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Coevolution of cytokines from the interleukin 10 family and their receptors
Koevoluce cytokinů z rodiny interleukinu 10 a jejich receptorů

DIPLOMA THESIS

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Prohlášení:

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Markéta Harazim

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ABSTRAKT

Rodina interleukinu 10 (FIL-10, family of interleukin 10) je důležitá skupina cytokinů regulující imunitní odpověď různého charakteru, od protizánětlivé odpovědi interleukinu (IL) 10, přes reakci regulující imunitní odpověď v epitelech podrodiny IL-19, IL-20 a IL-24 k IL-22 a IL-26, které ovliňují imunitní odpověď při infekcích. Celá rodina je příbuzná interferonům (IFN), z nichž několik (interferony λ) je zařazováno do FIL-10 z důvodu funkčních a strukturních podobností s členy FIL-10. Interleukiny této rodiny používají k signalizaci několik podjednotek receptorů, které v různých kombinacích a při vazbě různých interleukinů vyvolávají rozdílnou imunitní odpověď. Proteiny FIL-10 jsou produkovány již v evolučně velmi starých organismech jako paryby, proto studie předpokládala, že koevoluce probíhající mezi cytokiny této rodiny a receptory je detekovatelná v sekvencích genů a následně jimi kódovaných proteinů. Pomocí statistických metod studie popisuje evoluční vztahy v rámci FIL-10 a ve skupině jejich receptorů, Zaznamenali jsme koevoluci mezi některými studovanými interleukiny a jejich receptory. Evoluce IL10RB, sdíleného napříč proteinovou rodinou je zřejmě řízena evolucí IL-10, evoluce receptoru IL20RB, sdíleného IL-19, IL-20 a IL-24 je ovlivněna evolucí IL-20, koevoluce s IL-19 a IL-24 je ve většině případů nesignifikantní. Koevoluce reziduí aminokyselin v receptorech je lokalizována především v první extracelulární doméně receptoru.

KLÍČOVÁ SLOVA

rodina interleukinu 10, koevoluce, ligand-receptor

ABSTRACT

Interleukin 10 family (FIL-10) is an important family of cytokines triggering immune response of different outcome, from antiinflammatory factor interleukin (IL) 10 through epithelia related subfamily of IL-19, IL-20 and IL-24, to IL-22 and IL-26 with role in infection immunity. The family is closely related to interferons (IFNs), several of which (Interferon λs) are commonly placed into FIL-10 for its functional and structural similarities with FIL-10 proteins. FIL-10 interleukins share several receptors, which in different combinations of receptors and interleukin result in different immune response. As the family proteins are expressed in as evolutionary old taxa as cartilaginous fish, we presumed a coevolution in the protein family and the corresponding receptors would be detectable in the sequences of genes and subsequently proteins of FIL-10. Using statistical methods, evolutionary relations within the group of FIL-10 and group of their receptors were resolved. Coevolution of the cytokine-receptor combination in FIL-10 was detected in some of the expected cases. IL10RB seems to be consistently in coevolution with IL-10. Evolution of IL20RB receptor, which is shared within the group by IL-19, IL-20 and IL-24, is directed by IL-20 evolution, coevolution with IL-19 and IL-24 is in most cases statistically insignificant. Interestingly, coevolution of the amino acid residues is mainly located in the first extracellular domain of the receptors.

KEYWORDS

interleukin 10 family, coevolution, ligand-receptor

Contents

Abbreviations

aa	Amino acid, amino acids
Abbrev.	Abbreviation
AIC	Akaike information criterion
Aln.	alignment
Avg.	average
BIC	Bayesian information criterion
BLAST	Basic local alignment search tool
FIL−10	Interleukin 10 family
IFN	interferon
IFNGR1	Interferon gamma receptor 1
IFNGR2	Interferon gamma receptor 2
IFNLR1	Interferon lambda receptor 1
IL	interleukin
IL10RA	Interleukin 10 receptor 1, IL−10 receptor α
IL10RB	Interleukin 10 receptor 2, IL−10 receptor β
IL20RA	Interleukin 20 receptor 1, IL−10 receptor α
IL20RB	Interleukin 20 receptor 2, IL−10 receptor β
IL22BP	Interleukin 22 binding protein
IL22RA1	Interleukin 22 receptor α 1
IL22RA2	Interleukin 22 receptor α 2, also IL22BP
mDCs	Myeloid dendritic cells
mfDCA	Direct coupling analysis with mean−field approximation
MI	Mutual information
ML	maximum likelihood
MSA	Multiple sequence alignment
NJ	Neighbour joining

NK cell	Natural killer
PDB	Protein Data Bank
pDCs	Plasmacytoid dendritic cells
plmDCA	Direct coupling analysis with pseudo–likelihood maximisation
TLR	Toll–like receptor
UID	Unique identifier
UPGMA	Unweighted pair group method with arithmetic mean
WGD	Whole genome duplication
WSP	weighted sums of pairs

1 Introduction

1.1 Interleukin 10 family

Interleukins, in general, trigger immune response of different types by activating signalling pathways. Interleukin 10 is a representative of an important and large family of interleukins with diverse outcome of signalling in immune system. Interleukin 10 homologues have been found in as evolutionary old organisms as cartilaginous fish demonstrating thus a high level of conservation of their function and structure. Nevertheless, complexity of immune response is increasing in evolution, thus in lower vertebrates we can only find a few representatives of interleukin 10 family.

Interleukins of the family of interleukin 10, called hereafter FIL-10, signal through binding to cellular membrane receptors, whose combination implies their different functions. As with many signalling pathways in immune system, FIL-10 proteins use in many cases signal transfer through variants of Janus kinase – Signal Transducer and Activator of Transcription – (JaK/STAT) pathway. Regulation of transcription in such cases is yet not well described, although it is well known that effects of signal transduction of JaK/STAT pathways may be either of pro-inflammatory or antiinflammatory character.

Proteins of this family have very diverse biological effects, dependent on the type of interleukin as well as target tissue. The effects include immunosuppressive IL-10 and skin and mucosal immunity related IL-20 and IL-24 or proteins involved in antiviral response. Receptor subunits are often shared by several members of FIL-10 (Figure 1), which implies different signalling functions in diverse tissues and ligand-receptor pairs.

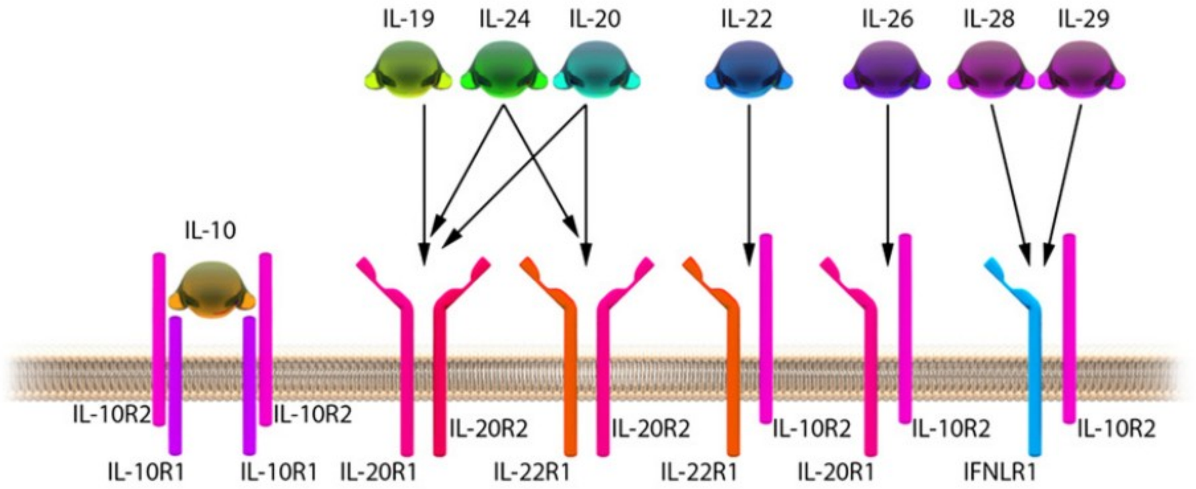

Figure 1. Combinations of interleukin 10 family proteins and their receptors. Subunits marked as 1 are referred to as A (e.g. IL10R1 = IL10RA), "2" subunits are marked B in the thesis. Image from Akdis et al. (2011) (edited).

1.1.1 Interleukin 10

Interleukin 10 (IL−10) is a homodimeric protein in length of 178 amino acids in each monomer (human IL−10). IL−10 is an important factor in antiinflammatory response and immune suppression.

IL−10 is produced by multiple immune cell types, major producers of IL−10 are monocytes, regulatory T and regulatory B cells (Del Prete et al. 1993; Holan et al. 2014), it is also secreted by macrophages and myeloid dendritic cells (mDCs), however there is no evidence of its expression in plasmacytoid dendritic cells (pDCs) (Boonstra et al. 2006).

IL−10 is a pleiotropic signalling molecule. It is involved in activation of JaK/STAT pathway, it is capable of blocking NF−κB transcription factor activity and is regulates differentiation of several cell types. One of its main activities is suppression of IFNγ production (Dandrea et al. 1993).

Its signalling through JaK/STAT pathway is not yet well described, since many cytokines share the similar signalling pathway to promote opposite effects (Jones et al. 2016). IL−10 inhibits production of pro−inflammatory IFNγ and IL−2, IL−5, IL−6 and IL−12. By regulation of MHC II expression in monocytes, IL−10 inhibits antigen presentation of microbial peptides (Commins et al. 2008). Treg cells, present throughout the body, produce IL−10, down−regulating immune response by Th1 and Th17 (Wang et al. 2016).

Maintenance of serum levels of IL−10 is crucial in pathology of several diseases. While deficiency inhibits tumour growth (Wang et al. 2016), impaired regulation leads to several diseases and symptoms. Antiinflammatory function implies role in pathological immune response, as is described primarily in respiratory tract and intestine.

IL−10 has crucial role in maintenance of allergen tolerance. Constant high levels of IL−10 are produced on mucosal tissues mainly in upper and lower airways. Allergen tolerance established by this mechanism is impaired in respiration allergy and asthma patients (Palomares et al. 2010).

Regulation of intestinal epithelial immunity is dependent on IL−10 as well. Mutant variants of both IL−10 gene and its receptor may cause severe colitis (Shah et al. 2012; Kole and Maloy 2014). Recombinant IL−10 has been used in experimental biological treatment of Crohn's disease and colitis (Braat et al. 2006; Marlow 2013).

IL−10 binds to receptor consisting of two IL10RA and two IL10RB chains (Kotenko et al. 1997).

1.1.2 Interleukin 19, interleukin 20 and interleukin 24 subfamily

Interleukins 19, 20 and 24 form protein subfamily, characterized primarily by its helical structure acting as a monomer and expression in epithelial tissues. Interleukin 19, or IL−19 is an IL−10 orthologue of 177 amino acids in length (human variant) (Gallagher et al. 2000). Interleukin 20 (IL−20) is a protein in length of 176 amino acids (Blumberg et al. 2001). IL−24, firstly described as melanoma differentiation associated 7 (mda7) is a 206 amino acid protein (Jiang et al. 1996; Caudell et al. 2002). 7−helical monomer structure is typical for the subfamily (Chang et al. 2003).

IL−19, IL−20 and IL−24 modulate inflammatory response in favour of Th2 type (Liao et al. 2004; Wu et al. 2014) and are also further expressed by Th2 cells. Primarily produced by monocytes induced by IL−4 in combination with LPS stimulation (Gallagher et al. 2000) members of this sub−family and their receptors are not only expressed in activated immune cells, but also in similar amounts in keratinocytes (Kunz et al. 2006). Furthermore, IL-19 is produced in central nervous system by microglia, for which it also serves as autocrine negative regulator (Horiuchi et al. 2015).

As mentioned above, subfamily of IL-19, IL-20 and IL-22 is expressed in high levels in skin and connective tissues. Proteins of the sub-family have positive effect on wound healing in skin and mucosal tissues, IL-19 effects in skin cell proliferation and wound healing by up-regulation expression of keratinocyte growth factor (Sun et al. 2013)

Similarly to IL-10, IL-19 serum levels and concentration in lungs are increased in asthma patients (Liao et al. 2004). Connection between kidney disease or injury and increased levels of IL-19 has been recently pointed out, but details of IL-19 function in kidney have not been described (Jennings et al. 2015). IL-19 is known to be involved in pathology of systemic lupus erythematodes (Lin et al. 2016) and together with other members of the sub-family, also in psoriasis (Otkjaer et al. 2005; Wang et al. 2012)

IL-24, apart from its immunity effects in skin, has specific tumor-suppresor activity. Through IL-20 receptors, independently of JaK/STAT pathway, IL-24 induces effectively apoptosis specifically to tumour cells (Zheng et al. 2006) by inducing autophagy, which in later stages switches to apoptosis (Yang et al. 2010; Bhutia et al. 2010).

The subfamily signals through different combinations of shared receptor subunits (some members are also shared with closely related IL-22 and IL-26, see Figure 1). IL-19 signals through IL20RA/IL20RB heterodimer. IL-20 binds to IL20RA/IL20RB heterodimer, nevertheless it is also able to signal through IL22RA/IL20RB heterodimer (Commins et al. 2008). IL-24 the uses same two receptor pairs as IL-20 (Wang et al. 2002). IL-20 and IL-24 N-terminus forms a β-hairpin structure distincting them in binding to receptor subunits from IL-19. Affinity to receptor chains is defined by their secondary structure (Logsdon et al. 2012)

1.1.3 Interleukin 22

IL-22 is 6 antiparallel helices protein with 179 amino acids. IL-22 is produced by activated immune cells, NK-22 (Cella et al. 2009), Th17 cells (Liang et al. 2006) and Th22 cells (express IL-22 without IL-17) (Duhen et al. 2009). IL-22 molecules secreted by NK-cells in lymphoid associated mucosal tissues (tonsils, Peyer's patches) provide innate protection against bacterial and viral infection (Cella et al. 2009).

As in its relatives, it was described that IL-22 facilitates wound healing in mucosal tissue by increase of fibroblast activity (McGee et al. 2013). Dysregulation in IL-22 signalling is observed in development of psoriasis and atopic dermatitis symptoms (Ma et al. 2008) and promotes hyperplasia of epidermis (Zheng et al. 2007).

Interleukin 22 binds to IL22RA/IL10RB heterocomplex (Kotenko et al. 2001). Soluble Interleukin 22 Binding Protein (IL22BP) competitively inhibits IL-22 activity (Xu et al. 2001).

1.1.4 Interleukin 26

Interleukin 26 or IL-26 is 171 amino acids long (human) and has similar primary structure to IL-19,20 and 24 family, however unlike them, forms a 6 helical homodimer. IL-26 is conserved throughout mammals, interestingly is lacking in mice and rats, it is present also in amphibian, bird (Donnelly et al. 2010) and fish (Igawa et al. 2006) models. Due to its absence in the most important model organisms – mice and rats, information about its *in vivo* function is quite limited.

IL-26 is produced by Th17 alongside with the other members of IL-10 family (Wilson et al. 2007). IL-26 main function seems to be in antimicrobial defence and Th17 antimicrobial activity is hugely dependent on IL-26 production. IL-26 activity in infection is based on its ability to form pores on extracellular bacteria membranes. IL-26 recognizes bacterial DNA released in lysis and forms insoluble complexes, afterwards presented, independently of IL-26 receptor, to pDCs to stimulate production of type I interferon (IFNα) (Meller et al. 2015).

Besides of its activity in innate infection, IL-26 signals through IL10R2 and IL20R1 combination of receptor subunits activating JaK/STAT pathway in epithelia. Pathway activation by IL-26 may be inhibited by heparin (Hor et al. 2004).

1.1.5 Interferon λ family

Interferon lambda (IFNλ) group is a group of 3 proteins in human consisting of IL 29 - Interleukin 29 / IFNλ1 - Interferon lambda 1, IL-28A or IFNλ2 and IL-28B (IFNλ3). Known as IL-28 and IL-29, IFNλs are commonly seen as IL-10 family members, due to their (monomer) structure, in particular to its resemblance of IL-22 structure and function - anti infection immunity (Gad et al. 2009). Members of this subgroup are further referred to as IFNλs, not ILs (IFNλ1, IFNλ2, IFNλ3) in this thesis. Similarity with IFN type I is presumably only of functional, not genetic character, as gene structure of IFNλ matches IL-10 5 exon - 4 intron pattern (Kotenko et al. 2003).

IFNλ group is categorized as type III interferons. Interferon-typical antiviral activity lies in blocking viral replication. IFNλs is known primarily for its activity against hepatitis viruses - it is responsible for inducing immune response against Hepatitis C (HCV) virus in liver (Marukian et al. 2011) predominantly by inhibition of both HBV and HCV replication (Robek et al. 2005). HCV clearance from organism is hugely dependent on IFNλ genotype (Sheahan et al. 2014).

Antiviral activity of IFNλ is not limited to hepatitis viruses. IFNλ is expressed together with IFN type I (IFNα) by wide variety of cell types after stimulation of toll-like receptor (TLR)3 and TLR9 (Ank et al. 2008). IFNλ is able to act against coronaviruses (Hamming et al. 2013) or norovirus (Baldridge et al. 2015; Nice et al. 2015) and possibly many other viruses.

IFNλ uses the complex of IFNRL1/IL10RA chains for immune signalling (Kotenko et al. 2003). IL10RA, being used by IL-10, IL-22 and IL-26 as well, is another similarity supporting IFNλ assignment into the family.

1.1.6 Receptors and signalling

Regulation of cytokine signaling is dependent on their interactions with the receptor chains and different expression in different tissues. Most receptors involved in signaling of IL-10 family are expressed in immune cells, primarily T, B and NK cells. In addition, their expression has also been detected in skin, liver or pancreas (Wolk et al. 2002). IL20RB is expressed in keratynocytes (Wolk et al. 2004) IL20RA expression, unlike other receptors, has not been detected in NK cells, T, B cells or monocytes, but it is present in high levels in skin (Wolk et al. 2002) The group of FIL-10 receptors includes IFNLR1 or IL28RA interacting with SH2 domain of JaK proteins (Zhang et al. 2016).

The receptors consist of two extracellular fibronectin type III domains, transmembrane domain and cytoplasmic domain (Figure 2) (Langer et al. 2004). The differences in structure and function are found mainly between the intracellular receptor domains, extracellular domains are in general sequentially more similar.

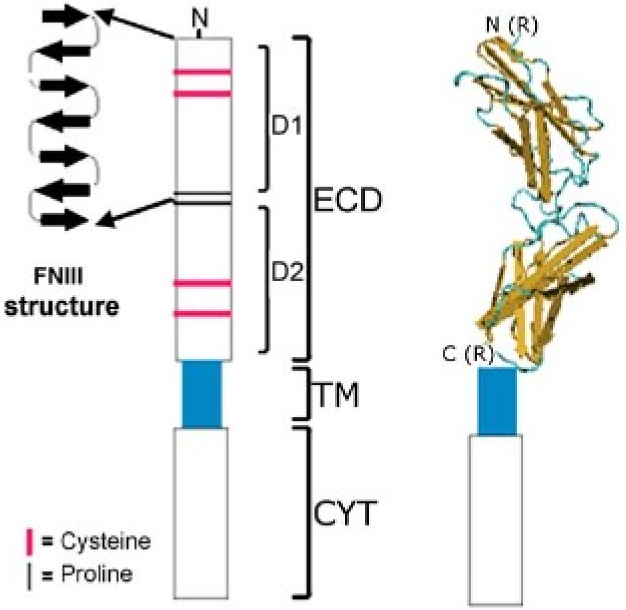

Figure 2. Structure of cytokine class 2 receptors. D1 and D2 mark fibronectin type III domains, TM (blue partition) transmembrane domain, CYT cytoplasmic domain. Image from Langer et al. (2004)

Based on structure and function in signalling, the receptor subunits may be divided into two groups, type 1 receptors and type 2 receptors (Figure 3) (Kotenko and Langer 2004). Type 1 receptor chain proteins include IL10RA (also called IL10R1, IL10Rα), IL20RA(also called IL20R1, IL20Rα), IL22RA1(also called IL22RA1, IL22Rα1) and IFNLR1 (also called IL28RA) all around 550 amino-acid in length. Type 2 receptor chain proteins, forming functional homodimers with type 1 chains, include IL10RB (also IL10R2, IL10Rβ) and IL20RB (also IL20R2, IL20Rβ), both around 320 amino-acids in length. Type 2 receptor subunits represent the shared subunits of the heterodimeric receptors used for signalling by the FIL-10 family (Kotenko and Langer 2004).

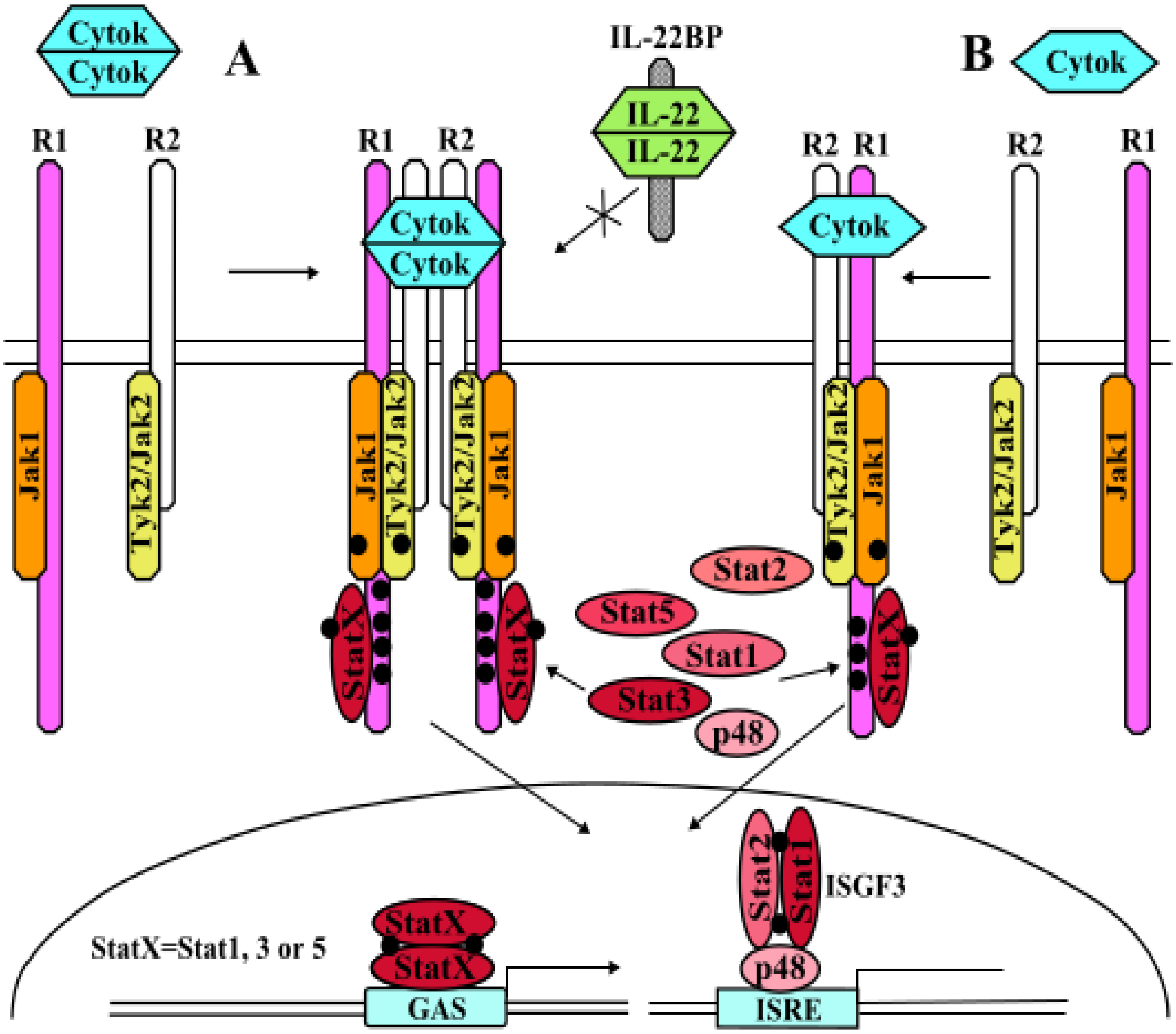

Figure 3. Receptor binding and signalling in monomeric and dimeric proteins of IL-10 family.
R1-type chain proteins: IL10RA, IL20RA and IL22RA. R2-type chain proteins IL10RB and IL20RB. Image from Kotenko and Langer (2004).

Several defects in signalling caused by mutations in receptor subunits are known. IL10RB mutation causes defect in JaK/STAT signalling connected with inflammatory bowel diseases such as Crohn's disease or colitis in adults (Glocker et al. 2009; Chaudhry et al. 2011) and a specific mutation in IL-10RA causes very early onset inflammatory bowel disease in children (Shim and Seo 2014).

Interleukin 22 binding protein

Interleukin 22 receptor A2, (IL22RA2) or interleukin 22 binding protein (IL22BP) is a soluble antagonist of IL-22 sharing amino acid homology with IL22RA1. It is highly expressed in placenta and spleen as well as digestive tract and immune system (Xu et al. 2001), particularly in mDCs (Martin et al. 2014).

IL22BP binds IL-22, but it is unable of inducing JaK/STAT signalling (Kotenko et al. 2001), functioning thus as IL-22 neutraliser. IL22BP is involved in regulation of pathological IL-22 response (Martin et al. 2014) and tumour-genesis in intestine (Huber et al. 2012).

Activity of IL22BP is affected by tissue damage sensing. Inflammasome down-regulates production of IL22BP. Disrupted ratio of IL-22 and its antagonist supports tumour development after previous damage by chronic inflammations of colon (Huber et al. 2012).

1.1.7 Gene clustering in Interleukin 10 family

In vertebrate genomes, interleukin genes commonly occur in clusters. This fact may be consequence of gene duplications in evolution and it effects regulation of gene expression. Interleukin 10 family genes in general have a 5 exon, 4 intron genomic pattern.

Human IL-10, IL-19, IL-20 and IL-24 genes are located on chromosome 1q31-32 (Kim et al. 1992; Blumberg et al. 2001). Human IFNγ, IL-26 and IL-22 genes are located on chromosome 12q15 (Donnelly et al. 2010). IL10RB and IFNGR2 are encoded on chromosome 21q33. IL20RA, IL22BP and IGNFR1 are clustered on human chromosome 6q23 (data from UCSC and NCBI databases). Vertebrate homologues of the mentioned groups are clustered in genome as well.

1.2 Coevolution of ligand-receptor pairs

Characterization of phylogenetic history of interleukin 10 family is valuable for understanding of the divergence of signalling pathways in the family and for general understanding of evolution of immunity. Evolutionary trees of the family and their receptors and analysis of their reciprocal relations may elucidate foundation of the interleukin diversity as known in human, as well as origins of ligand-receptor combination and their specificity.

Protein coevolution (correlated evolution) is a fundamental principle of evolution and occurs in every organism or group of organisms. By means of coevolution of protein and receptor pairs, signalling pathways are preserved despite changes in the genetic information of interacting partners and consequently in the protein structures. Coevolution of many ligand-receptor pairs in immune signalling molecules has been previously pointed out (Goh et al. 2000).

Coevolution occurs in single amino acid residues as well as in whole functionally related proteins. Protein signalling may be conserved throughout evolution either by negative selection, creating conserved sites, or by reciprocal changes in the active sites, their surroundings with effect on affinity or in sites influencing the structure of the protein. Therefore, physical interactions of proteins may be analysed by coevolutionary relations (Clark et al. 2011). In the family of IL-10, sharing receptor subunits, understanding of coevolutionary relations between the individual interleukin - receptor pairs may help the understanding of the specific signalling outcome of different interleukin - receptor combinations.

Coevolution may be studied on different levels, from inter-organism level to relations between amino acid or nucleotide residues. Protein-protein coevolutionary networks are complex and their study requires statistical methods, with respect to biological processes creating the coevolutionary relations. Current methods of protein coevolution analysis are based on comparison of distance matrices (Figure 4). For inter-protein evolution, distance matrices calculated from phylogenies are used, whereas inter-amino acid studies require a multiple sequence alignment (MSA) for calculation of the distance matrix (de Juan et al. 2013). Information given by the two methods of analysis describes both relation on protein and residue level, necessary for understanding both the protein relation network and structural basis of interactions. The amino acid level coevolution may be also studied by methods of covariation, describing mutual changes in the two amino acid sequences (Buslje et al. 2009; Iserte et al. 2015).

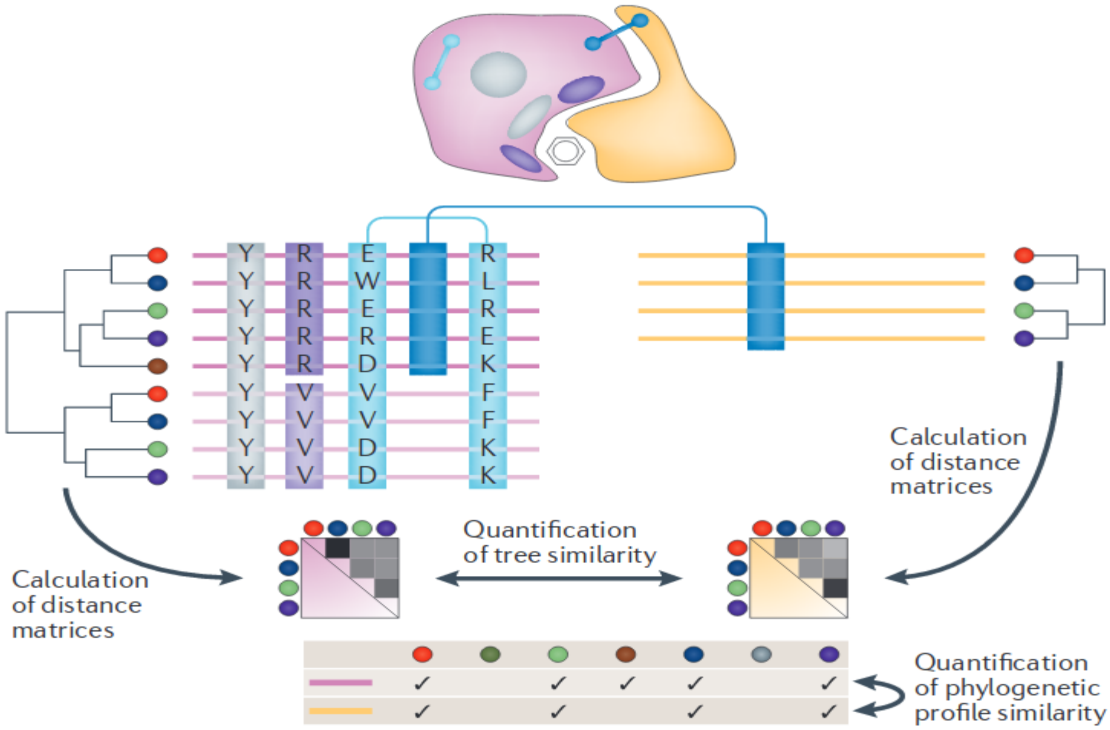

Figure 4. Analysis of protein receptor coevolution. Distance matrix is calculated from a phylogeny or multiple sequence alignment and analysed for pairwise correlation in protein evolution. Image from de Juan et al. (2013).

2 Aims

Interleukin 10 family proteins are an important family in immune regulation, however, the structural basis of receptor sharing and signalling is unclear. Coevolution of interleukins of the group of IL-10 and the corresponding receptor pairs is important for understanding complexity of the immune signalling pathways of the family. The thesis aims at describing evolutionary history of the FIL-10 proteins and their receptors.

The particular aims of the thesis are:

- to construct phylogenies of both interleukin 10 family cytokines and corresponding receptor proteins,
- using statistical methods, describe correlation between evolution of the interleukin family cytokines and their receptors,
    - at the level of the whole proteins
    - at the level of amino acid – amino acid coevolution.

3 Materials and methods

The methods used in this thesis and the processes are illustrated in Figure 5.

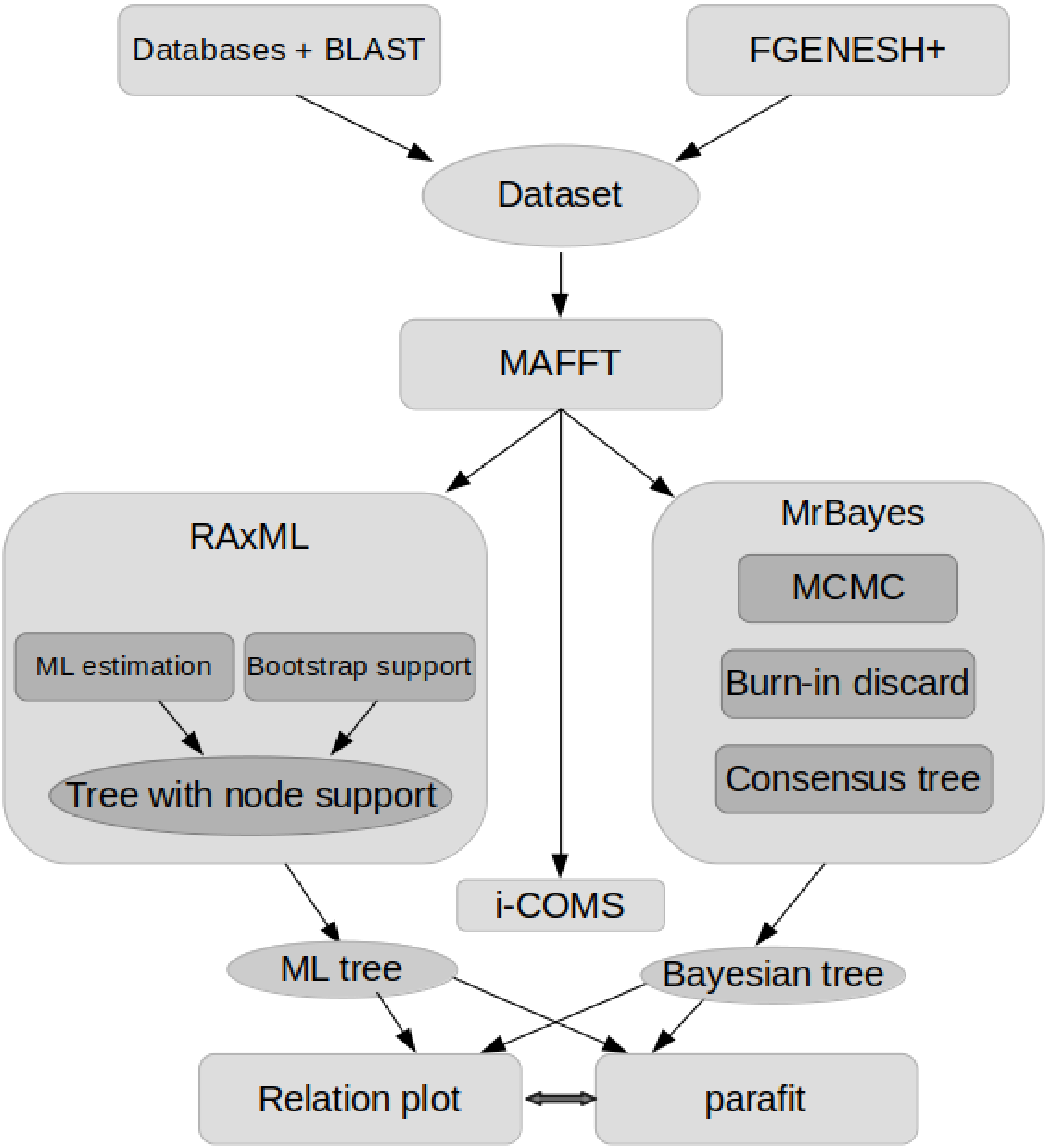

Figure 5. Workflow chart. Data (ellipses) obtained by methods (rectangular) on top are further analysed by RAxML software and MrBayes software. The coevolutionary analysis is preformed in Parafit tool of R and compared to relation plot of interleukins and corresponding receptors created by tools available in ape package in R. MAFFT alignment is further used for amino acid residue analysis by i−COMS.

3.1 Data collection

Interleukins of IL−10 family (FIL−10) need to be studied in species from distant taxonomic groups to determine their relationships. Species were selected based on their evolutionary distance, but also based on completeness and quality of genome sequence. Species from groups with expected changes in interleukin structure were preferred. Two of the most ancient chordate model organisms were selected, followed by cartilaginous fish. Apart from a traditional model species, *Danio rerio*, fish species were selected with consideration of the past whole genome duplications (WGD). *Tetraodon nigrovidis* represents teleost fish with 1 WGD (Jaillon et al. 2004) and *Oncorhynchus mykiss* (rainbow trout) was selected as a representative of *Salmonidae* with two WGDs (Berthelot et al. 2014). *Lepisosteus oculatus* was selected as a representative of an evolutionary old species in *Actinopterygii* group, *Latimeria chalumnae* as a representative of early *Sarcopterygii*. Representatives of reptiles and birds were selected from several groups as well as mammal species, from the most primitive species to human (Table 1).

Table 1: Binomial and English names of selected species. The species is mentioned as a representative of mentioned taxonomic group.

Binomial name	Taxonomic group	English name
Ciona intestinalis	*Tunicata*	−
Petromyzon marinus	*Petromyzontiformes*	Sea lamprey
Calorhinchus milii	*Chondrichthyes*	The Australian ghost shark
Lepisosteus oculatus	*Actinopterygii − Lepisosteiformes*	The Spotted gar
Danio rerio	*Actinopterygii − Cypriniformes*	The Zebrafish
Oncorhynchus mykiss	*Actinopterygii − Salmoniformes*	The Rainbow trout
Tetraodon nigroviridis	*Actiopterygii − Tetraodontiformes*	The Green spotted puffer
Latimeria chalumnae	*Sarcopterygii − Coelacanthiformes*	The West Indian ocean coelacanth
Xenopus tropicalis	*Amphibia*	The Western clawed frog
Anolis carolinensis	*Reptilia − Squamata*	The Carolina anole
Pelodiscus sinensis	*Reptilia − Testudines*	The Chinese softshell turtle
Alligator mississippiensis	*Crocodylia*	American alligator
Gallus gallus	*Aves*	The red junglefowl/domestic chicken
Ornithorhynchus anatinus	*Mammalia − Prototheria*	The Platypus
Monodelphis domestica	*Mammalia − Marsupialia*	The Grey short−tailed opossum
Pteropus vampyrus	*Mammalia − Pteropodidae*	The large flying fox
Mus musculus	*Mammalia − Rodentia*	The house mouse
Homo sapiens	*Mammalia − Primates*	Modern human

3.1.1 Database search and BLAST

The data was collected from the NCBI protein database at https://www.ncbi.nlm.nih.gov/protein/ and UniProt protein database at http://www.uniprot.org/. The databases were queried by the binomial name of selected model species (Table 1) and the name of a selected protein.

Sequences of proteins, which we not able to find in the databases were searched using Basic local alignment search tool (BLAST) at https://blast.ncbi.nlm.nih.gov/Blast.cgi. Protein databases were queried with the orthologous protein sequence from an evolutionary closest organism available from database search using BLASTp, delta-BLAST and tBLASTn algorithms.

Desired protein sequences with amino acid residue conservation higher than 30 % were exported from databases in FASTA format and further checked manually in multiple sequence for incorrect sequences.

3.1.2 FGENESH+

FGENESH+ (Solovyev 2001) is a prediction tool finding exon sequences in given genomic DNA. In this study, FGENESH+ was used for prediction of exon and protein sequences in genomes, where the protein sequence was neither available in the databases, nor was it identified by BLAST search, particularly in evolutionary older species, where genomes of nontraditional model species are not annotated completely or correctly.

FGENESH+ uses hidden Markov model and a related protein sequence to predict protein orthologues in eukaryotes. Required inputs for the program are: a homologous protein sequence from evolutionary close species, a species of prediction and genomic sequence of the species, that based on clustering of proteins should include coding sequence of the predicted protein. FGENESH+ is limited in ability to process genomic sequences longer than ~200 000 bp, therefore information about clustering of genes is necessary for a successful prediction.

3.1.3 Data formatting and processing

Data from databases were downloaded in FASTA format and alignments were preformed in FASTA format. For further analysis, data was converted to NEWICK format and PHYLIP formats using ReadSeq tool (Gilbert 2002).

UGENE (Okonechnikov et al. 2012) and Jalview (Waterhouse et al. 2009) were used for MSA visualization and control. The dataset was aligned and checked for error and duplicate sequences. In case of receptor alignment, extracellular parts of the sequence, interacting with the interleukin, were identified based on hydrophobicity scores in the alignment and used for further analysis.

For further visualisation of phylogenetic trees, R package ape (Paradis et al. 2004) or FigTree at http://tree.bio.ed.ac.uk/ software was used.

3.2 Multiple sequence alignment (MSA)

Critical step in a phylogenetic reconstruction is calculation of a multiple sequence alignment. Quality of the tree is hugely dependent on the quality of alignment, therefore high-quality alignment with well-defined positional homology is a necessary prerequisite. Several different tools for MSA of higher number of divergent sequences are commonly used. MAFFT (Katoh et al. 2002), MUSCLE (Edgar 2004a, 2004b), ClustalW (Thompson et al. 1994; Larkin et al. 2007), Clustal OMEGA (Sievers et al. 2014) and T-Coffee (Notredame et al. 2000), represent some of widely used tools for MSA. As the alignment quality is essential for phylogenetic inference, error sequences and overall quality of the alignment needs to be manually checked after aligning sequences. In this study, I used MAFFT tool to align the obtained sequences, since the tool provided the best results within the comparison of the mentioned tools. Comparison was based on sequence identity in the alignment, defining better identification of homologous positions.

3.2.1 MAFFT

MAFFT (**m**ultiple **a**lignment by **f**ast **F**urier **t**ransform) uses identification of homologous regions by fast Furier transform (FFT). MAFFT is claimed to be quicker than T-Coffee or ClustalW (Katoh and Standley 2013) while preserving high accuracy. FFT identifies homologous regions by grouping of amino acids in sequence by chemical and physical characteristics (Katoh et al. 2002). Afterwards, similarly to many other alignment tools, MAFFT refines the alignment to create better results. MAFFT uses 2 types of scores to determine the quality of alignment – WSP (weighted sums of pairs) and consistency score (also called *importance value* as described by Katoh et al. (2005)).

MAFFT is used in desktop version 7.307 (Katoh and Standley 2013). The `--globalpair` option for alignment of sequences of similar length is used. Pairwise alignments are computed with Needleman-Wunsch algorithm for global alignment (Needleman and Wunsch 1970). Maximum number of iterative refinement cycles is set to recommended 1000 (`--maxiterate 1000`). MAFFT uses BLOSUM62 scoring matrix for amino acid sequences by default.

After aligning the sequences, I reduced the dataset to analysable regions in trimAl tool (Capella-Gutierrez et al. 2009) on web interface on Phylemon2 web server at http://phylemon2.bioinfo.cipf.es/ using gappyout method, removing columns unsuitable for further analysis, while preserving highest percentage of the alignment among the available options

3.3 Amino acid evolution model selection

Inference of phylogeny requires selection of the best-fitting model of amino acid evolution for particular data. The sequence alignment was analysed by ProtTest 3 software (Abascal et al. 2005; Darriba et al. 2011).

The selection of amino acid evolution model was based on Akaike information criterion (AIC)

$$AIC = 2p - 2\ln(L)$$

and Bayesian information criterion (BIC)

$$BIC = 2 \cdot \ln(L) + p \cdot \ln(n)$$

where p corresponds to number of free parameters and L is maximum value of likelihood function. With higher penalization for number of parameters, BIC is less likely to propose an overfitted model.

Based on both criteria, JTT + Γ + F model was selected for both interleukin and receptor alignments. JTT is a protein evolution model based on substitutional matrix proposed by (Jones et al. 1992) and is used with the Γ distribution of parameters with shape parameter α estimated within the analysis. The F option assumes empirically determined amino acids frequencies.

3.4 Phylogenetic analysis

Phylogenetic trees may be constructed by several methods using different statistical approaches. Distance based methods use distance matrix of sequences in an alignment. The methods include neighbour joining (NJ) trees, finding the least related taxa and recalculating their tree nodes and branches, or UPGMA (Unweighted pair group method with arithmetic mean) trees, using hierarchical clustering based on pairwise similarity matrix creating rooted trees. Methods using optimality criteria include maximum parsimony, maximum likelihood (ML), or Bayesian inference. All these methods implement evolutionary models to establish the phylogeny.

Probability-based methods such as ML and Bayesian inference should be used for well supported trees, therefore this study uses software tools implementing these two methods.

3.4.1 Maximum Likelihood inference of phylogeny

Maximum likelihood is one of the basic methods of frequentist statistics (as opposed to Bayesian statistical framework) and it is widely used in bioinformatics.

Maximum likelihood (Whelan and Goldman 2001) methods are based on calculation of likelihood of parameters. Likelihood corresponds to probability of obtaining recorded data under the given model. The method is quite time consuming and dependent on computational resources. The calculation of likelihood is rather straightforward, yet the estimation of parameters used in the phylogeny is quite demanding. Parameters are estimated so that the likelihood of the data coming from the distribution defined by the values of parameters, reaches maximal possible values.

RAxML

For maximum likelihood phylogeny I used RAxML (Randomised Axelerated maximum likelihood) software (Stamatakis 2014). RAxML produces maximum parsimony trees, followed by calculation of likelihood of each tree by evaluating the tree parameters. Nodes are supported by bootstrap values from standard bootstrapping algorithm or rapid bootstrapping algorithm (Stamatakis et al. 2008), developed to lower computational demands of maximum likelihood phylogenetic inference.

ML estimations were calculated by RAxML MPI version 8.2.4. As the input, RAxML requires alignment in PHYLIP format. Protein evolution model is set according to ProtTest 3 results, gamma distribution shape parameter is calculated by RAxML:

```
raxmlHPC -f a -s alignment_file.phy -n il -m PROTGAMMAJTTX -p 12345 -x 12345 -#1000
```

−s	input alignment
−n	output files
−m	model used for estimation
	PROT − protein model
	GAMMA − Γ distribution of parameters
	X − empirical frequencies
−p	random seed for parsimony inference (important to reproduce results, required by RAxML)
−x	random seed for bootstrapping
−#	number of bootstrap replicates
−f a	execute rapid bootstrapping in one step with ML search

The above stated command executes rapid bootstrap analysis (Stamatakis 2014) with 1000 replicates and afterwards performs thorough search for best ML scoring tree. The output of the run is a tree with bootstrap values of the nodes, obtained by one command.

3.4.2 Bayesian inference of phylogeny

Bayesian statistical methods are widely used not only in biological applications, but also in physics and other fields. Bayesian estimation of phylogeny infers trees based on prior probability. Bayesian inference of phylogenies uses likelihood function to calculate posterior probability distribution of phylogenies (P(A|B)), implementing model of evolution. Following the Bayes' theorem:

$$P(A|B)=\frac{P(B|A)P(A)}{P(B)}$$

where P(A) represents posterior probability of the tree and P(B) likelihood of the data the trees are calculated. Nodal support of phylogeny is set by posterior probability of the node in the phylogenetic tree.

MrBayes

MrBayes is a software using above described principles to infer phylogenies. MrBayes implements Markov chain Monte Carlo algorithms (MCMC) to produce distribution of posterior probability of phylogenies. The resulting trees are concatenated from sampling of calculations of trees with high posterior probability (Felsenstein 2004). By default, MrBayes uses Metropolis coupling MCMC to run number of heated chains and a cold chain over adjusted distribution with fewer peaks and bigger steps, allowing crossing the "valleys" in probability, therefore it is less likely for the analysis to end in a local maximum, rather than finding global maximum probability.

MrBayes is used in version 3.2.6 (Ronquist et al. 2012; Ronquist and Huelsenbeck 2003). MrBayes works either in interactive mode, setting parameters one by one, or by inputting NEXUS format alignment in file with MrBayes command block for interleukins:

```
Begin MrBayes;
lset rates=gamma ngammacat=4;
prset statefreqpr=fixed(empirical) aamodelpr=fixed(jones) shapepr=fixed(2.946);

mcmcp ngen=5000000 nruns=2 nchains=4 printfreq=250 samplefreq=250 burnin=1000;
mcmc;
sump;
sumt;
end;
```

where lset and prset specify the model used for the analysis estimated by ProtTest 3, ngen parameter sets number of generations of MCMC estimations, nruns number of runs and nchains number of heated chains. Printfreq sets frequency of printed results, samplefreq frequency of trees, that will participate in final consensus tree. Burnin command sets number of initial samples (not probability estimations) that are discarded at the beginning of analysis for their lower posterior probability. In estimation of receptor phylogeny, shapepr parameter was set as calculated previously by ProtTest 3 to be 2.946 for interleukin phylogeny and 1.899 for receptor phylogeny.

3.5 Comparison of phylogenetic approaches

I compared maximum likelihood and Bayesian estimation of phylogenies visually, based on knowledge of relations of proteins. Afterwards, I calculated distance of tree topologies using `dist.topo` tool of package *ape* (Paradis et al. 2004) in R to quantify differences of phylogenies. The dist.topo calculates two types of scores, "PH85", defining difference of internal nodes and "score", derived from previous but incorporating internal branch lengths into the analysis – score calculated as square root of the sum of the squared differences of the internal branch lengths, defining different tip topologies.

3.6 Ligand-receptor coevolution analysis

For further analysis, only the best result tree was selected from the previous phylogenetic analyses. Analysis of coevolution, or correlated evolution, where topology of the tree and branch lengths should be correlated in related proteins, was preformed using several approaches, by MirrorTree (Ochoa et al. 2015), in R package *ape* by Parafit tool (Paradis et al. 2004; Legendre et al. 2002) and by i-COMS (Iserte et al. 2015).

3.6.1 MirrorTree

MirrorTree (Ochoa et al. 2015) is able to calculate coevolutionary relations between proteins using sequences, MSA or trees. Pearson's correlation coefficient between two distance matrices of orthologous protein family alignments or trees is calculated to describe the relations. The Pearson correlation coefficient is then assigned a p-value by comparing the distribution with a null distribution (distribution of proteins with no coevolutionary relations). I used both versions of MirrorTree (MT) that require an alignment and a phylogenetic tree.

MirrorTree version based on protein alignments analyses mostly inter-residue relations. MirrorTree accepts whole protein aligned sequences in FASTA format as input, constructs a NJ tree and calculates the correlation coefficient. MAFFT alignment of the interleukin family and of the extracellular domain of receptors was analysed. Version with complete trees accepts NEWICK format tree as input with bootstrap or probability values for calculation of Pearson's correlation coefficient. Both ML and Bayesian phylogenies were analysed by this method.

MirrorTree webserver (Ochoa and Pazos 2010) is available at http://csbg.cnb.csic.es/mtserver/.

3.6.2 Parafit

Analysis of evolutionary relations is performed with Parafit tool (Legendre et al. 2002) of the ape package. Parafit was originally developed for testing host-parasite coevolutionary relations, however is applicable to any related coevolving genes, proteins or organisms.

Parafit is able to calculate a global test of coevolution, indicating signs of relations within the two given trees or to test individual links between host and parasite, or in this case, ligand and receptor. For testing individual links Parafit requires an input of the two distance matrices created from unrooted phylogenies of interacting proteins, which I calculated in R. Additionally, for testing of individual protein link coevolution, a matrix of relations between proteins based on theoretical knowledge of interleukin-receptor interaction is needed. The input matrices are multiplied to create one matrix for the analysis.

Test statistics are calculated as a difference of sums of squares of values in the main diagonal of the combined matrix and a matrix without the particular interaction to determine the importance of the ligand-receptor relation (F1.stat) and in the second case, as difference standardised by the trace of non-permutated matrix (F2.stat). Permutation tests are based on random shuffling of values in rows of a relation matrix.

Results of Parafit search for coevolution were visualised by cophylo tool of *phytools* package in R (Revell 2012) in comparison with links of ligand-receptor pairs with no significant signs of coevolution.

3.6.3 i-COMS

i-COMS (interprotein COrrelated Mutations server) (Iserte et al. 2015)was used to analyse coevolutionary relations among the amino acid residues within the interleukin and the corresponding receptor extracellular domain.

The software is capable of analysing protein sequences, however I used an option of inputting previously created MAFFT MSA of the proteins as an input. The coevolving proteins to be analysed by i-COMS were selected based on the results of previous analysis. The protein alignments were created from the same sequences as used for the previous analysis, however, i-COMS requires the coevolving protein sequence pairs to be from the same species, therefore in case of either missing interleukin or receptor sequence, the corresponding unpaired sequence was excluded from the analysis. First sequence in the alignment used as a reference sequence was a human orthologue of the analysed protein.

I analysed the alignment by calculating a covariation score by several methods – mutual information (MI) (Buslje et al. 2009)direct coupling analysis with mean-field approximation (mfDCA) (Kaján et al. 2014) and direct coupling analysis with pseudo-likelihood maximisation (plmDCA) (Seemayer et al. 2014) All the mentioned tools calculate a covariation score of every column in the given MSAs.

The results were further visualised using the "circos" visualisation tool available in the interface. Visualisation of individual methods was preformed for 50 best scoring interactions for each method and an intersection of 100 best scoring relations of all methods and two selected methods based on consistency of results and evaluation of the distribution of covariance values.

The analysis was performed at the i-COMS webserver at http://i-coms.leloir.org.ar/.

4 Results

4.1 Data collection

The final dataset consists of the data found in the databases by BLAST variants and by database search (Supplement 1 and 2) and from protein sequences predicted by FGENESH+ software. No proteins from the group were found in the two most ancient species, tunicate *Ciona intestinalis* and sea lamprey *Petromyzon marinus*. The total number of 83 receptor interleukin sequences and 92 receptor sequences from 16 organisms was analysed. I successfully predicted sequences of 3 proteins (Table 3) not present in the databases, supported by homology with known proteins and intron-exon structure, namely IL-20 of *Anolis carolinensis*, IL-26 of *Gallus gallus* and IFNLR1 of *Pelodiscus sinensis.* The predicted protein sequences are presented in Supplement 3.

Table 2. FIL-10 proteins in vertebrates. ✓ marks presence of the protein in the dataset, ✗ marks proteins that are likely absent in the organism, - marks proteins, that were excluded from the dataset for being likely faulty or proteins that were not in the databases, but are likely to be expressed in the species.

	IL-10	IL-19	IL-20	IL-22	IL-24	IL-26	IFN λ 1	IFN λ 2	IFN λ 3	IFN λ 4
Ciona intestinalis	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Petromyzon marinus	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Calorhinchus milii	✓	✗	✓	✓	✗	✗	✗	✗	✗	✗
Lepisosteus oculatus	✓	✗	✓	-	✗	✗	✗	✗	✓	✗
Danio rerio	✓	✗	✓	✓	✗	✗	✗	✗	✓	✗
Oncorhynchus mykiss	✓	✗	✓	✓	✗	✗	✗	✗	✓	✗
Tetraodon nigroviridis	✓	✗	✓	✓	✗	✗	✗	✗	✓	✗
Latimeria chalumnae	✓	✗	✓	✓	✗	✗	✗	✗	-	✗
Xenopus tropicalis	✓	✗	✓	✓	✗	✓	✗	✗	✓	✗
Anolis carolinensis	✓	✗	✓	✓	✗	-	✗	✗	✓	✗
Pelodiscus sinensis	✓	✗	-	✓	✗	-	✗	✗	✓	✗
Alligator mississippiensis	✓	✗	✓	✓	✗	-	✗	✗	✓	✗
Gallus gallus	✓	✗	✓	✓	✗	✓	✗	✗	✓	✗
Ornithorhynchus anatinus	✓	✗	✓	✓	✗	✓	✗	✗	✓	✗
Monodelphis domestica	✓	✗	✓	✓	✓	✓	✗	✗	✓	✗
Pteropus vampyrus	✓	✓	✓	✓	✓	✓	✗	✗	✓	✗
Mus musculus	✓	✓	✓	✓	✓	✗	✓	✓	✓	✗
Homo sapiens	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 3. **FGENESH+ predicted protein sequences** with UIDs of homologue proteins used for prediction. Predicted sequences are presented in Supplement 3.

Protein	Homologue ID, species	Length, exons	Score
Anolis carolinensis IL-20	1011565931 *Alligator mississippiensis*	174 aa, 5 exons	1282.1282
Gallus gallus IL-26	558155504 *Pelodiscus sinensis*	178 aa, 4 exons	774.4939
Pelodiscus sinensis IFNLR1	1011571573 *Alligator mississippiensis*	587 aa, 7 exons	1370.3634

4.2 Phylogenetic inference

4.2.1 Maximum likelihood

ML search for optimal phylogeny was performed using RAxML software with JTT model with Γ distribution of parameters (shape parameter α estimated within the analysis) and empirically determined amino acids frequencies. Bootstrap values for nodal support were obtained by rapid bootstrapping algorithm implemented in RAxML. ML phylogenies of FIL-10 cytokines and receptors are presented in Figure 6 and Figure 7 respectively.

4.2.2 Bayesian inference

Bayesian inference of phylogeny is calculated in MrBayes software with JTT model with Γ distribution of parameters (shape parameter α previously estimated by ProtTest3 to be 2.946 for interleukins and 1.899 for receptors) and empirically determined amino acids frequencies. The result tree is concatenated from tree sampling from the distribution defined by command block given to MrBayes. Nodal support is provided by node posterior probability values. Figure 8 shows resolved relations of interleukins in FIL-10 in detail, Figure 9 shows detail of relations of extracellular parts of proteins within the receptor group, interacting with FIL-10 cytokines.

Both of the used methods show evolutionary closeness of IL-19, IL-20 and IL-24 subfamily, and common evolutionary basis of IL22BP and the functional receptor of IL-22, membrane bound IL22RA2. Type 2 (or B) receptors are quite clearly distinguished from functionally different type 1 (or A) receptors. Several proteins (notably IL-22 and IFN λ s) show divergence in fish from other species.

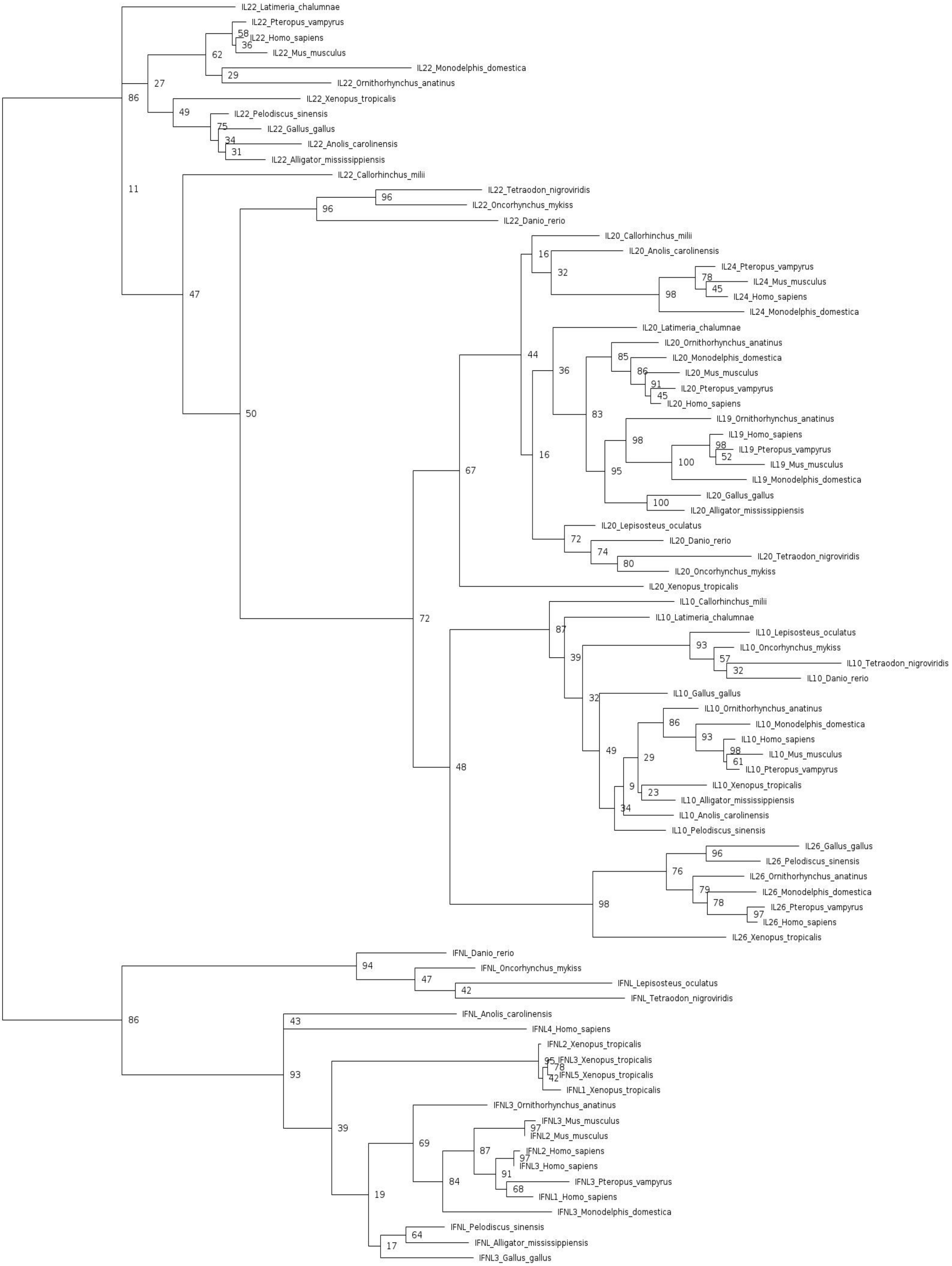

Figure 6. Maximum likelihood phylogeny of FIL-10 interleukins. Node labels represent bootstrap values obtained by rapid bootstrapping algorithm implemented in RAxML. Results were visualised by FigTree.

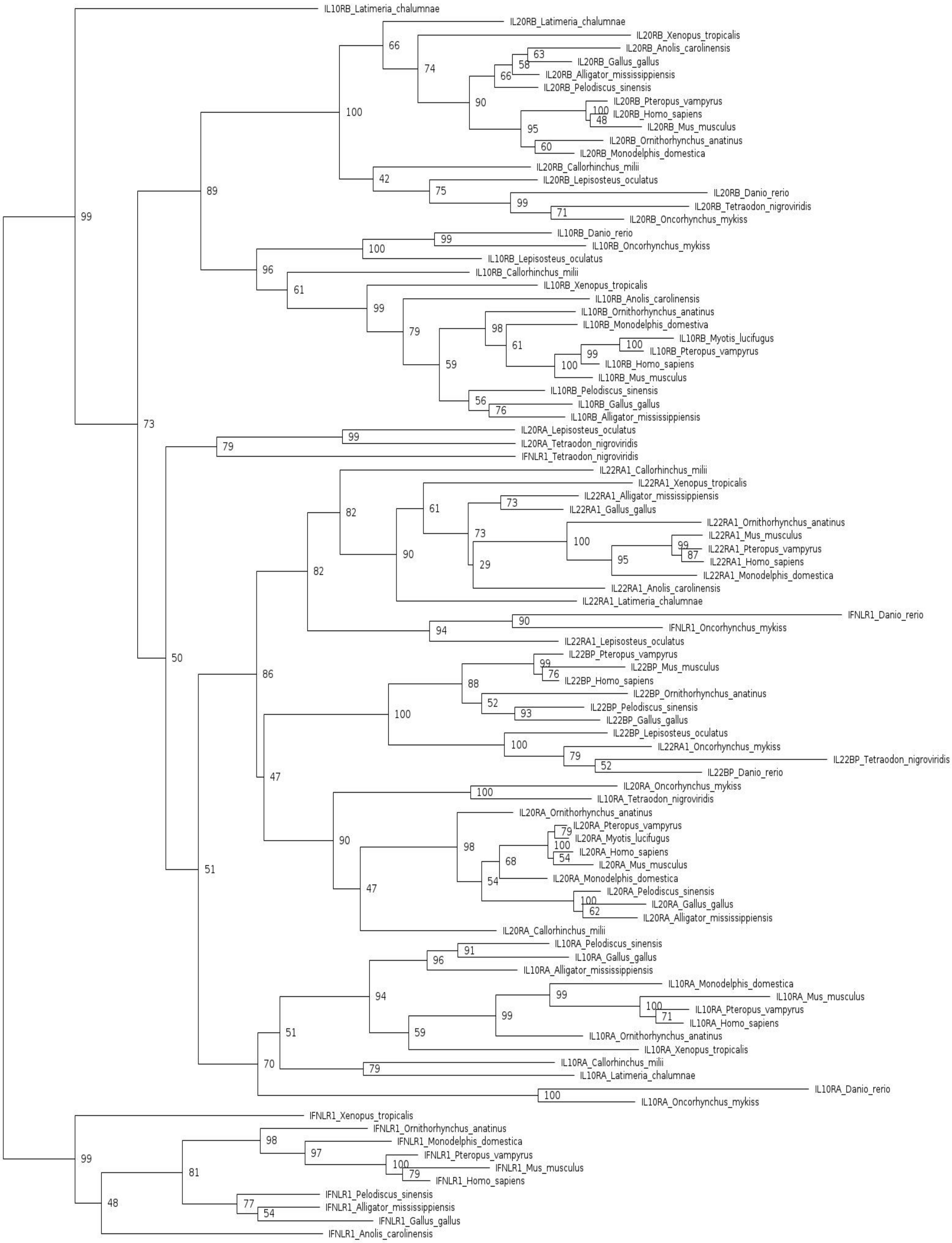

Figure 7. Maximum likelihood phylogeny of FIL-10 receptors. Node labels represent bootstrap values obtained by rapid bootstrapping algorithm implemented in RAxML. Results were visualised by FigTree.

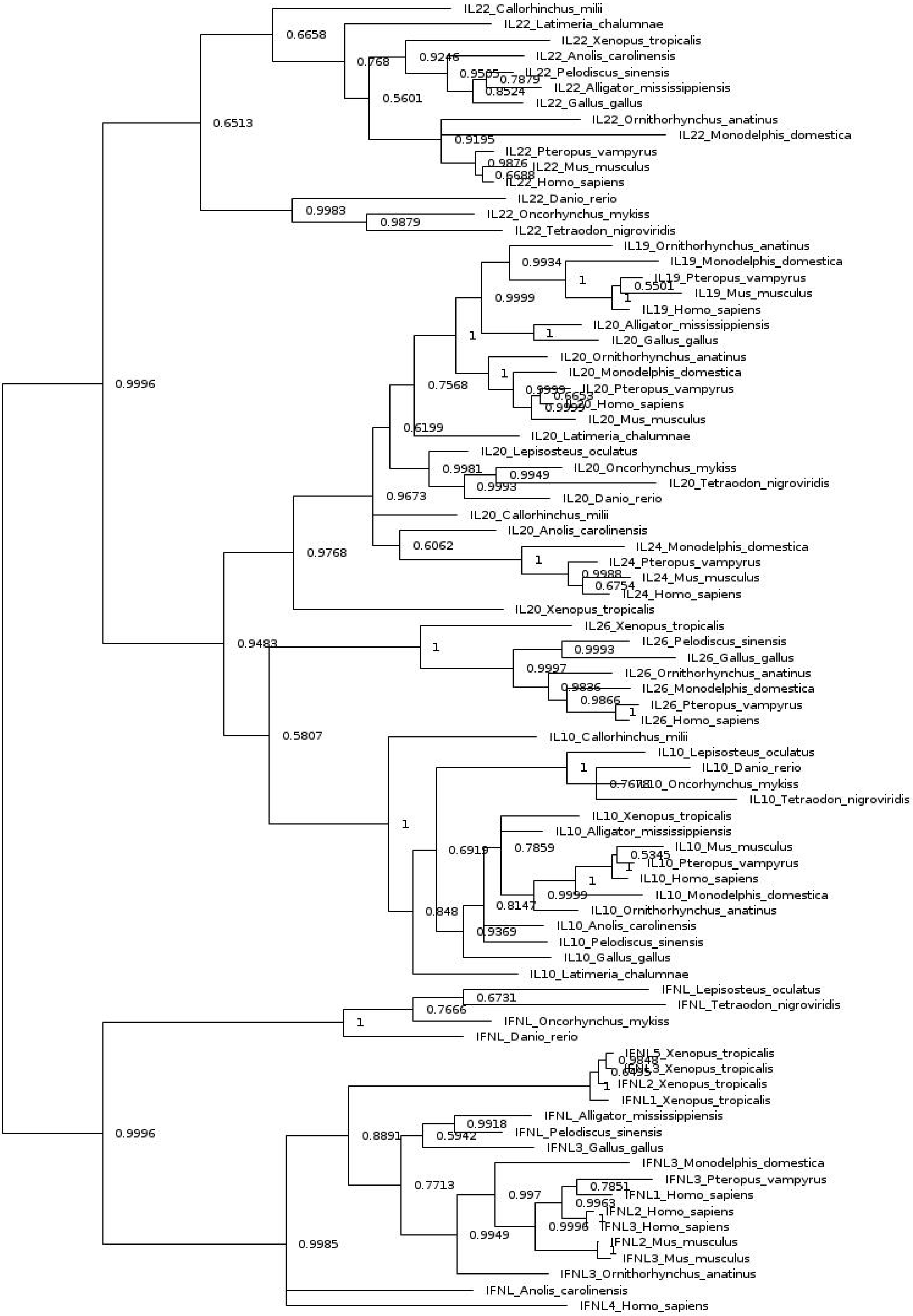

Figure 8. Bayesian phylogeny of interleukins of FIL-10. Node labels mark posterior probabilities. Result trees were visualised by FigTree.

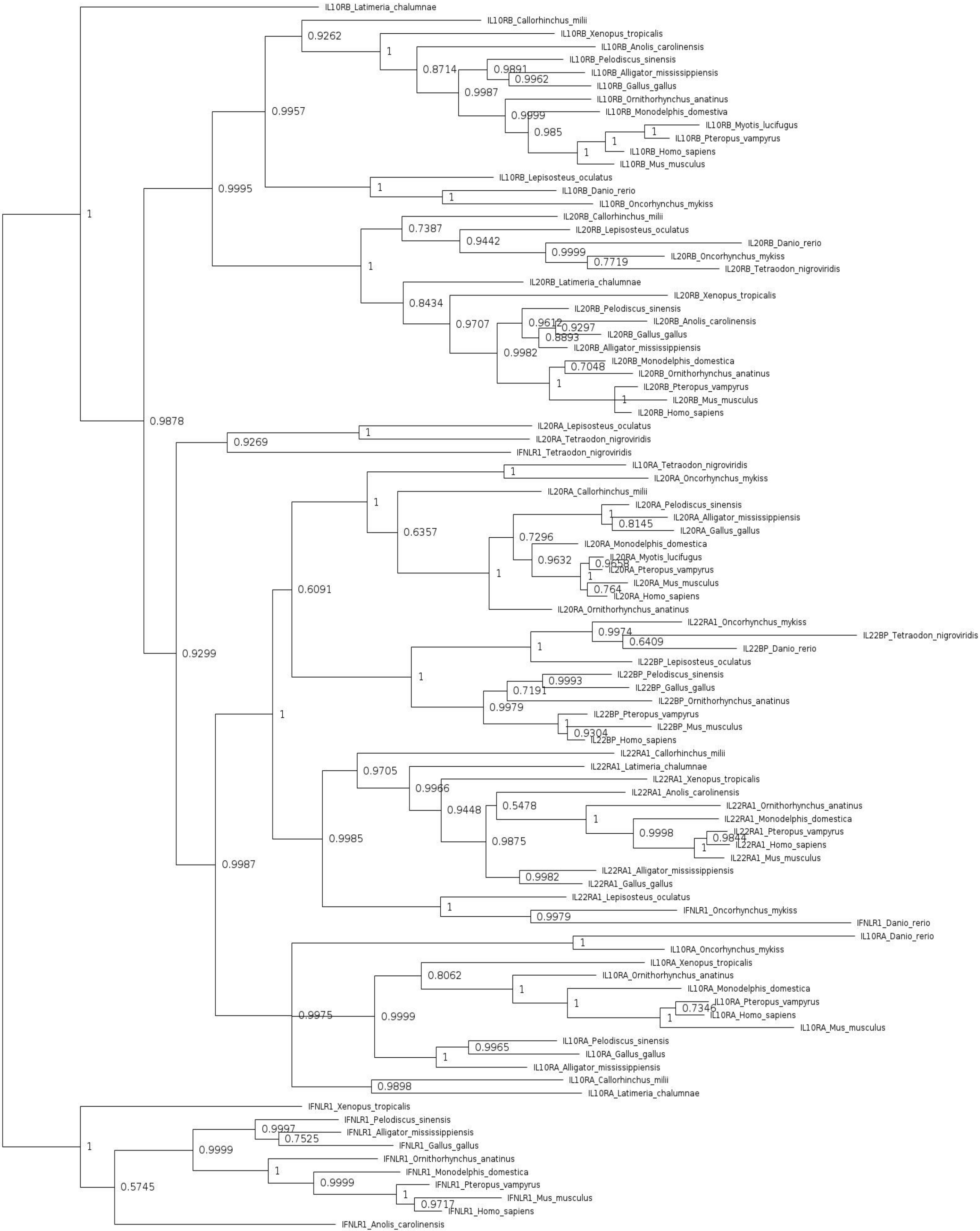

Figure 9. Bayesian phylogeny of receptors of FIL-10. Node labels represent posterior probabilities of the nodes. Result trees were visualised by FigTree.

4.2.3 Comparison of phylogenetic approaches

After inferring the phylogenies by two distinct methods, only one resulting phylogeny is to be used in further analysis. I tested similarity of the tree topologies created by RAxML and MrBayes with R *ape* package tool dist.topo.

In phylogenies of FIL-10, “PH85” method showing the difference of numbers of the internal branches was 18, and the “score” method incorporating branch lengths into the calculation was 1.1083. FIL-10 receptors showed differences in internal branch numbers to be 2 and “score” value impaired by branch length to be 0.1965. Based on visual comparison and analysis of differences of topologies of the trees, the RAxML result trees were selected for further analysis, because they had a wider range of support values therefore offering a more critical view of the node supports.

4.3 Interleukin-receptor coevolution analysis

Coevolution of the two protein groups, interleukins and extracellular domains of their receptors are analysed by several different approaches. MirrorTree (Ochoa et al. 2015) is searching for coevolution within the given MSA or tree, Parafit (Legendre et al. 2002), is testing global coevolution and given coevolutionary relations and i-COMS (Iserte et al. 2015) is analysing amino acid residue coevolution described by covariation in a related interleukin -extracellular receptor domain pair.

4.3.1 MirrorTree

MirrorTree was used in a version analysing a MSA created by MAFFT and in a version analysing a phylogenetic tree from both ML and Bayesian approaches. However, results of both analyses performed by MirrorTree on the available dataset were not consistent their interpretation was not unequivocal. I therefore do not present results of coevolution obtained by MirrorTree.

4.3.2 Parafit

I calculated 2 types of coevolutionary statistics using Parafit, test of global coevolution within the two trees and tests of individual links between receptors and ligands. Global test of coevolution showed significant coevolution within the two trees with p-value = 0.001.

In the test of individual interactions, total of 166 experimentally confirmed interactions were tested with two types of statistics calculated. Significant interleukin-receptor coevolution was found for 91 our of 166 (54,82 %) of the given relations (Figure 10, Figure 11) using both statistical tests (F1.stat and F2.stat) (Table 5, Figure 10), additional 5 interactions are significantly coevolving only based on F2.stat.

Significant coevolution was found in all tested interactions between IL-20 and IL20RB. IL20RB is shared within IL-19 and IL-24 (and coevolving IL-20) subfamily. IL-19 coevolution is on the edge of supported coevolutionary relations, in some cases supported only by F2.stat. IL-24 and its receptors show no significant signs of coevolution. In the case of IL10RB, the most promiscuous receptor within the family, significant coevolution was found with IL-10 and IFNλs, however, some of the interactions are confirmed as significant only by the F2.stat type of statistics. IL10RB is shared, apart from mentioned IL-10 and IFNλs, also by IL-22 and IL-26. IL 22 and IL-26 show no significant signs of coevolution with IL10RB (apart from one interaction, IL-26 of Gallus gallus with IL10RB, with p1 = 0.038, p2 = 0.022). A shared type 1 subunit, IL22RA1 is in coevolution with its ligands in most cases, except for fish interleukins. IL20RA orthologues, shared by the IL-19, IL-20 and IL-24 subfamily, are coevolving with some of its IL-20 orthologous ligands, however they show no signs of significant coevolution with IL-19 and IL-24, cytokines that are found only in mammals (Figure 11).

Table 4. Parafit results of coevolution of FIL-10 and corresponding receptors from trees created by Maximum Likelihood. Interactions with detected significant coevolution are on the top, sorted by p-value of F1.stat. Interactions without detected significant coevolution are under the separating lines, 1st by p.F1, second line for less strict p.F2. Types of used statistics are described in Chapter 3.7.1.

Interleukin	Receptor	F1.stat	p.F1	F2.stat	p.F2
IFNL2_Homo_sapiens	IFNLR1_Homo_sapiens	3236.431	0.001	0.0052	0.001
IFNL3_Gallus_gallus	IFNLR1_Gallus_gallus	2773.679	0.001	0.0045	0.001
IFNL3_Mus_musculus	IFNLR1_Mus_musculus	3731.223	0.001	0.0061	0.001
IFNL3_Pteropus_vampyrus	IFNLR1_Pteropus_vampyrus	3078.102	0.001	0.0050	0.001
IFNL4_Homo_sapiens	IFNLR1_Homo_sapiens	3315.070	0.001	0.0054	0.001
IFNL5_Xenopus_tropicalis	IFNLR1_Xenopus_tropicalis	4329.595	0.001	0.0070	0.001
IL10_Alligator_mississippiensis	IL10RA_Alligator_mississippiensis	3526.165	0.001	0.0057	0.001
IL10_Alligator_mississippiensis	IL10RB_Alligator_mississippiensis	2694.947	0.001	0.0044	0.001
IL10_Callorhinchus_milii	IL10RA_Callorhinchus_milii	2694.417	0.001	0.0044	0.001
IL20_Anolis_carolinensis	IL20RB_Anolis_carolinensis	5419.588	0.001	0.0088	0.001
IL20_Callorhinchus_milii	IL20RB_Callorhinchus_milii	5790.122	0.001	0.0094	0.001
IL20_Danio_rerio	IL20RB_Danio_rerio	5915.250	0.001	0.0096	0.001
IL20_Gallus_gallus	IL20RB_Gallus_gallus	5677.617	0.001	0.0092	0.001
IL20_Homo_sapiens	IL20RB_Homo_sapiens	4497.678	0.001	0.0073	0.001
IL20_Latimeria_chalumnae	IL20RB_Latimeria_chalumnae	4973.496	0.001	0.0081	0.001
IL20_Lepisosteus_oculatus	IL20RB_Lepisosteus_oculatus	6692.176	0.001	0.0109	0.001
IL20_Monodelphis_domestica	IL20RA_Monodelphis_domestica	5398.442	0.001	0.0088	0.001
IL20_Monodelphis_domestica	IL20RB_Monodelphis_domestica	6573.920	0.001	0.0107	0.001
IL20_Mus_musculus	IL20RA_Mus_musculus	6587.615	0.001	0.0107	0.001
IL20_Mus_musculus	IL20RB_Mus_musculus	6198.022	0.001	0.0101	0.001
IL20_Oncorhynchus_mykiss	IL20RA_Oncorhynchus_mykiss	7083.681	0.001	0.0115	0.001
IL20_Oncorhynchus_mykiss	IL20RB_Oncorhynchus_mykiss	2076.076	0.001	0.0034	0.001
IL20_Ornithorhynchus_anatinus	IL20RA_Ornithorhynchus_anatinus	6326.518	0.001	0.0103	0.001
IL20_Ornithorhynchus_anatinus	IL20RB_Ornithorhynchus_anatinus	2022.725	0.001	0.0033	0.001
IL20_Pteropus_vampyrus	IL20RA_Pteropus_vampyrus	6350.875	0.001	0.0103	0.001
IL20_Tetraodon_nigroviridis	IL20RA_Tetraodon_nigroviridis	6259.361	0.001	0.0101	0.001
IL20_Tetraodon_nigroviridis	IL20RB_Tetraodon_nigroviridis	2345.512	0.001	0.0038	0.001
IL22_Alligator_mississippiensis	IL20RB_Alligator_mississippiensis	7126.538	0.001	0.0116	0.001
IL22_Anolis_carolinensis	IL20RB_Anolis_carolinensis	7429.036	0.001	0.0120	0.001
IL22_Callorhinchus_milii	IL20RB_Callorhinchus_milii	7559.984	0.001	0.0123	0.001
IL22_Danio_rerio	IL20RB_Danio_rerio	7161.747	0.001	0.0116	0.001
IL22_Gallus_gallus	IL20RB_Gallus_gallus	6231.824	0.001	0.0101	0.001
IL22_Homo_sapiens	IL20RB_Homo_sapiens	4813.591	0.001	0.0078	0.001
IL22_Latimeria_chalumnae	IL20RB_Latimeria_chalumnae	5074.807	0.001	0.0082	0.001
IFNL_Pelodiscus_sinensis	IFNLR1_Pelodiscus_sinensis	2941.057	0.002	0.0048	0.002
IL10_Latimeria_chalumnae	IL10RA_Latimeria_chalumnae	2446.325	0.002	0.0040	0.002
IFNL_Oncorhynchus_mykiss	IFNLR1_Oncorhynchus_mykiss	3005.030	0.002	0.0049	0.001
IFNL1_Homo_sapiens	IFNLR1_Homo_sapiens	2640.014	0.002	0.0043	0.001
IFNL1_Xenopus_tropicalis	IFNLR1_Xenopus_tropicalis	3961.110	0.002	0.0064	0.001
IFNL2_Mus_musculus	IFNLR1_Mus_musculus	3754.421	0.002	0.0061	0.001
IFNL2_Xenopus_tropicalis	IFNLR1_Xenopus_tropicalis	4097.614	0.002	0.0066	0.001
IFNL3_Homo_sapiens	IFNLR1_Homo_sapiens	2992.654	0.002	0.0049	0.001

IFNL3_Monodelphis_domestica	IFNLR1_Monodelphis_domestica	3568.887	0.002	0.0058	0.001
IFNL3_Ornithorhynchus_anatinus	IFNLR1_Ornithorhynchus_anatinus	3701.690	0.002	0.0060	0.001
IFNL3_Xenopus_tropicalis	IFNLR1_Xenopus_tropicalis	4125.984	0.002	0.0067	0.001
IL10_Danio_rerio	IL10RA_Danio_rerio	2175.962	0.002	0.0035	0.001
IL20_Xenopus_tropicalis	IL20RB_Xenopus_tropicalis	2513.657	0.002	0.0041	0.001
IL22_Danio_rerio	IL22BP_Danio_rerio	2686.794	0.002	0.0044	0.001
IL22_Gallus_gallus	IL22BP_Gallus_gallus	2624.750	0.002	0.0043	0.001
IL22_Homo_sapiens	IL22BP_Homo_sapiens	2553.943	0.002	0.0041	0.001
IL22_Latimeria_chalumnae	IL22RA1_Latimeria_chalumnae	3501.874	0.002	0.0057	0.001
IFNL_Tetraodon_nigroviridis	IFNLR1_Tetraodon_nigroviridis	2265.071	0.003	0.0037	0.002
IL20_Lepisosteus_oculatus	IL22RA1_Lepisosteus_oculatus	3584.922	0.003	0.0058	0.002
IFNL_Danio_rerio	IFNLR1_Danio_rerio	2421.913	0.003	0.0039	0.001
IL10_Danio_rerio	IL10RB_Danio_rerio	2537.837	0.003	0.0041	0.001
IL20_Latimeria_chalumnae	IL22RA1_Latimeria_chalumnae	3483.183	0.003	0.0056	0.001
IFNL_Anolis_carolinensis	IFNLR1_Anolis_carolinensis	2053.121	0.004	0.0033	0.003
IL10_Homo_sapiens	IL10RA_Homo_sapiens	2033.530	0.004	0.0033	0.002
IL20_Monodelphis_domestica	IL22RA1_Monodelphis_domestica	2743.406	0.004	0.0044	0.002
IL22_Anolis_carolinensis	IL22RA1_Anolis_carolinensis	2184.352	0.004	0.0035	0.002
IFNL3_Gallus_gallus	IL10RB_Gallus_gallus	1779.993	0.004	0.0029	0.001
IL20_Pteropus_vampyrus	IL20RB_Pteropus_vampyrus	1929.527	0.004	0.0031	0.001
IL22_Homo_sapiens	IL22RA1_Homo_sapiens	3855.406	0.004	0.0063	0.001
IL22_Callorhinchus_milii	IL22RA1_Callorhinchus_milii	1918.201	0.005	0.0031	0.004
IL10_Anolis_carolinensis	IL10RB_Anolis_carolinensis	2497.864	0.005	0.0041	0.002
IL20_Homo_sapiens	IL22RA1_Homo_sapiens	3658.039	0.005	0.0059	0.001
IL10_Callorhinchus_milii	IL10RB_Callorhinchus_milii	2611.382	0.006	0.0042	0.002
IL22_Gallus_gallus	IL22RA1_Gallus_gallus	3111.661	0.006	0.0050	0.002
IL20_Gallus_gallus	IL22RA1_Gallus_gallus	2591.826	0.007	0.0042	0.005
IL22_Alligator_mississippiensis	IL22RA1_Alligator_mississippiensis	1992.918	0.007	0.0032	0.005
IFNL_Alligator_mississippiensis	IL10RB_Alligator_mississippiensis	2154.377	0.008	0.0035	0.005
IL20_Mus_musculus	IL22RA1_Mus_musculus	2293.081	0.008	0.0037	0.003
IFNL_Anolis_carolinensis	IL10RB_Anolis_carolinensis	1955.854	0.009	0.0032	0.005
IL20_Callorhinchus_milii	IL22RA1_Callorhinchus_milii	1449.568	0.011	0.0024	0.006
IL10_Gallus_gallus	IL10RA_Gallus_gallus	1948.427	0.011	0.0032	0.004
IL10_Monodelphis_domestica	IL10RA_Monodelphis_domestica	1640.203	0.011	0.0027	0.004
IL10_Gallus_gallus	IL10RB_Gallus_gallus	1640.400	0.012	0.0027	0.001
IL20_Anolis_carolinensis	IL22RA1_Anolis_carolinensis	1591.991	0.013	0.0026	0.009
IFNL_Danio_rerio	IL10RB_Danio_rerio	2101.211	0.015	0.0034	0.009
IFNL2_Homo_sapiens	IL10RB_Homo_sapiens	1028.153	0.021	0.0017	0.013
IL10_Latimeria_chalumnae	IL10RB_Latimeria_chalumnae	877.349	0.025	0.0014	0.011
IFNL3_Homo_sapiens	IL10RB_Homo_sapiens	917.767	0.026	0.0015	0.018
IFNL4_Homo_sapiens	IL10RB_Homo_sapiens	995.410	0.027	0.0016	0.015
IL10_Homo_sapiens	IL10RB_Homo_sapiens	953.848	0.031	0.0015	0.020
IL19_Ornithorhynchus_anatinus	IL20RA_Ornithorhynchus_anatinus	1615.789	0.032	0.0026	0.016
IL19_Pteropus_vampyrus	IL20RA_Pteropus_vampyrus	1559.953	0.033	0.0025	0.018
IL26_Gallus_gallus	IL10RB_Gallus_gallus	987.664	0.038	0.0016	0.022
IFNL1_Homo_sapiens	IL10RB_Homo_sapiens	869.737	0.039	0.0014	0.028

IL19_Monodelphis_domestica	IL20RA_Monodelphis_domestica	1325.097	0.040	0.0021	0.020
IL20_Alligator_mississippiensis	IL20RB_Alligator_mississippiensis	1732.304	0.041	0.0028	0.021
IL19_Mus_musculus	IL20RA_Mus_musculus	1599.322	0.043	0.0026	0.028
IL10_Lepisosteus_oculatus	IL10RB_Lepisosteus_oculatus	555.040	0.053	0.0009	0.030
IL19_Monodelphis_domestica	IL20RB_Monodelphis_domestica	1675.323	0.055	0.0027	0.034
IL19_Mus_musculus	IL20RB_Mus_musculus	1521.469	0.055	0.0025	0.032
IL19_Homo_sapiens	IL20RB_Homo_sapiens	1159.380	0.060	0.0019	0.043
IL22_Monodelphis_domestica	IL20RB_Monodelphis_domestica	1504.044	0.076	0.0024	0.042
IFNL_Lepisosteus_oculatus	IL10RB_Lepisosteus_oculatus	466.077	0.085	0.0008	0.053
IL26_Homo_sapiens	IL10RB_Homo_sapiens	549.354	0.111	0.0009	0.080
IFNL2_Xenopus_tropicalis	IL10RB_Xenopus_tropicalis	1420.982	0.126	0.0023	0.088
IFNL1_Xenopus_tropicalis	IL10RB_Xenopus_tropicalis	1366.711	0.133	0.0022	0.091
IFNL_Pelodiscus_sinensis	IL10RB_Pelodiscus_sinensis	1289.894	0.140	0.0021	0.101
IL20_Pteropus_vampyrus	IL22RA1_Pteropus_vampyrus	529.853	0.153	0.0009	0.103
IFNL_Oncorhynchus_mykiss	IL10RB_Oncorhynchus_mykiss	1096.896	0.154	0.0018	0.107
IFNL5_Xenopus_tropicalis	IL10RB_Xenopus_tropicalis	1255.547	0.159	0.0020	0.111
IL20_Ornithorhynchus_anatinus	IL22RA1_Ornithorhynchus_anatinus	506.120	0.161	0.0008	0.104
IFNL3_Xenopus_tropicalis	IL10RB_Xenopus_tropicalis	1260.959	0.164	0.0020	0.111
IFNL3_Pteropus_vampyrus	IL10RB_Pteropus_vampyrus	1249.824	0.172	0.0020	0.137
IL26_Pelodiscus_sinensis	IL10RB_Pelodiscus_sinensis	1040.549	0.179	0.0017	0.136
IFNL3_Ornithorhynchus_anatinus	IL10RB_Ornithorhynchus_anatinus	1056.413	0.183	0.0017	0.135
IFNL2_Mus_musculus	IL10RB_Mus_musculus	702.599	0.205	0.0011	0.153
IL26_Ornithorhynchus_anatinus	IL10RB_Ornithorhynchus_anatinus	942.828	0.216	0.0015	0.165
IL22_Mus_musculus	IL20RB_Mus_musculus	696.606	0.225	0.0011	0.172
IFNL3_Mus_musculus	IL10RB_Mus_musculus	596.481	0.226	0.0010	0.158
IL22_Monodelphis_domestica	IL22RA1_Monodelphis_domestica	455.606	0.227	0.0007	0.186
IL19_Pteropus_vampyrus	IL20RB_Pteropus_vampyrus	392.874	0.230	0.0006	0.169
IL19_Ornithorhynchus_anatinus	IL20RB_Ornithorhynchus_anatinus	432.287	0.274	0.0007	0.214
IL10_Mus_musculus	IL10RB_Mus_musculus	417.061	0.313	0.0007	0.256
IL24_Homo_sapiens	IL20RA_Homo_sapiens	473.270	0.317	0.0008	0.268
IL10_Ornithorhynchus_anatinus	IL10RB_Ornithorhynchus_anatinus	591.518	0.325	0.0010	0.278
IL10_Oncorhynchus_mykiss	IL10RB_Oncorhynchus_mykiss	542.488	0.332	0.0009	0.284
IL20_Alligator_mississippiensis	IL22RA1_Alligator_mississippiensis	258.092	0.339	0.0004	0.294
IL10_Mus_musculus	IL10RA_Mus_musculus	252.945	0.356	0.0004	0.305
IL10_Ornithorhynchus_anatinus	IL10RA_Ornithorhynchus_anatinus	246.118	0.382	0.0004	0.342
IFNL_Alligator_mississippiensis	IFNLR1_Alligator_mississippiensis	373.617	0.393	0.0006	0.336
IL10_Oncorhynchus_mykiss	IL10RA_Oncorhynchus_mykiss	280.035	0.403	0.0005	0.351
IL22_Mus_musculus	IL22BP_Mus_musculus	197.626	0.467	0.0003	0.434
IL19_Homo_sapiens	IL20RA_Homo_sapiens	161.324	0.493	0.0003	0.471
IL22_Mus_musculus	IL22RA1_Mus_musculus	136.135	0.515	0.0002	0.488
IL10_Xenopus_tropicalis	IL10RB_Xenopus_tropicalis	80.080	0.527	0.0001	0.517
IL22_Xenopus_tropicalis	IL22RA1_Xenopus_tropicalis	104.270	0.585	0.0002	0.554
IL20_Alligator_mississippiensis	IL20RA_Alligator_mississippiensis	−19.438	0.621	0.0000	0.623
IL10_Pelodiscus_sinensis	IL10RB_Pelodiscus_sinensis	−70.453	0.634	−0.0001	0.645
IL10_Pteropus_vampyrus	IL10RB_Pteropus_vampyrus	−94.933	0.642	−0.0002	0.647
IL22_Pteropus_vampyrus	IL20RB_Pteropus_vampyrus	−157.970	0.733	−0.0003	0.770

IL22_Pteropus_vampyrus	IL22BP_Pteropus_vampyrus	−190.456	0.755	−0.0003	0.787
IL22_Pelodiscus_sinensis	IL20RB_Pelodiscus_sinensis	−102.550	0.757	−0.0002	0.772
IL22_Pelodiscus_sinensis	IL22BP_Pelodiscus_sinensis	−192.513	0.764	−0.0003	0.782
IL26_Pelodiscus_sinensis	IL20RB_Pelodiscus_sinensis	−148.713	0.766	−0.0002	0.792
IL26_Ornithorhynchus_anatinus	IL20RB_Ornithorhynchus_anatinus	−226.675	0.800	−0.0004	0.824
IL22_Ornithorhynchus_anatinus	IL20RB_Ornithorhynchus_anatinus	−273.640	0.801	−0.0004	0.838
IL24_Pteropus_vampyrus	IL20RB_Pteropus_vampyrus	−201.312	0.801	−0.0003	0.829
IL22_Tetraodon_nigroviridis	IL20RB_Tetraodon_nigroviridis	−276.476	0.829	−0.0004	0.852
IL22_Ornithorhynchus_anatinus	IL22BP_Ornithorhynchus_anatinus	−334.534	0.847	−0.0005	0.876
IL22_Oncorhynchus_mykiss	IL20RB_Oncorhynchus_mykiss	−343.832	0.875	−0.0006	0.906
IL22_Ornithorhynchus_anatinus	IL22RA1_Ornithorhynchus_anatinus	−318.500	0.923	−0.0005	0.952
IL20_Callorhinchus_milii	IL20RA_Callorhinchus_milii	−965.059	0.932	−0.0016	0.958
IL10_Xenopus_tropicalis	IL10RA_Xenopus_tropicalis	−704.147	0.937	−0.0011	0.961
IL22_Pteropus_vampyrus	IL22RA1_Pteropus_vampyrus	−390.437	0.942	−0.0006	0.961
IL20_Gallus_gallus	IL20RA_Gallus_gallus	−1040.961	0.946	−0.0017	0.964
IL20_Lepisosteus_oculatus	IL20RA_Lepisosteus_oculatus	−263.114	0.948	−0.0004	0.963
IL10_Pteropus_vampyrus	IL10RA_Pteropus_vampyrus	−676.058	0.949	−0.0011	0.969
IL10_Tetraodon_nigroviridis	IL10RA_Tetraodon_nigroviridis	−713.393	0.954	−0.0012	0.971
IL10_Pelodiscus_sinensis	IL10RA_Pelodiscus_sinensis	−641.371	0.963	−0.0010	0.972
IL24_Homo_sapiens	IL20RB_Homo_sapiens	−1605.080	0.984	−0.0026	0.992
IL20_Homo_sapiens	IL20RA_Homo_sapiens	−1472.816	0.986	−0.0024	0.994
IL24_Mus_musculus	IL20RB_Mus_musculus	−1919.756	0.987	−0.0031	0.998
IL26_Homo_sapiens	IL20RB_Homo_sapiens	−1623.298	0.988	−0.0026	0.998
IL26_Monodelphis_domestica	IL20RB_Monodelphis_domestica	−2005.572	0.990	−0.0033	0.995
IL24_Homo_sapiens	IL22RA1_Homo_sapiens	−1936.916	0.992	−0.0031	0.999
IL24_Monodelphis_domestica	IL20RB_Monodelphis_domestica	−1930.833	0.992	−0.0031	0.997
IL24_Mus_musculus	IL20RA_Mus_musculus	−2215.830	0.992	−0.0036	0.997
IL24_Monodelphis_domestica	IL20RA_Monodelphis_domestica	−1653.180	0.993	−0.0027	0.998
IL24_Mus_musculus	IL22RA1_Mus_musculus	−1356.801	0.994	−0.0022	0.996
IL24_Pteropus_vampyrus	IL20RA_Pteropus_vampyrus	−2159.248	0.996	−0.0035	0.999
IL26_Gallus_gallus	IL20RB_Gallus_gallus	−2470.412	0.997	−0.0040	0.999
IL22_Tetraodon_nigroviridis	IL20RA_Tetraodon_nigroviridis	−2595.061	1.000	−0.0042	1.000

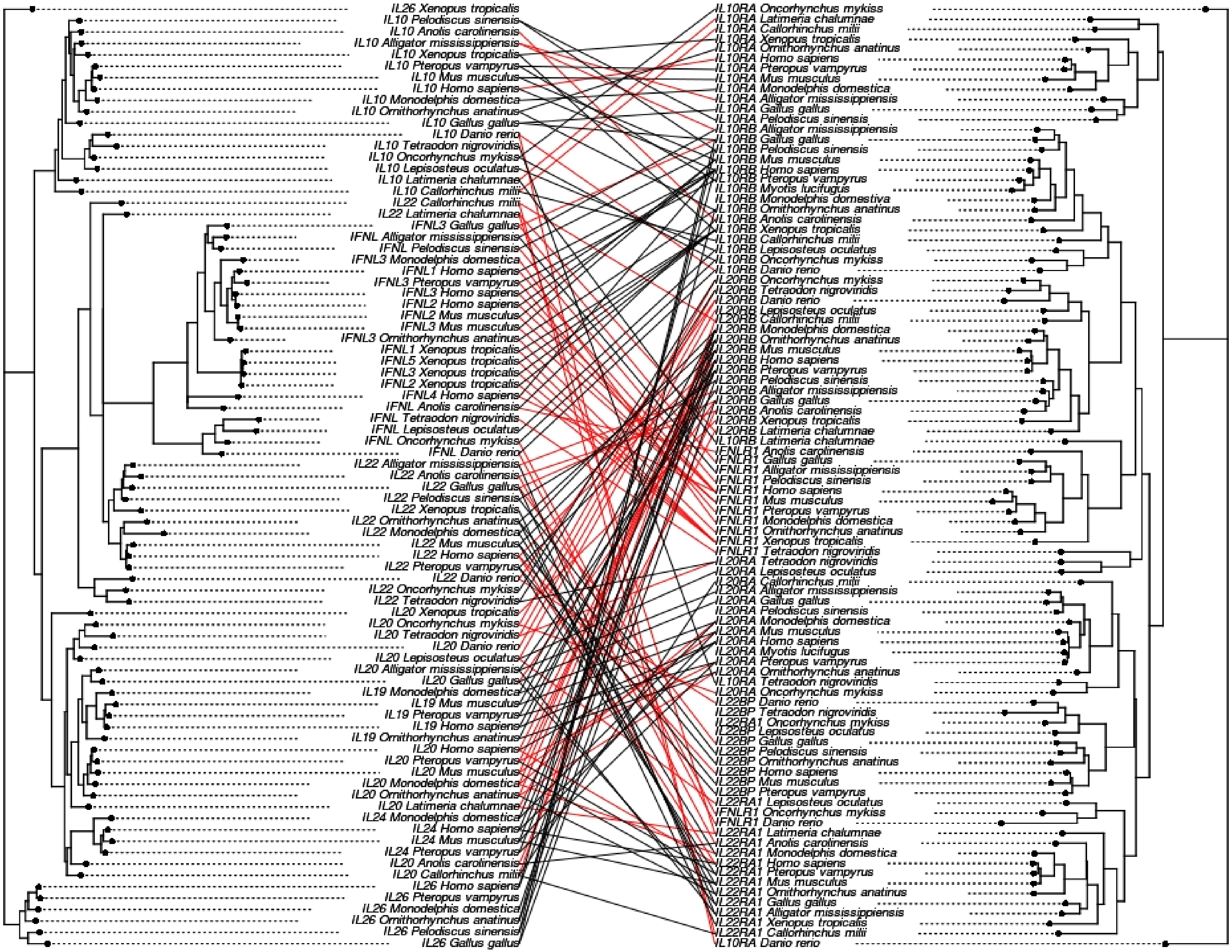

Figure 10. Graphical representation of coevolutionary relations and Parafit analysis. Red lines represent links between proteins with significant coevolution, black lines represent interactions with no significant coevolution.

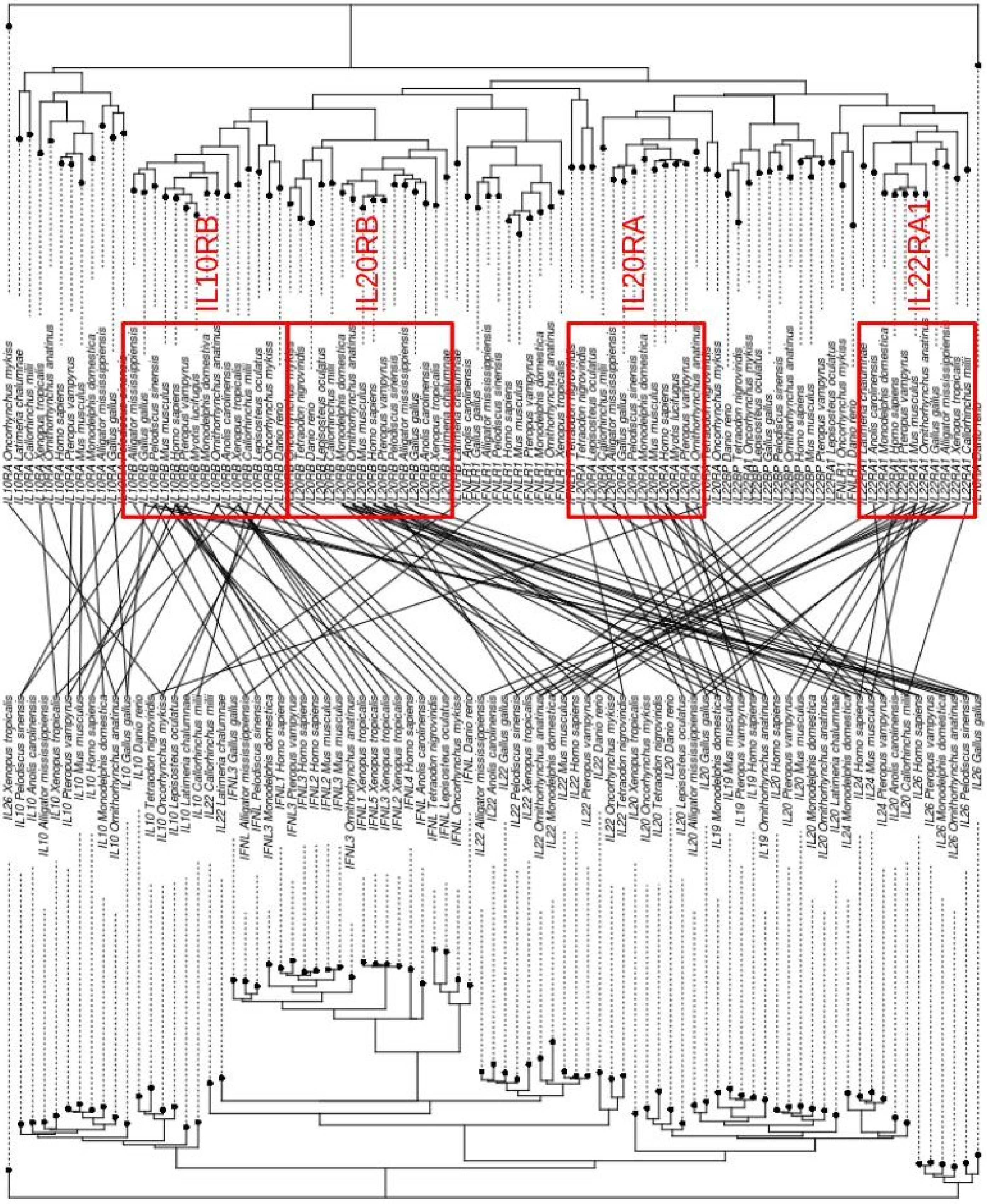

Figure 11. Proteins with consistently no significant signs of coevolution are highlighted by red frames. Black lines connect interactions without significant coevolution. Coevolution with its interaction partners has not been confirmed in IL10RB, IL20RB, IL20RA and IL22RA1.

4.3.3 i-COMS

Interleukin 10 – Interleukin 10 receptor β

IL-10 and IL10RB pair was tested for amino acid residue coevolution by 3 methods, MI, mfDCA and plmDCA (Figure 12). Total of 74691 links was tested based on 15 protein pairs with sequences aligned by MSA. The intersection of the results of the 3 methods included 12 interactions. Based on comparison of the distributions of the covariation scores (Figure 13) with inconsistent distribution of mfDCA, MI and plmDCA were further analysed in another intersection (20 interactions, Figure 12). For interleukins, the intersections show distribution of the coevolving amino acids along the whole sequence. In contrast, the coevolving amino acids are more clustered in the receptors. This conclusion is better distinguishable by the intersection of all methods. In addition to the cluster between amino acid residues L79 and T111 (R90, S98, W100), coevolution is significant for A17, N33 and Y149.

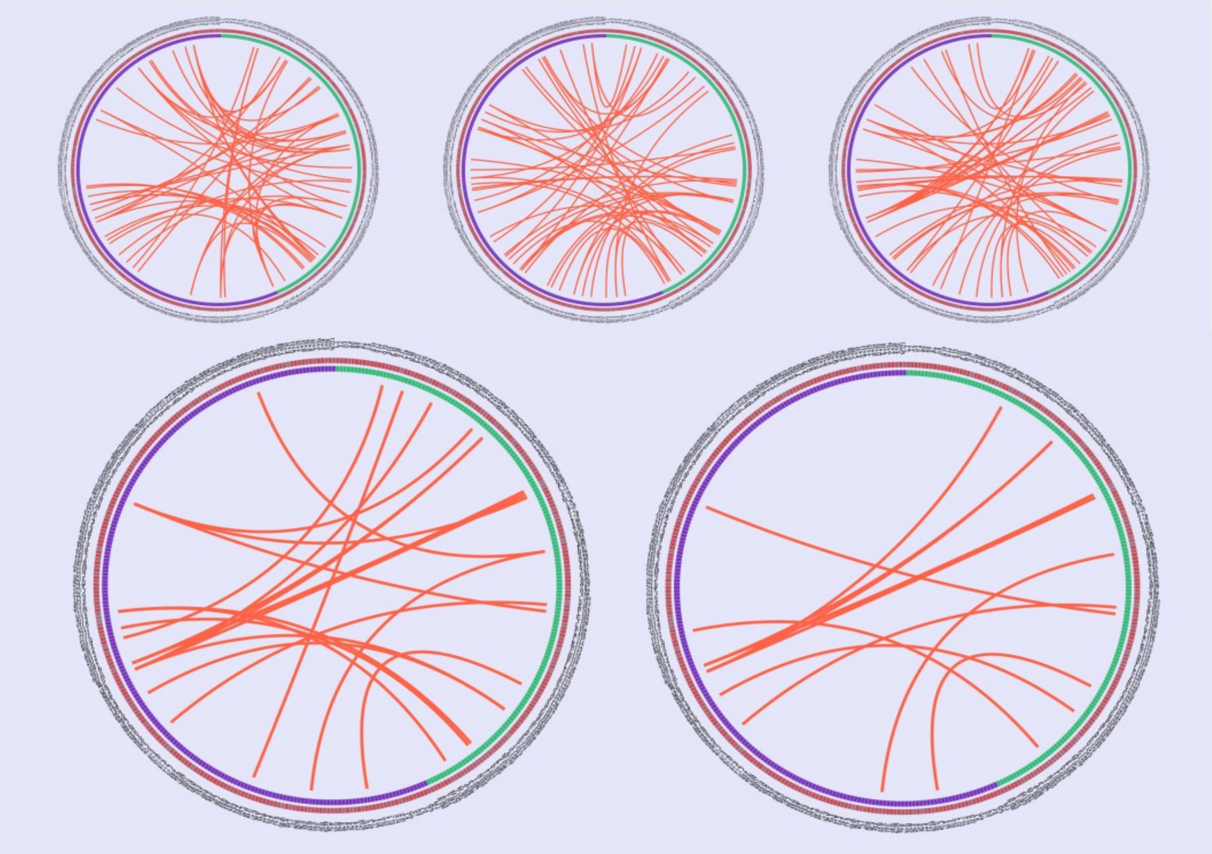

Figure 12. Circos visualisation of amino acid residue coevolution of IL-10 and IL10RB. Top circos represent (from the left) MI, mfDCA and plmDCA results with the 50 best scoring interactions. Bottom left shows the intersection of 100 best scoring interactions of MI and plmDCA (20 interactions), bottom right the intersection of of all the used methods (12 interactions). The interleukin is marked by green, receptor extracellular domain by purple, with clockwise direction of the reference sequence (from human, P22301 and Q08334).

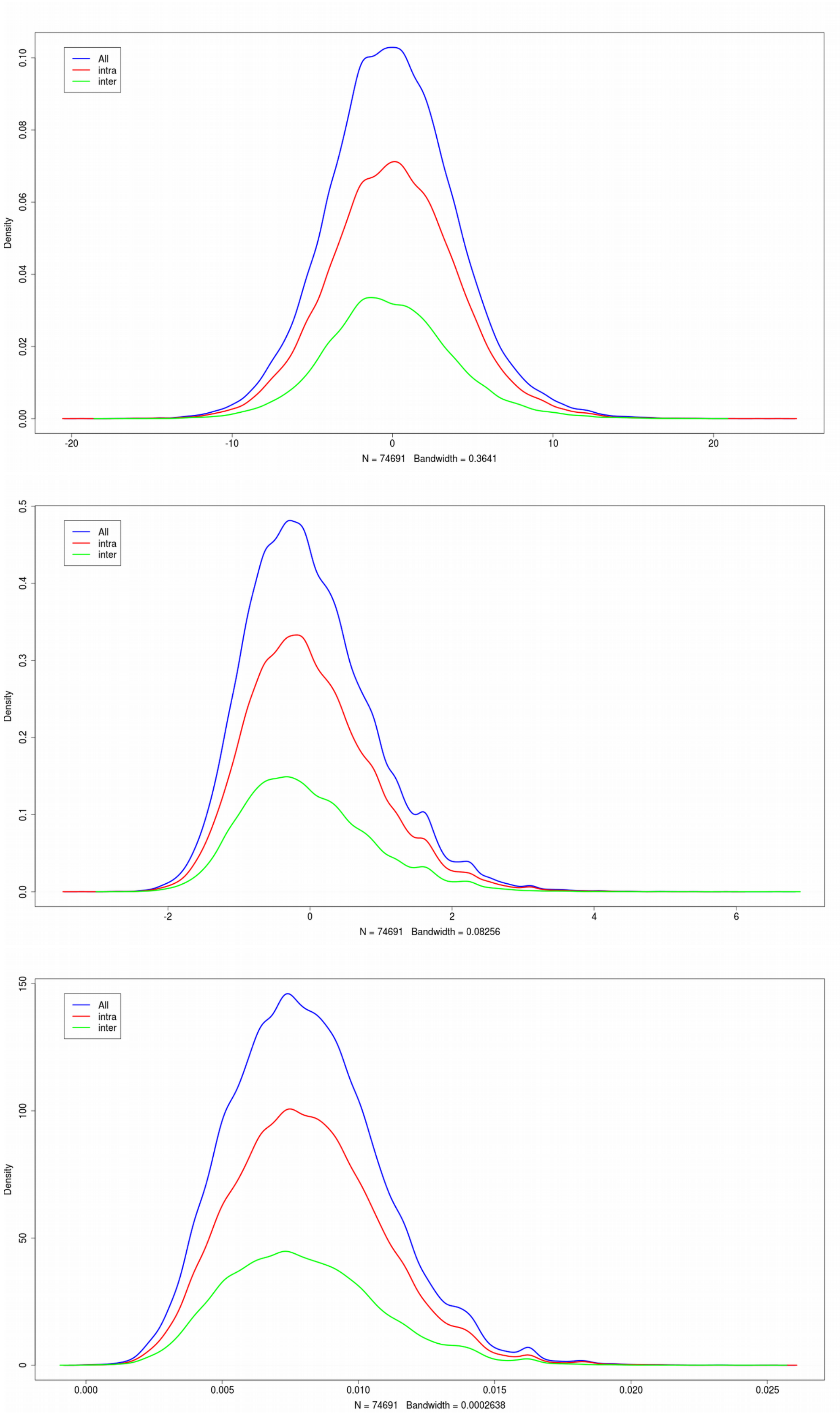

Figure 13. Distributions of the covariation scores between IL−10 and IL10RB by the used methods. MI (top), mfDCA (middle) and plmDCA (bottom).Blue lines represent all the interactions, red lines represent intra−protein relations, green lines inter−protein relations.

Interleukin 20 – Interleukin 20 receptor β

IL-20 and IL20RB was the second pair selected for the analysis of coevolution by the 3 methods as in the previous case, MI, mfDCA and plmDCA (Figure 14). Total of 82215 links was tested based on 14 protein pairs in the MSA. Intersect of all the methods was analysed and based on asymmetry of the distribution of the covariation scores of mfDCA (Figure 15), another intersection of only plmDCA and MI was analysed (Figure 14). The intersection of MI and plmDCA shows distribution of the coevolving amino acids in interleukin sequence in the range from C80 to W171. Receptor coevolving residues are clustered between M48 (H50 L52 W54) and P56. The cluster of coevolving residues is followed by close Y66, S67, Q71 and W83 with signs of coevolution and quite remote A115. A single coevolving interaction was confirmed by all the methods – A157 of interleukins with Y66 of the receptor domain.

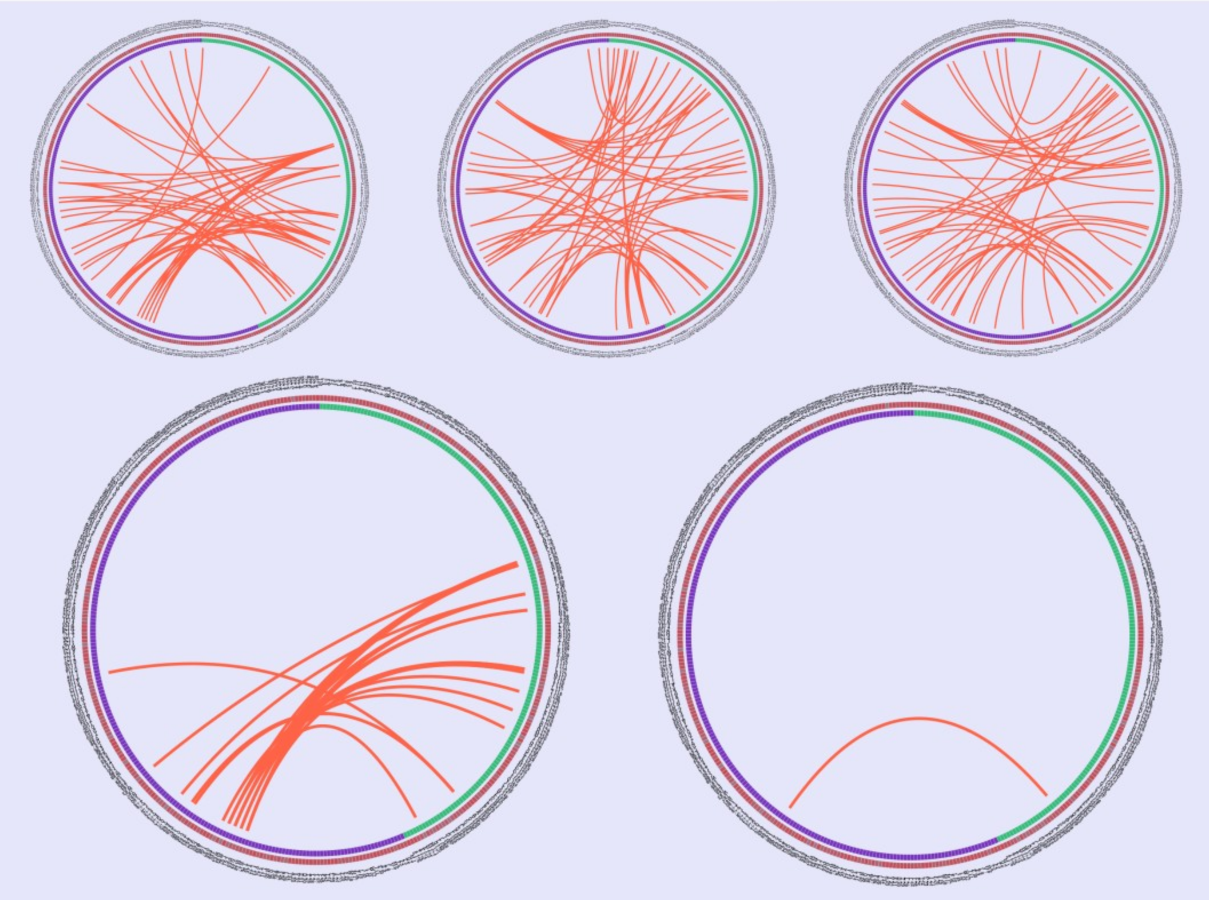

Figure 14. Circos visualisation of amino acid residue coevolution of IL-20 and IL20RB. Top circos represent (from the left) MI, mfDCA and plmDCA results with the best scoring 50 interactions visualised. Bottom left represents intersect of 100 best scoring interactions of MI and plmDCA (21 interactions), bottom right the intersect of of all the used methods (1 interaction). The interleukin is marked by green, receptor extracellular domain by purple, with clockwise direction of the reference sequence (from human,Q9NYY1 and Q6UXL0).

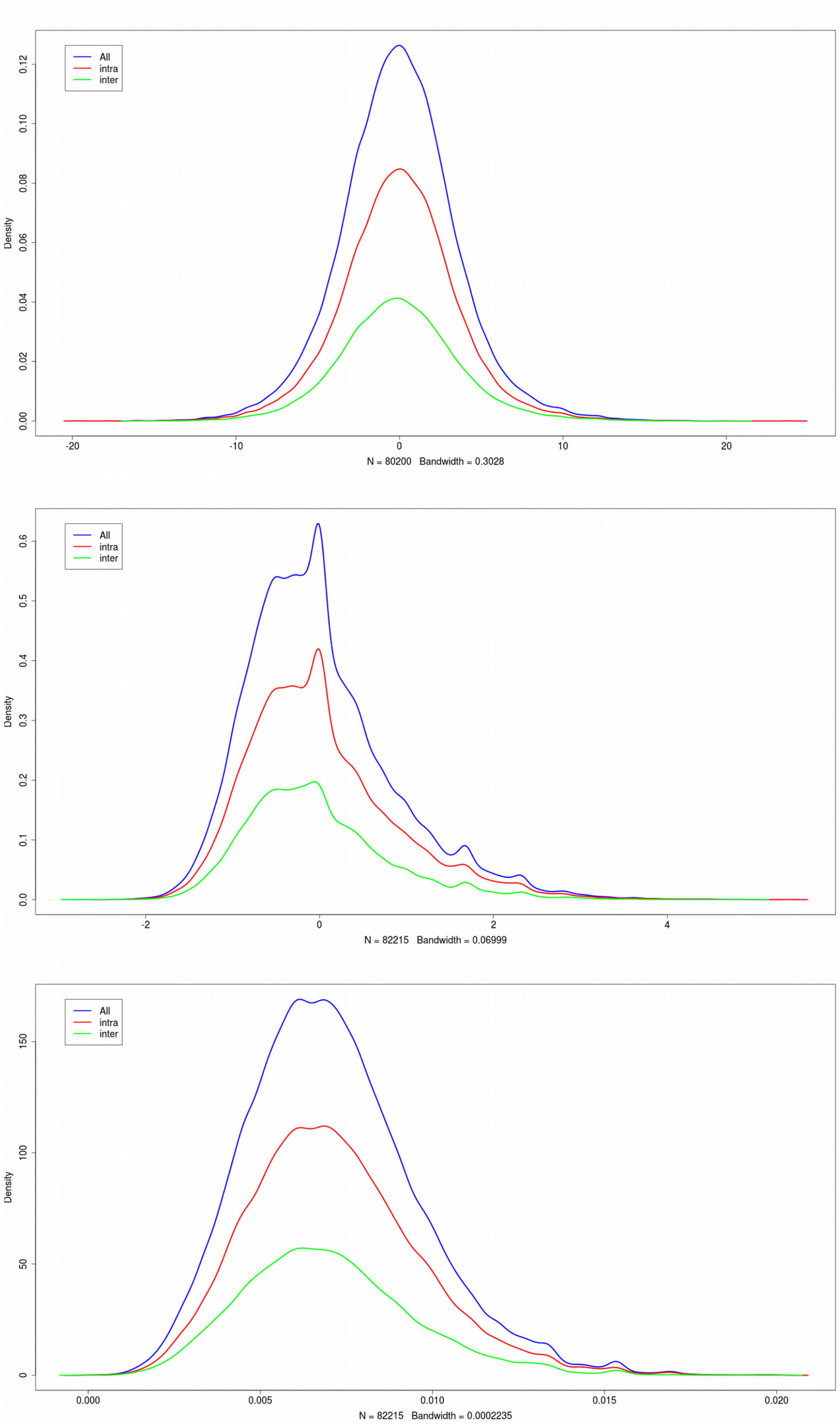

Figure 15. Distributions of the covariation scores between IL-20 and IL20RB by the used methods. MI (top), mfDCA (middle) and plmDCA (bottom).Blue lines represent all the interactions, red lines represent intra-protein relations, green lines inter-protein relations.

5 Discussion

Interleukin 10 family of cytokines represents an important group of signalling proteins causing divergent immune response. Phylogenetic relations of the proteins with different, in some cases even opposite effects is not yet well described, similarly to FIL-10 evolutionary relation to IFNs with mainly antiviral activity. FIL-10 with included IFNλs, as analysed in this study, uses combinations of shared receptor molecules causing a wide range of immune responses. Coevolution in sense of one to one substitution or convergent evolution of interleukins and receptors is detectable in evolving genome and subsequently in proteins and is analysable by statistical methods. This study confirms coevolution of some, but not all protein pairs with experimentally confirmed intermolecular interactions.

5.1 Presence of FIL-10 proteins in taxonomic groups

Insight into emergence of new interleukins from FIL-10 in evolution and correlation of evolution of interleukin-receptor pairs is provided by Krause and Pestka (2005) who assume that IL-10 and interferons and some other members of the family as well as their receptors diverged before formation of vertebrates. The closest homologue of the FIL-10 proteins and their receptors found in primitive species (*Petromyzon marinus* and *Ciona intestinalis*) is IL-17 orthologue and its receptors (information from NCBI database). Therefore, we assume, that the variety of interleukins diverged in early vertebrate evolution.

In *Callorhinchus milii,* IL-10, IL-20, IL-22, and the corresponding receptors, IL10RA, IL10RB, IL20RA and IL22RA1 were detected. The subfamily of IL-19, IL-20 and IL-24 with function in skin and epithelia (Wang et al. 2012; Sun et al. 2013) diverged from IL-20 in mammals, where skin structure is different from evolutionary older skin types.

5.2 Comparison of maximum likelihood and Bayesian inference

Evolution of sequences may be analysed by several approaches. I analysed proteins by maximum-likelihood approach and Bayesian approach. As the dataset consists of a fairly high number of proteins of divergent species, length and variability of analysable regions is crucial for the successful definition of the relations among the family members.

ML and Bayesian trees both provide node support, in case of ML phylogeny, a bootstrap value, in case of Bayesian phylogeny, a posterior probability value. Both values in the trees quite notably differ. The difference of the nodal support of the methods may be caused by mechanism of inference of phylogeny, as well as different requirements for the data diversity. The maximum likelihood analysis creates several phylogenies, where the best scoring ML tree is found and afterwards used to map bootstrap values onto it (Stamatakis 2014). The best scoring ML tree may not describe the biological evolution exactly, since it is highly dependent on selection of a model describing amino acid evolution appropriate for the given data, shape parameter of the distribution and evaluation of parameters in ML search for the best scoring tree. In the Bayesian approach, several trees from predefined random sampling from the posterior distribution of the trees are used to create a consensus tree (Ronquist and Huelsenbeck 2003). The consensus tree, created by sampling of a number of trees is less likely to propose a phylogeny that is not well describing the evolution of the protein family, while second or third to the best scoring ML phylogeny may propose better result of tree phylogeny with subsequently higher bootstrap values.

The difference of the two approaches was compared visually. Differences in the topology are clear, but the difference was also quantified as the difference of number of internal nodes and as the difference with a correction as described in Chapter 4.1.3. The differences in the topologies were higher in the trees of interleukins, than in receptors. The difference in topologies may be explained by a higher variability or length of analysable region in one of amino acid residues in one of the analysed alignments or the mechanism of inference of the phylogenies.

The topologies of the trees are fairly similar. For further study, I used ML trees, since it offers more critical view on the node support by its wider range of bootstrap values.

5.3 Evolutionary history of interleukin 10 family cytokines and their receptors

In the resulting trees, we can observe grouping of functionally similar proteins. IL-19 and IL-20 are shown to be very closely related, with IL-19 diverged from IL-20 very late in evolution. IL-19 is currently, according to the information in sequence databases, known to be expressed only in mammalian species, and the resulting phylogeny supports the divergence from mammalian IL-20. The branch of the tree with IL-19 and IL-20 also contains IL-24 expressed in mammals, closely related with its function in skin and epithelia.

IL22BP is a soluble protein, that binds IL-22 and competitively inhibits activation of JaK/STAT pathway by blocking interaction of IL-22 with IL22RA1 and IL10RB (Kotenko et al. 2001). Unlike (Langer et al. 2004) who describe closer relation of IL22BP to IL10RA1, in this study, IL22BP diverged from subgroup of IL20RA and IL22RA1, which supports hypothesis, that IL22BP, an IL-22 antagonist, has evolved from IL22RA1, which is used by IL-22 for signalling, by loss or mutation in the membrane domain.

The resulting phylogenies describe relations within the groups of interleukins in one phylogeny and receptors in the second, however due to unique combinations of the shared receptor subunits, coevolution of interleukins and their receptors is not obvious from the phylogenies at the first sight. Therefore, coevolution between the ligands and receptors needs to be analysed from the phylogenies using numerical methods.

5.4 Coevolution of interleukins and their receptors

Coevolutionary relations are a well known term in pairs of parasites and hosts, where coevolution is often mediated by positive selection pressures (Anderson and May 1982), but coevolutionary relations are also present in inter-protein interactions of various types in one organism. In the coevolution of ligand-receptor pairs, purifying selection is expected, together with interrelated changes in amino acid structure in active sites (Fraser et al. 2002), since preservation of active amino acid sites is necessary to maintain the signalling function. Changes in evolutionary relations between functionally connected proteins are detectable at the genetic level and subsequently the encoded protein structure. Coevolution was analysed using method originally developed for comparison of evolution in parasites and hosts, since the analytic method implements presumptions of the evolution applicable likewise to ligand-receptor coevolution (Legendre et al. 2002).

Previous research on IL-10 family evolution and evolution of receptors of the family showed possible relations among the interleukins of the family, however, methods of analysis are not well specified (Kotenko and Langer 2004) or structure of the used data provides results with information insufficient to identify the relations and the common ancestor (Lutfalla et al. 2003) and relation of receptors and proteins is assumed, but not tested. Relation of type I and type II receptors and their ligands has been described on structural level in other groups of interleukins (LaPorte et al. 2008), however information about FIL-10 coevolutionary relations and structural basis of the interaction is still lacking.

In this study, correlated evolution of interleukins of FIL-10 was detected in some tested interactions, however, several inter-protein interactions were statistically insignificant. The most versatile receptor in the family - IL10RB - is shared by IL-10, IL-22, IL-26 and IFNλ of all subtypes. However, in case of interaction with IL-22 and IL-26, no significant coevolution with IL10RB was detected. Both IL-22 and IL-26 are crucial for antiviral and antimicrobial response of the organism (Meller et al. 2015; Mulcahy et al. 2016), unlike the other members of the family involved mainly in skin and epithelial immune reactions. IL-26 signals through a shared subunit of IL10RB and subunit of IL20RA used by fewer closely related interleukins for signalling (IL−19, IL−20 and IL−24 subfamily and IL−26), therefore evolution of IL−26 is likely more affected by IL20RA than IL10RB.

IL−22 uses IL22RA1 and IL−10RB as receptors, however shows significant coevolution with IL22RA1 only in evolutionary older species. In the tested mammal interactions (4 out of 5) the coevolution was not detected. Mammals are the youngest group in the tested dataset, however promiscuity of receptors described in modern human is observed first even before divergence of bony fish (in cartilaginous fish). Thus, correlation of evolution of the signalling pairs should be strong in ancestral sequence. Nevertheless, despite the early divergence of receptors, certain level of similarity needs to be maintained by ongoing processes of negative selection in the interleukins and their receptors, rather than by their coevolution, to maintain the protein function.

IL10RB is coevolving with most orthologues of IL−10 and IFNλs. Evolution of proteins with interactions with more proteins are generally slower than those of proteins with unique interaction partners (Fraser et al. 2002). Therefore, the promiscuous receptors, such as IL10RB or IL20RB, and subsequently the coevolving interleukins are generally expected to be more conserved than proteins with more unique signalling subunits.

The interleukins mentioned above − IL−10 and IFNλs − use type 1 receptor, which is unshared with other interleukins (IL10RA for IL−10) or shared by functionally very close interleukins (IFNLR1 for IFNλ subfamily), therefore with fewer interactions, the interleukins have better potential to adapt to the shared subunit IL10RB, than the interleukins using both shared subunits, such as IL−19, IL−20, IL−22, IL−24 and IL−26. However, IL20RB is from all its ligands in coevolution mainly with IL−20 which signals through both type 1 and type 2 shared subunits. This may show that there is an evolutionary pressure on the receptors of both type 1 and 2 to evolve while preserving the function of the ancestral interleukin.

Coevolution on amino acid level was studied in the pairs of IL−10 and IL10RB and IL−20 and IL20RB, since based on the previous analysis, strongest coevolutionary relations between the shared type 2 receptors IL10RB and IL20RB and IL−10 and IL−20 respectively are expected.

The analysis was based on several approaches, however the mfDCA method (Kaján et al. 2014) showed asymmetries in the distribution of obtained covariance values, mainly in the analysis of IL−20 – IL20RB coevolution, therefore the results excluding the mfDCA method were also analysed.

All the residues of IL20RB coevolving with IL−20 are located in the first fibronectin type III domain (structural information from the UniProt database, 2017). The same pattern is present in IL10RB residues coevolving with IL−10, where most of them are also present in the first fibronectin type III domain (62 % of interactions confirmed by MI and plmDCA, 75 % of the interactions confirmed by all the used methods), although the coevolving residue clustering is not obvious on the first sight (Figure 16).

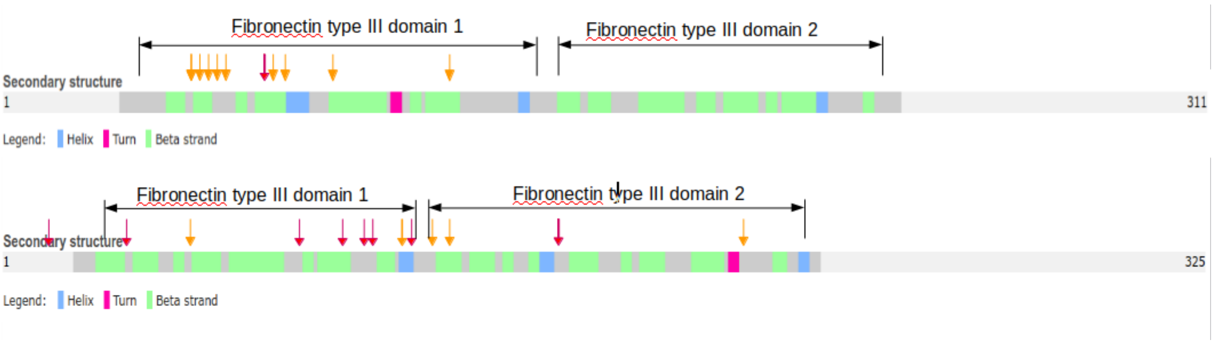

Figure 16. Scheme of the position of the coevolving residues in IL10RB (top) and IL20RB. The secondary structure (UniProt Q08334 and Q6UXL0 respectively) is marked with green for beta strands, pink for turns, blue for helices. The orange darts mark sites with coevolution by the intersection of plmDCA and MI methods, the red darts show amino acid residues confirmed to be coevolving also by mfDCA. Blue strips mark fibronectin type III domains in the receptor.

The type 2 receptors are likely coevolving mainly with the ancestral interleukin by reciprocal mutation in the first fibronectin type III domain and interleukin residues pointing out an important coevolutionary role of the first fibronectin type III domain of type 2 cytokine receptors.

6 Conclusions

This study investigates evolutionary history of an important group of immune regulators, interleukin 10 family proteins, and its relation to evolutionary history of their receptors, using statistical methods and data available at public databases. The evolutionary relations of the FIL-10 and its related proteins from groups of interferons are not yet well described, although the relations are presumed. The presented results help to understand the relation of the cytokines and receptors within the groups.

FIL-10 cytokine and receptor orthologues were found in as evolutionary old species as cartilaginous fish. Despite the presumption of divergence of FIL-10 proteins before divergence of vertebrates, no representatives of the family were found in primitive chordate species, therefore the divergence of the family is likely dated to early vertebrates.

The relations between sequences within the interleukin family show distinguishing of IL-19, IL-20 and IL-24 subfamily. The receptor phylogeny shows relation of an antagonist IL22BP to membrane bound functional receptor IL22RA1 and subgrouping of the related interleukin receptors.

Evolution of interleukin-receptor pairs is in 54,82 % of the tested cases correlated, with a few important exceptions: coevolution of IL10RB, the receptor used by several FIL-10 cytokines, was not observed with all its binding partners it is supposed to recognize, most notably IL-26. Also a shared receptor subunit IL20RB is in significant coevolution with one of its ligands IL-20, but does not show significant signs of coevolution with most of the other ligands, IL-26, IL-19 and IL-24 orthologues. Coevolutionary interactions between IL-10 and IL10RB and IL-20 and IL20RB are localised in the first fibronectin type III domain of the receptor.

The observed relations between interleukins in the group may also provide a guide for search for interleukins of FIL-10 in taxonomic groups, especially in fish taxa, where similarity to the protein orthologues is limited in several cases. The results also provide a basis for comparative study of evolutionary relationships by comparing three dimensional structures between fish, bird and mammal species.

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7.1 Online resources

FigTree software available from http://tree.bio.ed.ac.uk/

i-COMS webserver available at http://i-coms.leloir.org.ar/

MirrorTree webserver available at http://csbg.cnb.csic.es/mtserver/.

NCBI BLAST available at https://blast.ncbi.nlm.nih.gov/Blast.cgi

NCBI database at https://www.ncbi.nlm.nih.gov/protein/

Phylemon 2 webserver at http://phylemon2.bioinfo.cipf.es/

Protein Data Bank available at http://www.rcsb.org/pdb

UCSC Genome browser at https://genome-euro.ucsc.edu/

UniPROT database at http://www.uniprot.org/

Supplementary material

Supplement 1. Interleukin sequences from databases. Table mentions binomial species name, protein name as stated in the database, database from which the sequence has been obtained and sequence UID.

Callorhinchus milii	interleukin-10	NCBI	632963185
Callorhinchus milii	interleukin-20	NCBI	632963320
Callorhinchus milii	interleukin-22	NCBI	632973502
Lepisosteus oculatus	interferon-lambda	NCBI	973187678
Lepisosteus oculatus	interleukin-10	NCBI	573880859
Lepisosteus oculatus	Interleukin-20	NCBI	972961186
Danio rerio	interferon-lambda	UniProt	A0FJI5
Danio rerio	Interleukin 10	NCBI	190337256
Danio rerio	interleukin 22	NCBI	66472836
Danio rerio	interleukin-20	NCBI	130508100
Oncorhynchus mykiss	interferon-lambda	NCBI	209972108
Oncorhynchus mykiss	Interleukin 20	NCBI	311771762
Oncorhynchus mykiss	interleukin 22	NCBI	242098052
Oncorhynchus mykiss	interleukin-10	NCBI	47678893
Tetraodon nigroviridis	interleukin 10	NCBI	29125864
Tetraodon nigroviridis	interleukin-20	NCBI	31747223
Tetraodon nigroviridis	Interleukin-22	NCBI	31747227
Latimeria chalumnae	interleukin 22	UniProt	H3A664
Latimeria chalumnae	interleukin-10	NCBI	556991959
Latimeria chalumnae	Interleukin-20	UniProt	H3A3C0
Xenopus tropicalis	interferon lambda1	NCBI	256860232
Xenopus tropicalis	interferon lambda2	NCBI	256860234
Xenopus tropicalis	interferon lambda3	NCBI	847153596
Xenopus tropicalis	interferon lambda5	NCBI	256860238
Xenopus tropicalis	interleukin-10	NCBI	284813591
Xenopus tropicalis	interleukin-20	NCBI	847096564
Xenopus tropicalis	interleukin-22	NCBI	213983241
Xenopus tropicalis	interleukin-26	NCBI	212549595
Anolis carolinensis	interferon lambda	NCBI	629633502
Anolis carolinensis	interleukin-10	NCBI	327278623
Anolis carolinensis	interleukin-22	NCBI	637331876
Pelodiscus sinensis	interferon lambda-3	NCBI	946664579
Pelodiscus sinensis	interleukin-10	NCBI	558226408
Pelodiscus sinensis	interleukin-22	NCBI	558155500
Pelodiscus sinensis	interleukin-26	NCBI	558155504

Alligator mississippiensis	Interferon lambda-3	UniProt	A0A151N9Y8
Alligator mississippiensis	interleukin-10	NCBI	1011565932
Alligator mississippiensis	interleukin-20	NCBI	1011565931
Alligator mississippiensis	interleukin-22	NCBI	1011613344
Gallus gallus	Interferon lambda-3	NCBI	184186388
Gallus gallus	interleukin-10	NCBI	51173888
Gallus gallus	interleukin-20	NCBI	118102427
Gallus gallus	interleukin-22	NCBI	571255083
Ornithorhynchus anatinus	interferon lambda-3	NCBI	345314447
Ornithorhynchus anatinus	interleukin-10	NCBI	620945898
Ornithorhynchus anatinus	interleukin-19	UniProt	F6SIJ2
Ornithorhynchus anatinus	interleukin-20	NCBI	345320613
Ornithorhynchus anatinus	interleukin-22	NCBI	149632275
Ornithorhynchus anatinus	interleukin-26	NCBI	149632273
Monodelphis domestica	interferon lambda-3	NCBI	612025825
Monodelphis domestica	interleukin-10	NCBI	334322284
Monodelphis domestica	interleukin-19	UniProt	F7CQL8
Monodelphis domestica	interleukin-20	UniProt	F6UG76
Monodelphis domestica	interleukin-22	NCBI	612049968
Monodelphis domestica	Interleukin-24	UniProt	F6XDH7
Monodelphis domestica	interleukin-26	NCBI	126339160
Mus musculus	Interferon lambda-2	UniProt	Q4VK74
Mus musculus	Interferon lambda-3	UniProt	Q8CGK6
Mus musculus	Interleukin-10	UniProt	P18893
Mus musculus	Interleukin-19	UniProt	Q8CJ70
Mus musculus	Interleukin-20	UniProt	Q9JKV9
Mus musculus	Interleukin-22	UniProt	Q9JJY9
Mus musculus	Interleukin-22b	UniProt	Q9JJY8
Mus musculus	Interleukin-24	UniProt	Q925S4
Pteropus vampyrus	interferon lambda-3	NCBI	759197444
Pteropus vampyrus	interleukin-10	NCBI	759192357
Pteropus vampyrus	interleukin-19	NCBI	759192345
Pteropus vampyrus	interleukin-20	NCBI	759192342
Pteropus vampyrus	interleukin-22	NCBI	759099166
Pteropus vampyrus	interleukin-24	NCBI	759192339
Pteropus vampyrus	interleukin-26	NCBI	759099163
Homo sapiens	Interferon lambda-1	UniProt	Q8IU54
Homo sapiens	Interferon lambda-2	UniProt	Q8IZJ0
Homo sapiens	Interferon lambda-3	UniProt	Q8IZI9

Homo sapiens	Interferon lambda-4	UniProt	K9M1U5
Homo sapiens	Interleukin-10	UniProt	P22301
Homo sapiens	Interleukin-19	UniProt	Q9UHD0
Homo sapiens	Interleukin-20	UniProt	Q9NYY1
Homo sapiens	Interleukin-22	UniProt	Q9GZX6
Homo sapiens	Interleukin-24	UniProt	Q13007
Homo sapiens	Interleukin-26	UniProt	Q9NPH9

Suppelement 2. Receptor sequences from databases. Table mentions binomial species name, protein name as stated in the database, database from which sequence has been obtained and sequence unique identifier (UID).

Callorhinchus milii	Interleukin-10 receptor alpha	UniProt	V9KUB8
Callorhinchus milii	Interleukin-10 receptor beta	UniProt	V9KWB3
Callorhinchus milii	Interleukin-20 receptor alpha	UniProt	V9LAR4
Callorhinchus milii	interleukin-20 receptor beta	NCBI	632934306
Callorhinchus milii	Interleukin-22 receptor alpha-1	UniProt	V9KQD1
Lepisosteus oculatus	Interleukin-10 receptor beta	NCBI	973190915
Lepisosteus oculatus	Interleukin-20 receptor alpha	UniProt	W5M9F1
Lepisosteus oculatus	interleukin-20 receptor beta	UniProt	W5MSL1
Lepisosteus oculatus	Interleukin-22 receptor alpha-1	UniProt	W5MEH8
Lepisosteus oculatus	interleukin-22 receptor alpha-2	UniProt	W5NEC6
Danio rerio	interferon lambda receptor 1	NCBI	308275358
Danio rerio	interleukin 10 receptor alpha	NCBI	117606403
Danio rerio	interleukin 10 receptor beta	NCBI	76563837
Danio rerio	interleukin-22 receptor alpha-2	NCBI	113674671
Danio rerio	interleukin-20 receptor beta	NCBI	300490528
Oncorhynchus mykiss	IL-22 binding protein	UniProt	K0J8Z9
Oncorhynchus mykiss	interferon lambda receptor 1	NCBI	642126604
Oncorhynchus mykiss	Interleukin-10 receptor alpha	NCBI	642084689
Oncorhynchus mykiss	Interleukin-10 receptor beta	NCBI	526252816
Oncorhynchus mykiss	interleukin-20 receptor alpha	NCBI	185133176
Oncorhynchus mykiss	interleukin-20 receptor beta	NCBI	642096911
Tetraodon nigroviridis	interferon lambda receptor 1	UniProt	H3C6M4
Tetraodon nigroviridis	Interleukin-10 receptor alpha	NCBI	28475293
Tetraodon nigroviridis	Interleukin-20 receptor alpha	UniProt	Q7ZT35
Tetraodon nigroviridis	interleukin-20 receptor beta	UniProt	H3CAT4
Tetraodon nigroviridis	interleukin-22 receptor alpha-2	UniProt	H3DHY6
Latimeria chalumnae	interleukin-10 receptor alpha	NCBI	942119549
Latimeria chalumnae	interleukin-10 receptor beta	NCBI	556973645
Latimeria chalumnae	interleukin-20 receptor beta	NCBI	942207957
Latimeria chalumnae	Interleukin-22 receptor alpha 1	NCBI	942123523

Xenopus tropicalis	Interferon lambda receptor 1	NCBI	284795282
Xenopus tropicalis	Interleukin-10 receptor alpha	UniProt	F7C5L9
Xenopus tropicalis	Interleukin-10 receptor beta	NCBI	284521656
Xenopus tropicalis	interleukin-20 receptor beta	NCBI	512866588
Xenopus tropicalis	interleukin-22 receptor alpha-1	NCBI	847101222
Anolis carolinensis	Interferon lambda receptor 1	UniProt	A0A0A0Q037
Anolis carolinensis	Interleukin-10 receptor beta	NCBI	629633506
Anolis carolinensis	interleukin-20 receptor beta	UniProt	H9GNK8
Anolis carolinensis	interleukin-22 receptor alpha-1	UniProt	H9GAE8
Pelodiscus sinensis	Interleukin-10 receptor alpha	UniProt	K7F248
Pelodiscus sinensis	Interleukin-10 receptor beta	UniProt	K7G2L8
Pelodiscus sinensis	Interleukin-20 receptor alpha	UniProt	K7GCV4
Pelodiscus sinensis	interleukin-20 receptor beta	UniProt	K7G359
Pelodiscus sinensis	interleukin-22 receptor alpha-2	UniProt	K7GCA0
Alligator mississippiensis	interferon lambda receptor 1	NCBI	1011571573
Alligator mississippiensis	Interleukin-10 receptor alpha	UniProt	A0A151NFH0
Alligator mississippiensis	Interleukin-10 receptor beta	UniProt	A0A151ME26
Alligator mississippiensis	Interleukin-20 receptor alpha	UniProt	A0A151M3P1
Alligator mississippiensis	interleukin-20 receptor beta	UniProt	A0A151LYK3
Alligator mississippiensis	interleukin-22 receptor alpha-1	UniProt	A0A151MML2
Gallus gallus	Interferon lambda receptor 1	UniProt	K9JA28
Gallus gallus	interleukin 10 receptor 1	NCBI	83999156
Gallus gallus	Interleukin-10 receptor beta	NCBI	84618077
Gallus gallus	Interleukin-20 receptor alpha	UniProt	F1NYV0
Gallus gallus	interleukin-20 receptor beta	UniProt	E1BW22
Gallus gallus	interleukin-22 receptor alpha-1	UniProt	E1BRV0
Gallus gallus	interleukin-22 receptor alpha-2	UniProt	F1NYV1
Ornithorhynchus anatinus	interferon lambda receptor 1	NCBI	620962825
Ornithorhynchus anatinus	Interleukin-10 receptor alpha	UniProt	F6SGX7
Ornithorhynchus anatinus	Interleukin-10 receptor beta	NCBI	620974654
Ornithorhynchus anatinus	Interleukin-20 receptor alpha	UniProt	F6VJN7
Ornithorhynchus anatinus	interleukin-20 receptor beta	NCBI	620955148
Ornithorhynchus anatinus	interleukin-22 receptor alpha-1	UniProt	F6UJT9
Ornithorhynchus anatinus	interleukin-22 receptor alpha-2	UniProt	F6VJR3
Monodelphis domestica	interferon lambda receptor 1	NCBI	612024252
Monodelphis domestica	Interleukin-10 receptor alpha	UniProt	F7BIG7
Monodelphis domestica	Interleukin-10 receptor beta	UniProt	F6V1X5
Monodelphis domestica	Interleukin-20 receptor alpha	UniProt	F6ZVC6
Monodelphis domestica	interleukin-20 receptor beta	UniProt	F7GD26

Monodelphis domestica	interleukin-22 receptor alpha-1	UniProt	F7G154
Mus musculus	Interferon lambda receptor 1	UniProt	Q8CGK5
Mus musculus	Interleukin-10 receptor alpha	UniProt	Q61727
Mus musculus	Interleukin-10 receptor beta	UniProt	Q61190
Mus musculus	Interleukin-20 receptor alpha	UniProt	Q6PHB0
Mus musculus	interleukin-20 receptor beta	UniProt	E9Q9A6
Mus musculus	interleukin-22 receptor alpha-1	UniProt	Q80XZ4
Mus musculus	interleukin-22 receptor alpha-2	UniProt	Q80XF5
Pteropus vampyrus	interferon lambda receptor 1	NCBI	759112709
Pteropus vampyrus	Interleukin-10 receptor alpha	NCBI	759193413
Pteropus vampyrus	Interleukin-10 receptor beta	NCBI	759136111
Pteropus vampyrus	Interleukin-20 receptor alpha	NCBI	759108563
Pteropus vampyrus	interleukin-20 receptor beta	NCBI	759158090
Pteropus vampyrus	interleukin-22 receptor alpha-1	NCBI	759112706
Pteropus vampyrus	interleukin-22 receptor alpha-2	NCBI	759108749
Homo sapiens	Interferon lambda receptor 1	UniProt	Q8IU57
Homo sapiens	Interleukin-10 receptor alpha	UniProt	Q13651
Homo sapiens	Interleukin-10 receptor beta	UniProt	Q08334
Homo sapiens	Interleukin-20 receptor beta	UniProt	Q6UXL0
Homo sapiens	Interleukin-20 receptor alpha	UniProt	Q9UHF4
Homo sapiens	interleukin-22 receptor alpha-1	UniProt	Q8N6P7
Homo sapiens	interleukin-22 receptor alpha-2	UniProt	Q969J5

Supplement 3. FGENESH+ predicted sequences

〉FGENESH： Anolis carolinensis | IL20
MAFGAFSCLVLVAFLFAKTVVAEGRRLSLGQCELNSVSFRELRDNFDAIKENVQTQDIRTDVILLKESVLREVPM
SESCCLLRHLLRFYVESIFKHYEPTSNLLRRKTSTLANAFLSIKAKLRECHNQNKCSCGEETNRRFKLVLDEYQK
LDKTTAAIKSLGEMDVLFAWMEGF

〉FGENESH： Gallus gallus | IL26
MKVYSIFRSGHLLVLLCLFTVEGKKSPTGKHTCRKGLLSQVTENLYTKASSLKSSVPKDLIKNTRLLKKTTKMLF
MTNCNVRDQLLSFYMKNVFSHLGMESEKLFVISAFRVLQENMNACLPCAPSTRLTSAVKNIKKTFLKVRVGGV
GSCFGGVGGFTSNTFIFLTAWGEGGLQGHQ

〉FGENESH： Pelodiscus sinensis | IFNLR1
MSAGSRAVLVALCSFQQLLGSVALGQPGVPLPPPRNVKLLSKDFGVAVTWLPGEGSPPDVLYSVRYQTLYHQSN
WKQVRHCKNISHVTCNLTCGPDPYNKFSTRVKALAAGRQSPWVESNSLEYHLDVHLAPPALAVSVAETTINVS
ATFPLASCVKSVFIGLKYDLDFWKAGTGDKVPFHDRMKWENVTISTLALSGNYCLSARASYQAIQLKHSQFSR
PLCMLLTPRAKGWEFLITMAVPLLILLFFCTAPGTVLEELIERDLFICVVQPASAGRWRSDASRTARNDTSLVARN
NASPVARNDASPVARNDTSPVARNDTSLTASLLSLSEEEDDDSGGRPYTEMPLFLRRAPNCSGASMSQEGSHSGS
ELSGSHLAGGPVPDLAGLGFSRLVWRGGPAEEDASGFPDSEKSSSFSESSSVGEFSLSEAPCPVTCGGERQGWEAD
TGQEDPFLQVSVLAEGLKGGSPAEEWGVPRRGPRKTDPQRHLHPDPSVCVARGVSEAADGFPLEEQLVRFQTV
KLALDEGVASDSESLAGGAERDPPPLSAALSETGGAEAWGKGGGLWPARDPAWQCRGYQHMRYMPRT