



Antibody reactivity to the major fish allergen parvalbumin is determined by isoforms and impact of thermal processing



Shruti R. Saptarshi¹, Michael F. Sharp¹, Sandip D. Kamath, Andreas L. Lopata^{*}

School of Pharmacy and Molecular Science, Centre for Biodiscovery and Molecular Development of Therapeutics, James Cook University, Townsville, Queensland, Australia

ARTICLE INFO

Article history:

Received 23 June 2013

Received in revised form 17 August 2013

Accepted 7 October 2013

Available online 18 October 2013

Keywords:

Parvalbumin

Allergen

Asia-Pacific fish

Seafood

IgE epitopes

Monoclonal antibody

ABSTRACT

The EF-hand calcium binding protein, parvalbumin, is a major fish allergen. Detection of this allergen is often difficult due to its structural diversity among various fish species. The aim of this study was to evaluate the cross-reactivity of parvalbumin in a comprehensive range of bony and cartilaginous fish, from the Asia-Pacific region, and conduct a molecular analysis of this highly allergenic protein. Using the monoclonal anti-parvalbumin antibody PARV-19, we demonstrated the presence of monomeric and oligomeric parvalbumin in all fish analysed, except for gummy shark a cartilaginous fish. Heat processing of this allergen greatly affected its antibody reactivity. While heating caused a reduction in antibody reactivity to multimeric forms of parvalbumins for most bony fish, a complete loss of reactivity was observed for cartilaginous fish. Molecular analysis demonstrated that parvalbumin cross-reactivity, among fish species, is due to the molecular phylogenetic association of this major fish allergen.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Fish is one of the eight prominent foods known to cause allergy (Lopata & Lehrer, 2009). Being an excellent source of proteins and omega fatty acids, the consumption of fish has increased greatly. However, allergy to fish is also increasing, affecting up to 0.2% of the general population. Fish allergy is an important concern in the seafood processing environment. A recent study reported that prevalence of fish allergies can be as high as 8% among fish processing workers (Jeebhay et al., 2008). Along with consumption and handling of fish allergens, patients can also react to aerosolized fish proteins generated during cooking or processing (Sharp & Lopata, 2013; Sicherer & Teuber, 2004). Clinical manifestations of fish allergy may include symptoms ranging from wheezing, tightness of the throat, urticaria, vomiting, diarrhea etc. to the life threatening reaction called anaphylaxis.

The major fish allergen has been identified as parvalbumin, an EF hand calcium binding protein (Beale, Jeebhay, & Lopata, 2009; Bugajska-Schretter et al., 1998). Parvalbumins are globular

proteins about 12 kDa in size and are abundant in lower vertebrates such as amphibians and fish (Girija & Rehbein, 1988). These are water soluble and remarkably stable over a broad temperature and pH range (Arif, 2009; Arif, Jabeen, & Hasnain, 2007; Untersmayr et al., 2006). Parvalbumins are abundant in the white muscle tissue of fish, however lower concentrations have also been reported in fish dark muscle tissue (Kobayashi et al., 2006; Wilwert, Madhoun, & Coughlin, 2006). Fish exhibit differences in their environmental habitats and their overall muscle composition. Multiple isoforms of parvalbumin can be expressed in a single fish species during its different developmental stages (Brownridge et al., 2009; Huriaux, Collin, Vandewalle, Philippart, & Focant, 1997; Van Do, Hordvik, Endresen, & Elsayed, 2003). For example, fresh water carp has been reported to express up to eight isoforms of parvalbumin, differing slightly in molecular weight and isoelectric properties (Brownridge et al., 2009).

The detection of fish parvalbumin is challenging compared to other food allergens; this can be attributed to the high biochemical and immunological variability among the different fish species (Gajewski & Hsieh, 2009; Sharp & Lopata, 2013). Fish consumption strongly depends on regional availability. Most studies on characterization of parvalbumins have been conducted on fish commonly consumed in the northern hemisphere. Several fish species such as barramundi, flathead, gummy shark are indigenous to the Asia-Pacific region. However, data on fish allergens in these species is limited. Moreover, not much research has been done on the comparison of the diversity of parvalbumin isoforms across different orders of fish or effect of heat processing on their antibody reactivity.

^{*} Corresponding authors. Address: Molecular Immunology Group, School of Pharmacy and Molecular Science, Centre for Biodiscovery and Molecular Development of Therapeutics, Building 21, Molecular Sciences, James Cook Drive, Douglas Campus, James Cook University, Townsville, Queensland 4811, Australia. Tel.: +61 (07) 47814563; fax: +61 (07) 47816078.

E-mail addresses: shruti.saptarshi@jcu.edu.au (S.R. Saptarshi), michael.sharp2@my.jcu.edu.au (M.F. Sharp), sandip.kamath@jcu.edu.au (S.D. Kamath), andreas.lo-pata@jcu.edu.au (A.L. Lopata).

¹ These authors contributed equally to this manuscript.

The main aim of our study was to compare parvalbumin distribution profiles, specific antibody reactivity and cross-species recognition across 12 different orders of fish and study the impact of heat-processing on the mono and polymeric forms of these parvalbumins. Using bioinformatics tools, we have shown that phylogenetic classification of fish based on the amino acid sequence of parvalbumin, can be linked to the immunological cross reactivity of this allergen. Furthermore, we deduced different antibody binding sites of parvalbumin for bony and cartilaginous fish which can aid in designing specific antibodies for better detection of parvalbumin.

2. Materials and methods

2.1. Fish samples

Nineteen species of fish commonly consumed in the Asian-Pacific region (see Table 1) were analysed. Fresh fillets of each species were purchased from the local fish market and transported on ice to the laboratory. All samples were stored at -80°C prior to processing.

2.2. Preparation of protein extracts

Fifty grams of fish white muscle was homogenized in 100 ml of phosphate buffered saline (PBS, 10 mM, pH 7.2) using an Ultra Tur-rax blender (IKA, Staufen, Germany) and extracted overnight with gentle tumbling at 4°C . The crude extract was centrifuged at 5000g for 30 min at 4°C and filter sterilized using $0.2\ \mu\text{m}$ cellulose acetate filter membranes (Sartorius, Germany) and this is referred to as 'raw extract'. To standardize the heat-processing an aliquot of raw extracts were heated at 95°C for 15 min in a water bath.

Precipitated proteins were removed by centrifugation at 5000g for 15 min and the resulting aliquot referred to as 'heated extract'. The prepared protein extracts were stored at -80°C until further analysis.

2.3. Protein quantification

Protein concentrations were determined for the raw and heated extracts using the Quick Start™ Bradford assay kit (Bio-Rad, USA).

Readymade bovine serum albumin standards (Bio-Rad, USA, 0.125–2.00 mg/ml) were used and the absorbance was determined at 595 nm using a Multiskan Ascent®, (Pathtech, Australia) micro plate reader.

2.4. SDS–PAGE analysis

Protein profiles for raw and heated fish extracts were obtained using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Fish proteins (25 μg) were diluted in a $5\times$ Laemmli sample buffer containing 2-mercaptoethanol, heated for 5 min and loaded on a 12% Tris–Glycine gel. Precision Plus protein standards (Bio-Rad, USA) were used to estimate the molecular weights of individual proteins, using the Mini-PRO-TEAN® Tetra Cell (Bio-Rad, USA) system at 170 V. Proteins were visualized by Coomassie Brilliant Blue R-250 (Bio-Rad, USA) staining.

2.5. Immunoblotting

The presence of different parvalbumins in raw and heated samples was determined by immunoblotting. Fish protein extracts (5 μg) were separated using SDS–PAGE and then transferred to an activated PVDF membrane (Bio-Rad, USA) using the Semi-dry Trans Blot electrophoretic transfer system (Bio-Rad, USA) for 10 min at 10 V. After blocking with 5% (w/v) skim milk (in TBS) for 1 h at room temperature, membranes were incubated for 1 h with the primary monoclonal anti-parvalbumin antibody (PARV-19; Sigma, USA) diluted 1:3000 in 1% skim milk and TBS. Membranes were subsequently washed in TBS with 0.5% Tween-20 (TBS-T) and incubated with the secondary anti-mouse HRP labelled antibody (Sigma, USA). The protein–antigen interaction was visualised using Enhanced Chemiluminescence substrate (Sigma, USA) followed by exposure to photographic film (GE Healthcare, Australia).

2.6. Inhibition-ELISA

To analyse the cross-species antibody reactivity for the different fish parvalbumins an in-house developed inhibition ELISA was performed. Briefly, 10 $\mu\text{g}/\text{ml}$ of heated carp extract was coated on a 96-well high binding plate (Costar, USA) and

Table 1
Biological classification and scientific names of fish species analysed in this study.

Common name	Order	Family	Scientific name
<i>Bony fish</i>			
Snapper	Perciformes	Sparidae	<i>Pagrus auratus</i>
Silver bream	Perciformes	Sparidae	<i>Acanthopagrus australis</i>
Yellowtail kingfish	Perciformes	Carangidae	<i>Seriola lalandi</i>
Barramundi	Perciformes	Latidae	<i>Lates calcarifer</i>
Blue fin tuna	Perciformes	Scombridae	<i>Thunnus maccoyii</i>
Slimy mackerel	Perciformes	Scombridae	<i>Scomber australasicus</i>
Orange roughy	Beryciformes	Trachichthyidae	<i>Hoplostethus atlanticus</i>
Tiger flathead	Beryciformes	Platycephalidae	<i>Neoplatycephalus richardsoni</i>
Atlantic salmon	Salmoniformes	Salmonidae	<i>Salmo salar</i>
Rainbow trout	Salmoniformes	Salmonidae	<i>Onchorynchus mykiss</i>
Carp	Cypriniformes	Cyprinidae	<i>Cyprinus carpio</i>
Pilchard	Clupeiformes	Clupeidae	<i>Sardinops neopilchardus</i>
Rock ling	Ophidiiformes	Ophidiidae	<i>Genypterus blacodes</i>
Atlantic cod	Gadiformes	Gadidae	<i>Gadus morhua</i>
<i>Cartilaginous fish</i>			
Skate	Rajiformes	Rajidae	<i>Raja cerva</i>
Gummy shark	Carchariniformes	Triakidae	<i>Mustelus antarcticus</i>
Sparsely spotted stingaree	Myliobatiformes	Urolophidae	<i>Urolophus paucimaculatus</i>
Blacktip shark	Carchariniformes	Carcharhinidae	<i>Carcharias limbatus</i>
Elephant shark	Chimaeriformes	Callorhynchidae	<i>Callorhynchus milii</i>

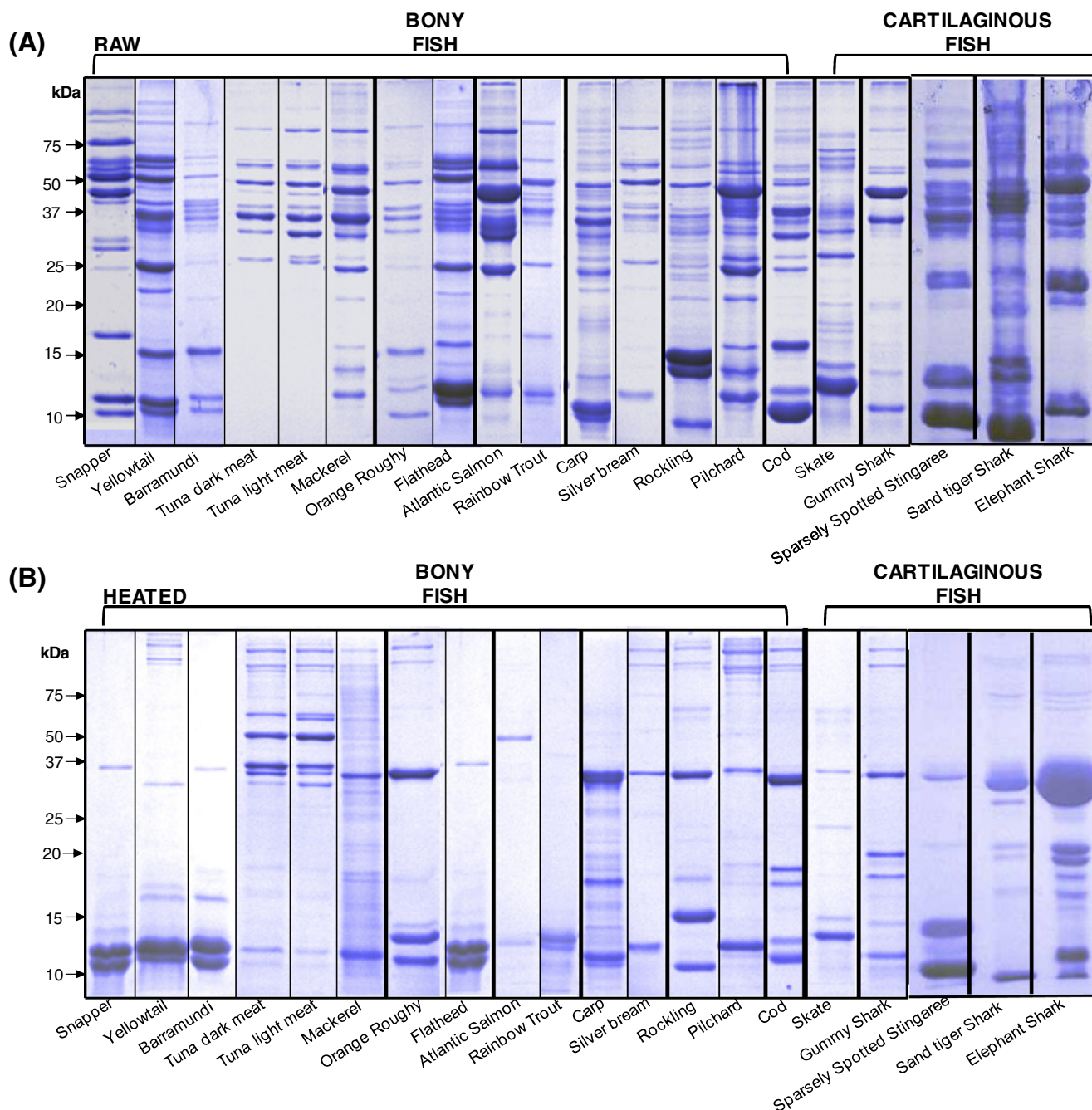


Fig. 1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of raw (A) and heat-processed (B) protein extracts of bony and cartilaginous fish. The common names are depicted under each lane. See Table 1 for complete list of scientific names.

incubated overnight at 4 °C and subsequently blocked with 5% skim milk in TBS, for 1 h, at room temperature. The Primary antibody PARV-19 (diluted 1:1000) was mixed with increasing concentrations of inhibitors, ranging from 0 to 1000 µg/ml for 1 h at 37 °C in separate microcentrifuge tubes. This antibody–antigen mixture was then added to the 96-well plate and incubated for a further 1 h at 37 °C. Following three washing steps with TBS-T, the plates were incubated with the secondary antibody (Rabbit anti-mouse monoclonal antibody; Sigma, USA) for 1 h at room temperature and washed three times and binding visualized using 3,3',5,5'-tetramethylbenzidine (TMB) substrate for HRP (BD Biosciences, USA). The reaction was stopped using 2 M sulphuric acid and the absorbance measured at 450 nm.

The percent inhibition was calculated as $100 - [\text{O.D. 450 nm of antibody with inhibitor} / \text{O.D. 450 nm of antibody without inhibitor}] \times 100$.

2.7. Amino acid sequence alignment of parvalbumin and epitope analysis

Two multiple alignments of parvalbumin amino acid sequences were performed using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). The first alignment consisted of 9 bony fish from 4 different orders (Gadiformes, Perciformes, Cypriniformes and Salmoniformes), which generated a bony fish consensus sequence. The second alignment used the two known cartilaginous fish par-

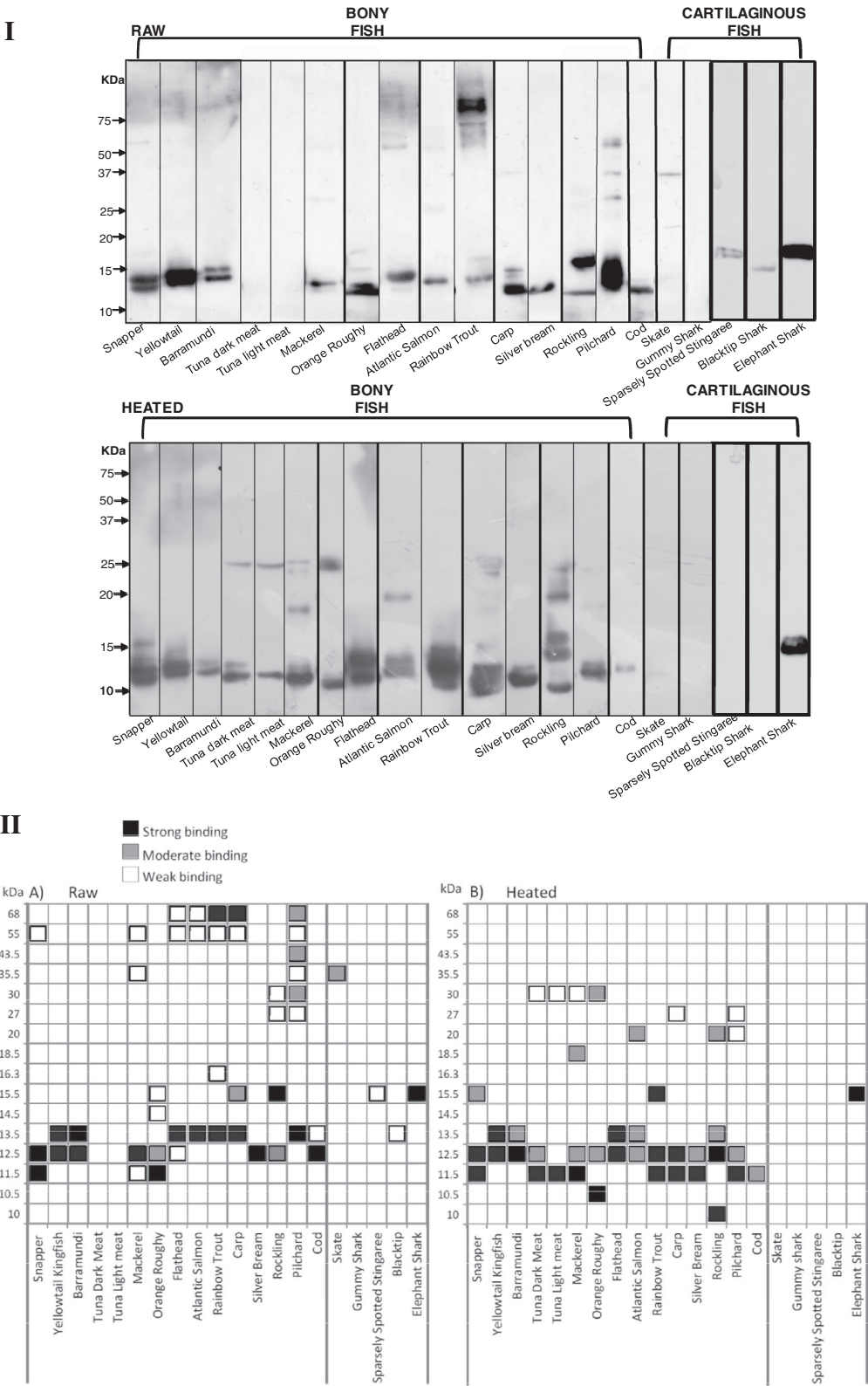


Fig. 2. Immunoblot analysis of raw (A) and heat-processed (B) protein extracts from bony and cartilaginous fish using monoclonal anti-parvalbumin antibody (I). The common names of each fish species are shown under each lane. See Table 1 for complete list of scientific names. Calculated molecular weights of monomeric and polymeric forms of parvalbumin are presented in the allergogram (II) for raw (A) and heat-processed (B) fish, facilitating the analysis of parvalbumin. Antibody binding is graded as weak, moderate and strong binding.

valbumin amino acid sequences that also generated a consensus sequence. The difference between the two consensus sequences was calculated using BLOSUM 62 scoring. Possible antigenic sites

in the bony fish consensus sequence were estimated using the epitope-predicting tool Antigenic (<http://emboss.bioinformatics.nl/cgi-bin/emboss>).

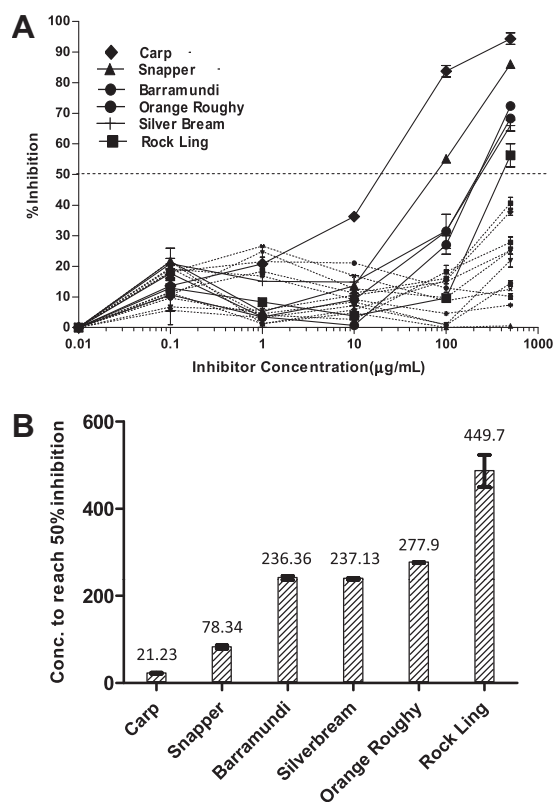


Fig. 3. Inhibition ELISA for the quantification of the monoclonal anti-parvalbumin antibody cross-reactivity between carp parvalbumin (coating antigen) and 19 different fish parvalbumins (A). The inhibition dose response curves for the 19 fish demonstrate that only 5 species achieved over 50% inhibition, with homologous carp parvalbumin being the strongest inhibitor. The protein concentrations to reach 50% inhibition show great differences with rock ling requiring over 20 times more parvalbumin compared to homologous carp (B).

2.8. Phylogenetic analysis of fish parvalbumin amino acid sequences

Phylogenetic analysis of 38 fish parvalbumin isoforms was conducted using the Neighbour-Joining method using the software MEGA5. The bootstrap consensus tree inferred from 10,000 replicates was taken to represent the evolutionary history of fish parvalbumins.

3. Results

3.1. Protein profiles of fish extracts and effect of heat treatment

Proteins present in raw and heat-processed extracts of 19 fish species (see Table 1) were separated effectively using SDS-PAGE (Fig. 1). Most raw fish extracts showed presence of two or more protein bands in the molecular weight range of 10–15 kDa, except silver bream and elephant shark extracts which displayed a single band. Both light and dark tuna extracts showed no bands. The profile for the higher molecular weight protein bands in the range of 37–75 kDa was similar for most fish species.

To analyse the effects of heat-processing, the fish protein extraction parameters such as osmolality, pH extraction buffer volume, specimen weight, heating temperature and heating time were kept constant. This allowed direct comparison of the effect of heating on different shellfish species without any bias. Heating of the fish extracts denatured many heat labile proteins although all fish extracts showed presence of heat stable proteins around

37 kDa and multiple heat stable proteins at 10–15 kDa. However, mackerel, Atlantic salmon, silver bream and blacktip shark displayed only one heat stable protein band in the 10–15 kDa weight range. Interestingly, heated dark and light tuna protein extracts had two distinct bands at 12–13 kDa, which were not present in the raw extracts.

3.2. Monoclonal antibody reactivity to parvalbumin in raw and heated fish extracts

Immunoblotting with the monoclonal antibody against the raw extracts of the various fish showed several bands in the molecular weight range of 10–15 kDa, demonstrating the presence of parvalbumin isoforms (Fig. 2A). Some fish species, such as snapper, yellowtail, barramundi and carp, demonstrated parvalbumin isoforms with very similar molecular weights, while other species seemed to have only one dominant isoform, as observed for mackerel, flathead, Atlantic salmon and cod. Few species, such as rainbow trout and pilchard showed presence of additional higher molecular weight parvalbumin isoforms. In contrast, no bands were observed for Yellow fin tuna (light meat and dark meat) and gummy shark. The heated fish extracts demonstrated prominent monoclonal antibody binding to most; lower molecular weight parvalbumin forms (Fig. 2B). Heating had the most profound effect on yellow fin tuna, which demonstrated strong bands. In contrast, cartilaginous fish lost all antibody reactivity after heating and none of the five species showed parvalbumin bands except elephant shark that showed presence of one band. Comparison of the location and band intensity of the different parvalbumin forms by densitometric analysis resulted in a detailed allergogram, allowing the specific evaluation of impact of heat-processing on antibody recognition. The higher molecular weight antibody reactivity, particularly in raw extracts, was most likely due to the presence of polymeric forms of monomeric parvalbumins, in the molecular weight range of 11.5–13.5 kDa

3.3. Monoclonal antibody cross-reactivity of carp parvalbumin against heated fish extracts

The cross-reactivity of 19 fish species was evaluated by performing a quantitative inhibition ELISA using heated carp extract as the coating antigen (Fig. 3A). The decrease in reactivity of the monoclonal antibody to the immobilized carp allergen extract, comprising of two strong parvalbumin forms, was used as a measure of immunological cross-reactivity against other heated fish extracts used as inhibitors. The carp extract as expected, was able to completely abolish the antibody reactivity at about 500 µg/ml, while only 5 of the 19 other fish species were able to inhibit the antibody reactivity by over 50% in a dose depended manner. The concentrations needed to reach 50% inhibition varied significantly from about 20 µg/ml for carp to about 450 µg/ml for rockling (Fig. 3B). In contrast, none of the other 14 fish extracts were able to achieve inhibitions above 40%, even at the highest inhibitor concentration.

3.4. Epitope analysis of monoclonal antibody between bony and cartilaginous fish

The multiple alignments of fish parvalbumin amino acid sequences demonstrated regions of high consensus in bony and cartilaginous fish, situated at the two calcium binding sites. However, the amino acid sequences are less conserved at the N-terminal region, demonstrated by values under 4 in the BLOSUM 62 score graph of Fig. 4. The program 'Antigenic' predicted the most antigenic site, the most likely target for this monoclonal antibody to be between positions T13 and 39 and K39

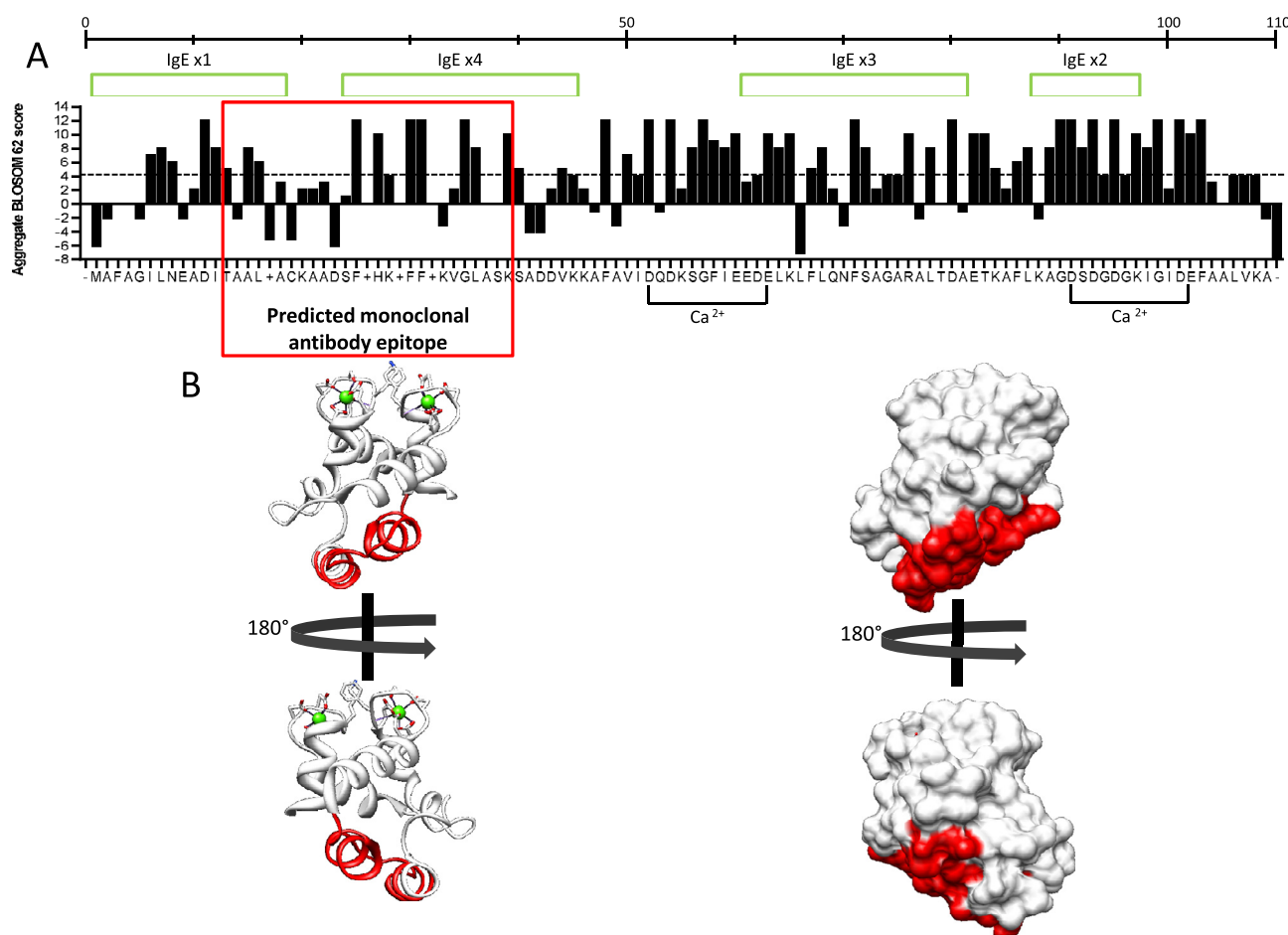


Fig. 4. (A) The potential PARV-19 epitope predicted using 'Antigenic' analysis is boxed in red. The aggregate BLOSUM 62 score was calculated from the two consensus sequences generated for bony and cartilaginous fish. Bony fish consensus is shown under the chart with the four known IgE epitopes highlighted in green. (B) Ribbon and space fill models of carp parvalbumin (PDB ID: 4cpv), with the predicted epitope coloured in red. Bound calcium ions are shaded in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Phylogenetic relationships of fish parvalbumins

The phylogenetic analysis of fish parvalbumin demonstrated relationships between isoforms from specific taxonomic orders. For example, Gadiformes, Perciformes and Cypriniformes form two distinct clades each and three out of four Salmoniformes form another clade (Fig. 5). All the α -parvalbumins, which also include the only two cartilaginous fish parvalbumins with known amino acid sequences, grouped close to the higher vertebrate parvalbumins.

4. Discussion

The major fish allergen parvalbumin is a highly diverse protein and exists as multiple isoforms in different fish species (Sharp & Lopata, 2013). The present study analyses for the first time, parvalbumins in a comprehensive range of fish from the Asia-Pacific region. We have made use of the anti-frog parvalbumin antibody PARV-19, widely used for the identification of this major allergen (Gajewski & Hsieh, 2009; Weber, Steinhart, & Paschke, 2009). Immunoblotting experiments confirmed presence of multiple bands of parvalbumin isoforms in the molecular weight range of 10–15 kDa for most raw and heated fish extracts. Characteristic parvalbumin oligomers were also detected by the monoclonal antibody in raw extracts of eight fish including mackerel, flathead, trout, carp, rock ling, pilchard and skate. Multimeric formation of parvalbumin can be attributed to the presence of readily reducible

cysteine residues in the protein (Ross, Tilghman, Hartmann, & Mari, 1997). Heat processing of the fish extracts affected the antibody recognition of parvalbumins for some fish species. For example, while no bands were observed in raw extracts of tuna, monomeric as well as oligomeric parvalbumin was detected in the heated extracts. Similarly, heating also led to oligomer formation of parvalbumin in the orange roughy extract. Oligomeric parvalbumin from flathead, rainbow trout and skate was found to be heat labile. These results highlight the difference in thermal stability of parvalbumin across different genera of fish and confirm that, heat processing has a profound effect on antibody reactivity of different parvalbumins.

The 32,400 different types of fish can be largely grouped as bony (Osteichthyes) which comprises of most edible fish and cartilaginous (Chondrichthyes) which include sharks and rays (Froese & Pauly, 2012). Parvalbumin has been described as a pan-allergen in a majority of bony fish varieties such as cod, carp and salmon (Beale et al., 2009; Elsayed & Apold, 1983; Lindstrom, van Do, Hordvik, Endresen, & Elsayed, 1996; Perez-Gordo et al., 2011; Swoboda et al., 2002; Untersmayr et al., 2006; Van Do et al., 2003). Interestingly reports on allergenic parvalbumin from cartilaginous fish are limited (Cai et al., 2010; Rodríguez, Gracia, Iriarte, & Bartolomé, 2003). Parvalbumins from sharks have been suggested to be structurally different to those of bony fish (Roquet, Declercq, Tinant, Rambaud, & Parello, 1992). The monoclonal antibody reactivity to the cartilaginous fish extracts used in our study was found to be minimal with no binding detected for gummy shark. This

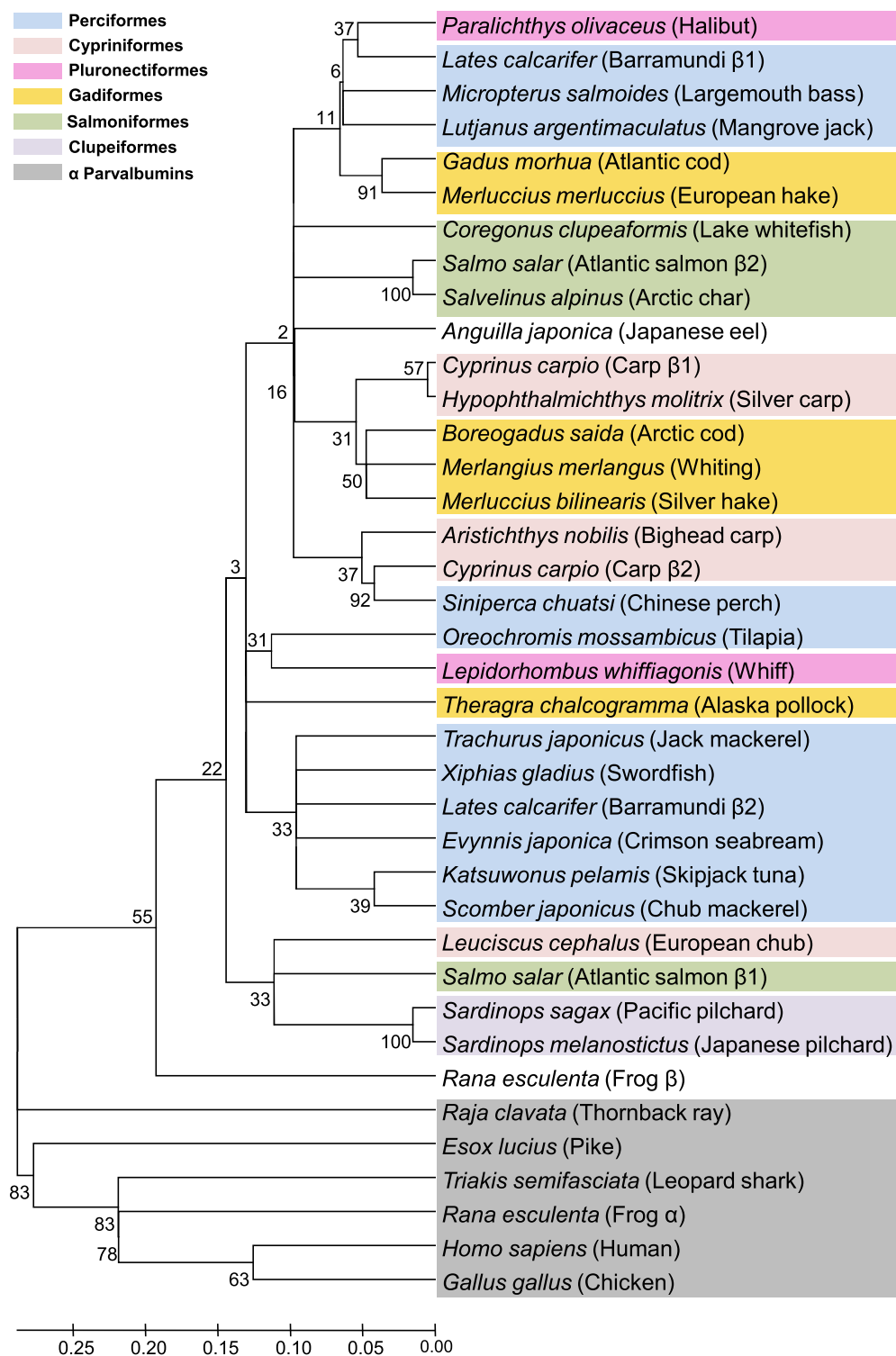


Fig. 5. The evolutionary history of a variety of parvalbumins was inferred using the Neighbour-Joining method. The bootstrap consensus tree inferred from 10,000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 38 parvalbumin amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 99 positions in the final dataset. Evolutionary analyses were conducted in MEGA5. For convenience each order is shaded a separate colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reactivity was completely lost for the heated extracts demonstrating that shark parvalbumin may be heat labile. The inhibition assay further confirmed the low cross reactivity of this allergen between cartilaginous and bony fish. Interestingly, the antibody

was capable of binding to parvalbumins in raw as well as heated extract of the elephant shark. The elephant shark is a chimera fish. A recent study suggested that parvalbumin from elephant shark may be more similar to bony fish parvalbumin (Venkatesh et al.,

2007). The inability of the monoclonal antibody to detect shark parvalbumin may be due to the dissimilarity in the amino acid sequence homology of parvalbumins from bony and cartilaginous fish.

The cross-reactivity among fish is most likely due to molecular phylogenetic association of parvalbumin, though Fæste and Plassen (2008) in their study with anti-cod antibodies have suggested that cross reactivity may be based on the habitat (fresh water or saltwater) of fish. Detailed molecular analysis of parvalbumins from fish used in the present study, and phylogenetic grouping of 38 available parvalbumin amino acid sequences was used to understand the cross-reactive nature of parvalbumin in different fish. An example includes, the correlation between the amino acid sequences of the order Perciformes group together (Fig. 5) and the three species of fish (barramundi, snapper, silver bream) that cross-reacted with carp parvalbumin in the inhibition assay. These fish showed up to 50% inhibition of the monoclonal antibody to carp parvalbumin. Currently, there is a lack of parvalbumin sequences available from the orders Ophidiiformes (rockling) and Beryciformes (orange roughy), and this made it difficult to analyse the molecular relationships and cross-reactivity of these species with the Perciformes or carp. Interestingly the two known cartilaginous fish parvalbumins (leopard shark and thornback ray) clustered close to the α -parvalbumins of higher vertebrates such as humans and chicken away from other bony fish. This not only explains the lack of binding of the monoclonal antibody to cartilaginous fish parvalbumin in our study, but may also explain the current reports on their low allergenicity (Koyama et al., 2006).

In summary, we have shown that the monoclonal antibody, used in the present study, reacted to all fish species except gummy shark, demonstrating that the parvalbumin epitope for this antibody must be in a relatively conserved region of this allergenic protein. The effect of heating on fish allergens must be taken into account when designing future diagnostics. This study also identified a specific antibody target at the N-terminal region of parvalbumin, allowing the distinction of bony from cartilaginous fish. Further characterisation of different parvalbumins will therefore help to create a comprehensive molecular phylogenetic association between fish species. This can aid in predicting immunological cross-reactivity and generate fish order-specific detection tools to further our resources in detecting this major fish allergen in foods.

Acknowledgement

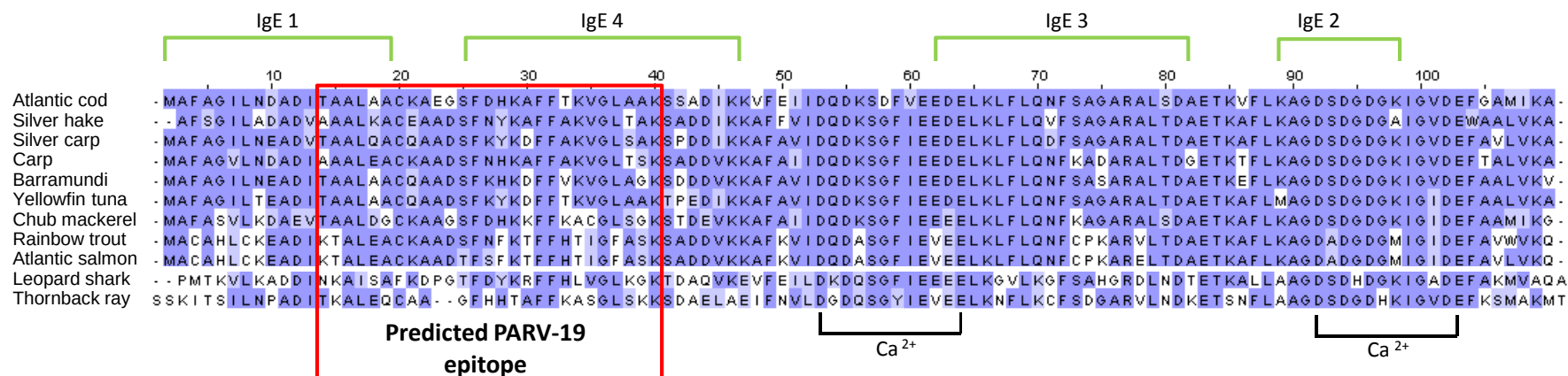
A.L. is holder of an Australian Research Council-Future Fellowship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2013.10.035>.

References

- Arif, S. H. (2009). A Ca^{2+} -binding protein with numerous roles and uses: Parvalbumin in molecular biology and physiology. *BioEssays*, 31(4), 410–421.
- Arif, S. H., Jabeen, M., & Hasnain, A.-U. (2007). Biochemical characterization and thermostable capacity of parvalbumins: The major fish food allergens. *Journal of Food Biochemistry*, 31(1), 121–137.
- Beale, J. E., Jeebhay, M. F., & Lopata, A. L. (2009). Characterisation of purified parvalbumin from five fish species and nucleotide sequencing of this major allergen from Pacific pilchard, *Sardinops sagax*. *Molecular Immunology*, 46(15), 2985–2993.
- Brownridge, P., de Mello, L. V., Peters, M., McLean, L., Claydon, A., Cossins, A. R., et al. (2009). Regional variation in parvalbumin isoform expression correlates with muscle performance in common carp (*Cyprinus carpio*). *Journal of Experimental Biology*, 212(2), 184–193.
- Bugajska-Schretter, A., Elfman, L., Fuchs, T., Kapiotis, S., Rumpold, H., Valenta, R., et al. (1998). Parvalbumin, a cross-reactive fish allergen, contains IgE-binding epitopes sensitive to periodate treatment and Ca^{2+} depletion. *Journal of Allergy and Clinical Immunology*, 101(1), 67–74.
- Cai, Q.-F., Liu, G.-M., Li, T., Hara, K., Wang, X.-C., Su, W.-J., et al. (2010). Purification and characterization of parvalbumins, the major allergens in red stingray (*Dasyatis akajei*). *Journal of Agricultural and Food Chemistry*, 58(24), 12964–12969.
- Elsayed, S., & Apold, J. (1983). Immunochemical analysis of cod fish allergen M: Locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy*, 38(7), 449–459.
- Fæste, C. K., & Plassen, C. (2008). Quantitative sandwich ELISA for the determination of fish in foods. *Journal of Immunological Methods*, 329(1–2), 45–55.
- Froese, R., & Pauly, D. (2012). *Fish base*. World Wide Web electronic publication, <www.fishbase.org> (version 08/2012).
- Gajewski, K. G., & Hsieh, Y. H. (2009). Monoclonal antibody specific to a major fish allergen: Parvalbumin. *Journal of Food Protection*, 72(4), 818–825.
- Girija, N., & Rehbein, H. (1988). Comparison of parvalbumin patterns from different fish species by isoelectric focusing of muscle extracts. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 91(4), 723–728.
- Huriaux, F., Collin, S., Vandewalle, P., Philippart, J. C., & Focant, B. (1997). Characterization of parvalbumin isotypes in white muscle from the barbel and expression during development. *Journal of Fish Biology*, 50(4), 821–836.
- Jeebhay, M. F., Robins, T. G., Miller, M. E., Bateman, E., Smuts, M., Baatjes, R., et al. (2008). Occupational allergy and asthma among salt water fish processing workers. *American Journal of Industrial Medicine*, 51(12), 899–910.
- Kobayashi, A., Tanaka, H., Hamada, Y., Ishizaki, S., Nagashima, Y., & Shiomi, K. (2006). Comparison of allergenicity and allergens between fish white and dark muscles. *Allergy*, 61(3), 357–363.
- Koyama, H., Kakami, M., Kawamura, M., Tokuda, R., Kondo, Y., Tsuge, I., et al. (2006). Grades of 43 fish species in Japan based on IgE-binding activity. *Allergology International*, 55(3), 311–316.
- Lindstrom, C. D., van Do, T., Hordvik, I., Endresen, C., & Elsayed, S. (1996). Cloning of two distinct cDNAs encoding parvalbumin, the major allergen of Atlantic salmon (*Salmo salar*). *Scandinavian Journal of Immunology*, 44(4), 335–344.
- Lopata, A. L., & Lehrer, S. B. (2009). New insights into seafood allergy. *Current Opinion in Allergy and Clinical Immunology*, 9(3), 270–277.
- Perez-Gordo, M., Lin, J., Bardina, L., Pastor-Vargas, C., Cases, B., Vivanco, F., et al. (2011). Epitope mapping of Atlantic salmon major allergen by peptide microarray immunoassay. *International Archives of Allergy Immunology*, 157(1), 31–40.
- Rodríguez, V., Gracia, M. T., Iriarte, P., & Bartolomé, B. (2003). Allergy to dogfish. *Allergy*, 58(12), 1315–1317.
- Roquet, F., Declercq, J.-P., Tinant, B., Rambaud, J., & Parello, J. (1992). Crystal structure of the unique parvalbumin component from muscle of the leopard shark (*Triakis semifasciata*): The first X-ray study of an α -parvalbumin. *Journal of Molecular Biology*, 223(3), 705–720.
- Ross, C., Tilghman, R. W., Hartmann, J. X., & Mari, F. (1997). Distribution of parvalbumin isotypes in adult snook and their potential applications as species-specific biomarkers. *Journal of Fish Biology*, 51(3), 561–572.
- Sharp, M. F., & Lopata, A. L. (2013). Fish allergy: In review. *Clinical Reviews in Allergy and Immunology*, 1–14.
- Sicherer, S. H., & Teuber, S. (2004). Current approach to the diagnosis and management of adverse reactions to foods. *Journal of Allergy and Clinical Immunology*, 114(5), 1146–1150.
- Swoboda, I., Bugajska-Schretter, A., Verdino, P., Keller, W., Sperr, W. R., Valent, P., et al. (2002). Recombinant carp parvalbumin, the major cross-reactive fish allergen: A tool for diagnosis and therapy of fish allergy. *Journal of Immunology*, 168(9), 4576–4584.
- Untersmayr, E., Szalai, K., Riemer, A. B., Hemmer, W., Swoboda, I., Hantusch, B., et al. (2006). Mimotopes identify conformational epitopes on parvalbumin, the major fish allergen. *Molecular Immunology*, 43(9), 1454–1461.
- Van Do, T., Hordvik, I., Endresen, C., & Elsayed, S. (2003). The major allergen (parvalbumin) of codfish is encoded by at least two isotypic genes: cDNA cloning, expression and antibody binding of the recombinant allergens. *Molecular Immunology*, 39(10), 595–602.
- Venkatesh, B., Kirkness, E. F., Loh, Y. H., Halpern, A. L., Lee, A. P., Johnson, J., et al. (2007). Survey sequencing and comparative analysis of the elephant shark (*Callorhynchus milii*) genome. *PLoS Biology*, 5(4), e101.
- Weber, P., Steinhart, H., & Paschke, A. (2009). Competitive indirect ELISA for the determination of parvalbumins from various fish species in food grade fish gelatins and isinglass with PARV-19 anti-parvalbumin antibodies. *Journal of Agricultural and Food Chemistry*, 57(23), 11328–11334.
- Wilwert, J. L., Madhoun, N. M., & Coughlin, D. J. (2006). Parvalbumin correlates with relaxation rate in the swimming muscle of sheephead and kingfish. *Journal of Experimental Biology*, 209(2), 227–237.



Supplementary Figure 1