
- [+] SLC55 Mitochondrial cation/proton exchangers
- [+] SLC56 Sideroflexins
- [+] SLC57 NiPA-like magnesium transporter family
- [+] SLC58 MagT-like magnesium transporter family
- [+] SLC59 Sodium-dependent lysophosphatidylcholine symporter family
- [+] SLC60 Glucose transporters
- [+] SLC61 Molybdate transporter family
- [+] SLC62 Pyrophosphate transporters
- [+] SLC63 Sphingosine-phosphate transporters
- [+] SLC64 Golgi Ca^{2+}/H^+ exchangers
- [+] SLC65 NPC-type cholesterol transporters

65 families
458 genes

Adapted from:
www.bioparadigms.org

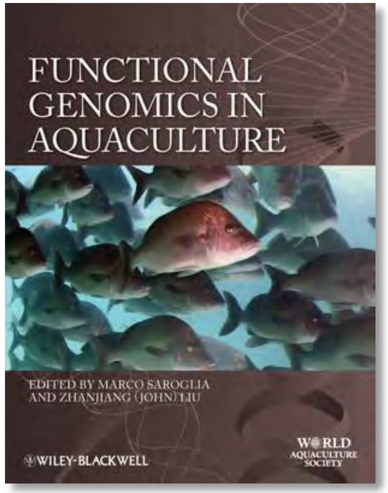
The Solute Carrier (SLC) family series in teleost fish

The SLC family series in teleost fish



Chapter 10
The SoLute Carrier (SLC) Family Series in Teleost Fish

Tiziano Verri, Genciana Terova, Alessandro Romano, Amilcare Barca, Paola Pisani, Carlo Storelli, and Marco Saroglia

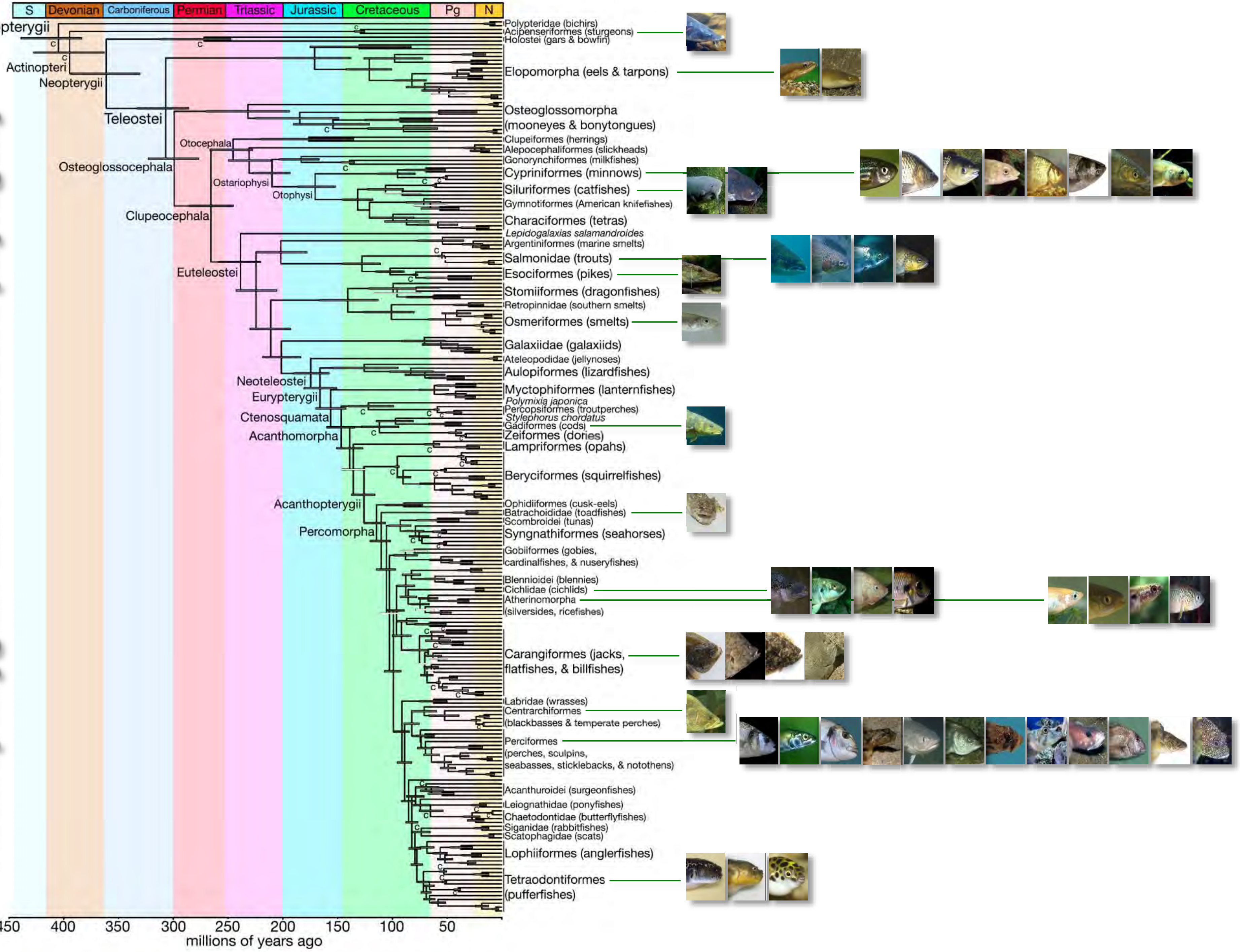


TOP 12 (85% genes)

- *2 minnows*
- *2 trouts*
- *2 pufferfishes*
- *2 eels*
- *1 catfish*
- *1 tilapia*
- *1 bass*
- *1 smelt*

GenBank database consulting (December 2010-March 2011)

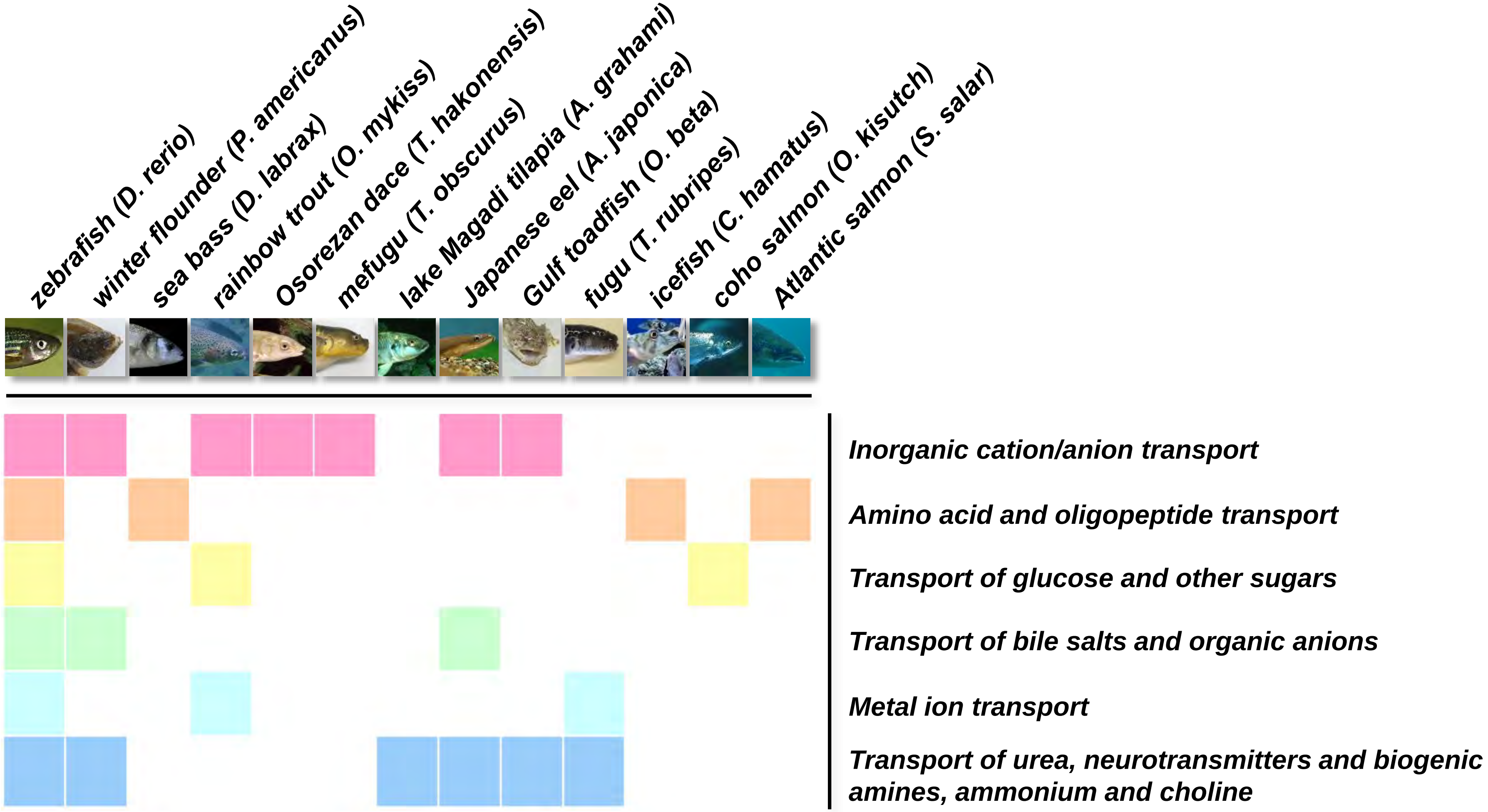
Actinopterygian time-calibrated phylogeny



Adapted from:
Near et al., *PNAS* 109:13698 (2012)

The SLC family series in teleost fish

Functional expression of SLC proteins in teleost fish



GenBank database consulting (December 2010-March 2011)

Table 1. List of the solute carrier (SLC) families in zebrafish (*Danio rerio*) compared to human

Family	Name	Human Total genes	Zebrafish Total genes	Non-duplicated genes	Duplicated genes designated as 'a' and 'b'	Duplicated genes designated as 'tandem duplicate'	Possibly duplicated genes and/or genes not obviously designated
SLC1	High affinity glutamate and neutral amino acid transporter family	7	9	5	4	0	0
SLC2	Facilitative GLUT transporter family	14	12	6	2	0	4
SLC3	Heavy subunits of the heteromeric amino acid transporters	2	2	1	1	0	0
SLC4	Bicarbonate transporter family	10	6	3	3	0	0
SLC5	Sodium glucose cotransporter family	12	8	7	0	0	1
SLC6	Sodium- and chloride-dependent neurotransmitter transporter family	20	13	8	4	0	1
SLC7	Cationic amino acid transporter/glycoprotein-associated family	14	10	6	0	0	4
SLC8	Na^+–Ca^{2+} exchanger family	4	4	1	3		
SLC9	Na^+–H^+ exchanger family	13	7	5	1		
SLC10	Sodium bile salt cotransport family	7	4	4	0		
SLC11	Proton-coupled metal ion transporter family	2	1	1	0		
SLC12	Electroneutral cation-coupled Cl cotransporter family	9	8	5	1		
SLC13	Human Na^+-sulfate–carboxylate cotransporter family	5	4	3	0		
SLC14	Urea transporter family	8	1	1	0		
SLC15	Proton oligopeptide cotransporter family	4	3	2	1		
SLC16	Monocarboxylate transporter family	14	10	7	2		
SLC17	Vesicular glutamate transporter family	9	5	3	2		
SLC18	Vesicular amine transporter family	4	3	2	1		
SLC19	Folate/thiamine transporter family	3	3	2	0		
SLC20	Type III Na^+-phosphate cotransporter family	2	2	1	1	0	0
SLC21	Organic anion transporting family	11	11	11	0	0	0
SLC22	Organic cation/anion/zwitterion transporter family	23	5	4	1	0	0
SLC23	Na^+-dependent ascorbic acid transporter family	4	2	2	0	0	0
SLC24	Na^+/(Ca^{2+}–K^+) exchanger family	5	4	3	1	0	0
SLC25	Mitochondrial carrier family	53	35	26	8	0	1
SLC26	Multifunctional anion exchanger family	11	8	7	0	0	1
SLC27	Fatty acid transport protein family	6	4	2	2	0	0
SLC28	Na^+-coupled nucleoside transport family	3	0	0	0	0	0
SLC29	Facilitative nucleoside transporter family	4	3	2	0	0	1
SLC30	Zinc efflux family	10	9	8	1	0	0
SLC31	Copper transporter family	2	2	2	0	0	0
SLC32	Vesicular inhibitory amino acid transporter family	1	1	1	0	0	0
SLC33	Acetyl-CoA transporter family	1	1	1	0	0	0

Table 1. Continued

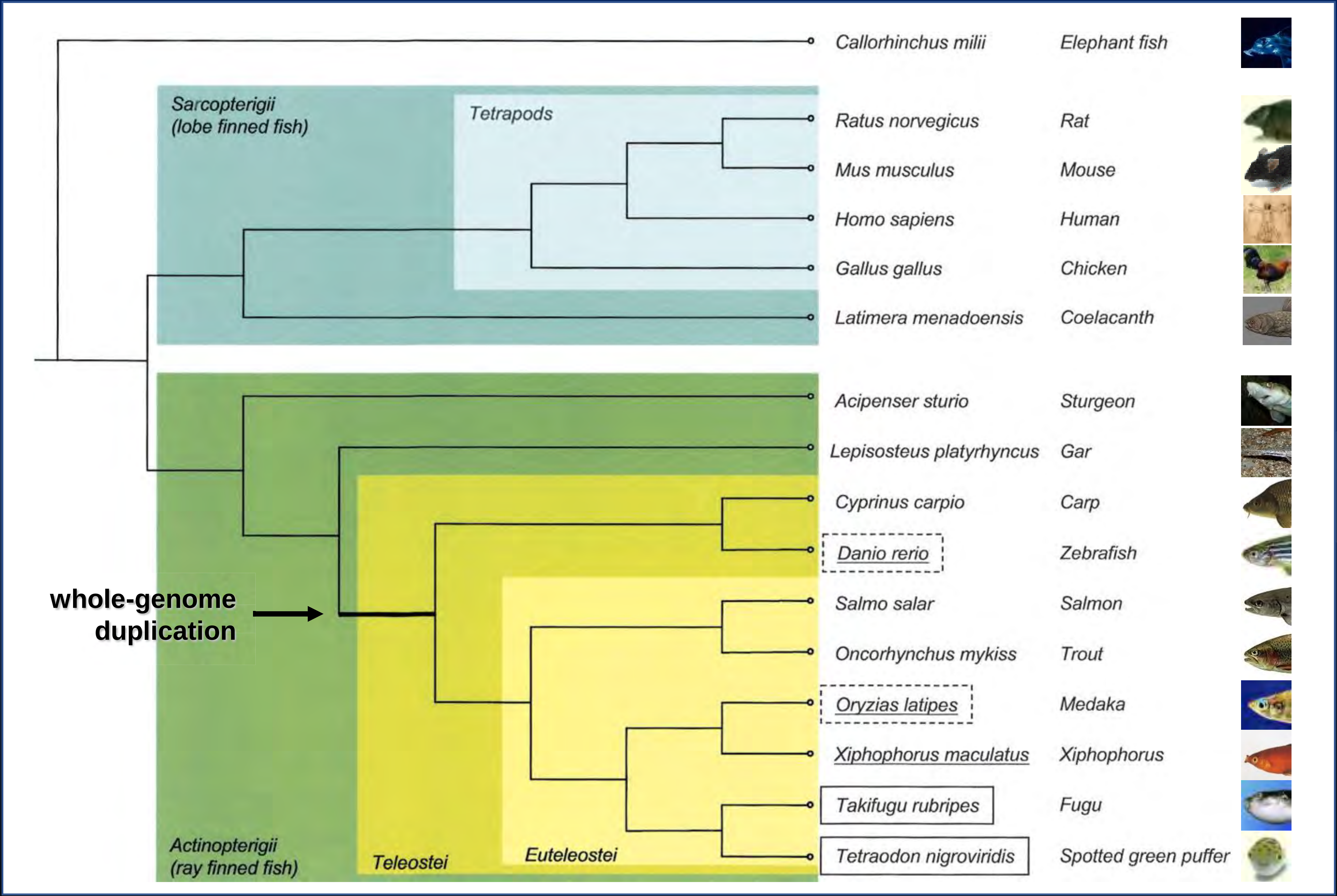
Family	Name	Human Total genes	Zebrafish Total genes	Non-duplicated genes	Duplicated genes designated as 'a' and 'b'	Duplicated genes designated as 'tandem duplicate'	Possibly duplicated genes and/or genes not obviously designated
SLC34	Type II Na^+-phosphate cotransporter family	3	2	0	1	0	1
SLC35	Nucleoside-sugar transporter family	30	19	16	3	0	0
SLC36	Proton-coupled amino acid transporter family	4	1	1	0	0	0
SLC37	Sugar-phosphate/phosphate exchanger family	4	3	2	1	0	0
SLC38	System A & N sodium-coupled neutral amino acid transporter family	11	8	6	1	0	1
SLC39	Metal ion transporter family	14	10	10	0	0	0
	…amily	1	1	1	0	0	0
	…rter	3	1	1	0	0	0
	…mily	3	6	5	0	0	1
	…e amino	3	3	0	3	0	0
	…y	5	4	2	2	0	0
	…mily	4	4	4	0	0	0
		3	2	2	0	0	0
	…(MATE)	2	2	2	0	0	0
		1	1	0	1	0	0
	…ily	4	4	3	0	0	1
		1	1	1	0	0	0
	…d	2	1	1	0	0	0
	…RFVT/SLC52	3	2	2	0	0	0
Total		398	275	201	51	2	21

The total number of 'official members' (i.e. the members for which an official symbol has been assigned) in each family is shown for human and zebrafish. The number of zebrafish non-duplicated, duplicated and possibly duplicated/not obviously designated genes is also shown. Data have been obtained by GenBank database search on the Reference Zv9 Primary Assembly (July 2013).

52 families
275 genes

The SLC family series in zebrafish (*Danio rerio*)

Adapted from:
Romano et al., *J. Physiol.* 592:881 (2014)



Whole genome duplication event(s)