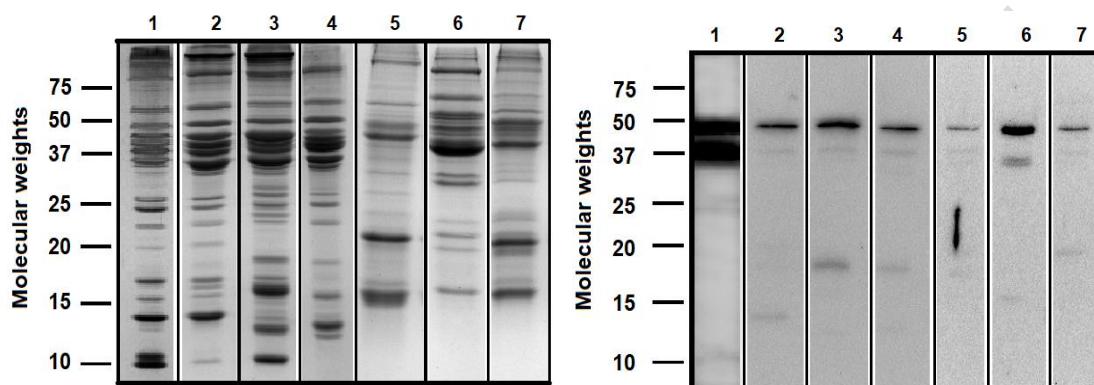


SUPPLEMENTARY MATERIAL

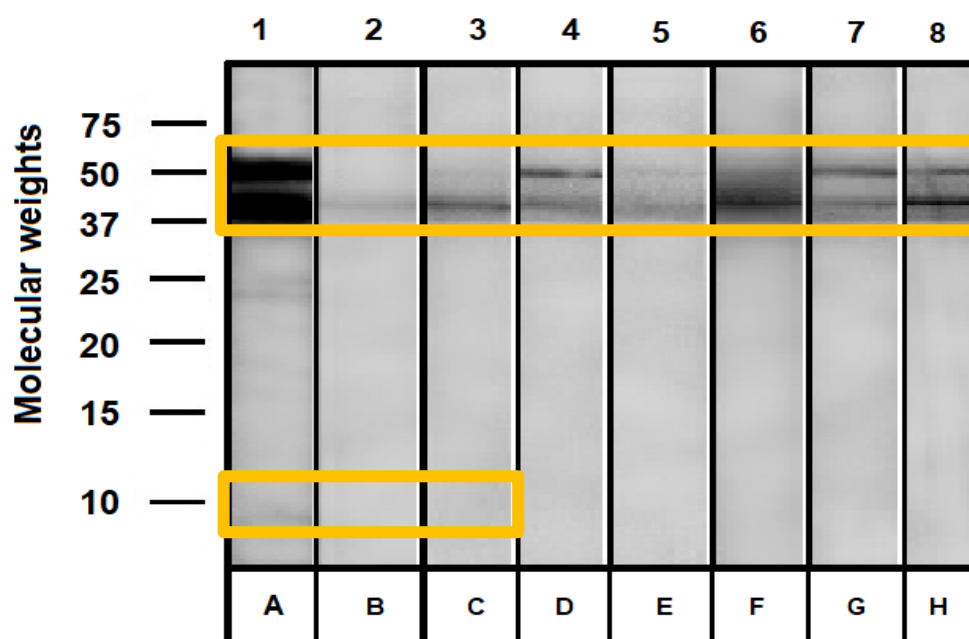
Supplementary Figure 1. SDS-PAGE and IgE Western Blot performed under reducing conditions with the following raw extracts: Lanes 1, *Dicologlossa spp.*; 2, *Sole spp.*; 3, *Lepidorhombus spp.*; 4, *Scophthalmus spp.*; 5, *Merluccius spp.*; 6, *Salmo spp.*; 7, *Gadus spp.*.



Left, SDS-PAGE shows multiple bands of proteins in all of them, ranging from 10 kDa to more than 75 kDa. Right Western Blot shows a detection of protein bands, including:

- 1. Low weight molecular bands (10-14kDa):** showing a weak recognition in Lanes 1, *Dicologlossa spp.*; 2, *Sole spp.*, but did not appear in the other lanes.
- 2. High weight molecular bands (ranging from 37 to 50 kDa):** clearly recognized in all the lanes, but with a higher intensity in Lane 1 *Dicologlossa spp.*.

Supplementary Figure 2. IgE Western Blot inhibition performed under reducing conditions with raw *Dicologlossa spp.* in solid phase and the patient serum was preincubated with the following fish extracts at 1 μ g/ml before the IgE-immunodetection: Lanes 1, without inhibition (negative control); 2, *Dicologlossa spp.* (positive control); 3. *Solea spp.*; 4, *Lepidorhombus spp.*; 5, *Scophthalmus spp.*; 6. *Merluccius spp.*; 7. *Salmo spp.*; 8. *Gadus spp.*.

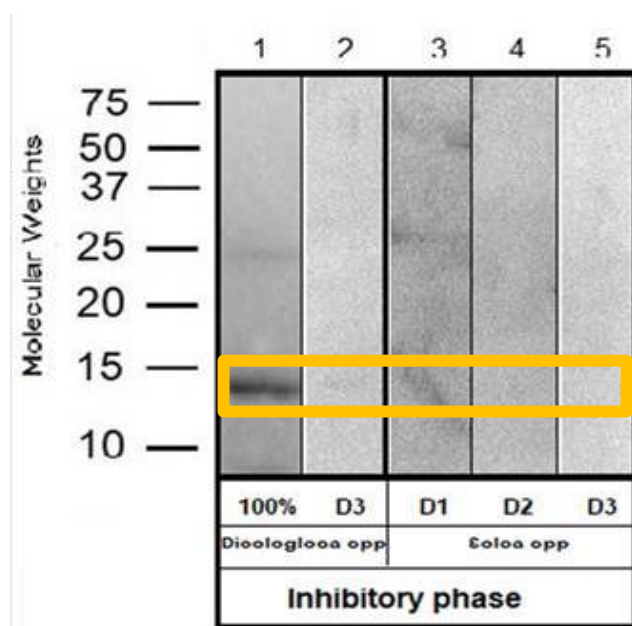


It can be highlighted that in the IgE Western Blot inhibition, the patient serum recognized several protein bands, including the following:

1. Low weight molecular bands (10-14kDa): weak recognition in Lane 1, *Dicologlossa spp.* without inhibition (negative control); but it is completely inhibited in Lane 2 *Dicologlossa spp.* (positive control) and Lane 3 *Solea*.

2. High weight molecular bands (ranging from 37 to 50 kDa): strong recognition in lane 1, *Dicologlossa spp.* without inhibition, partially inhibited in the rest.

Supplementary Figure 3. IgE Western Blot inhibition performed under reducing conditions with *Dicologlossa spp.* in solid phase and preincubating patient serum before IgE immunodetection with: Lane 1, without inhibition (negative control); 2, *Dicologlossa spp.* 1 mg/ml (positive control); 3, *Sole spp.* 0.032 mg/ml; 4, *Sole spp.* 0.125 mg/ml; 5, *Sole spp.* 0.5 mg/ml.



Patient serum recognized low weight molecular bands (10-14kDa) in Lane 1, without inhibition, which completely disappeared in Lane 2. This same band, was completely inhibited by *Sole spp.* at all concentrations tested, demonstrating that *Sole spp.* and *Dicologlossa spp.* share a protein with similar epitopes.

Supplementary Figure 4 Mass spectrometry sequencing revealed the identity of a parvalbumin beta-2-like, with a monoisotopic mass of 12185 Da, a calculated pI of 4.81, and a peptide sequence of 109 amino acids (Swissprot, NCBI database). This new protein has a protein sequence coverage of 28% with another parvalbumin beta-2-like of *Hippoglossus stenolepis*, a subspecies of *Solea*, included in the order pleuronectiformes.

MATRIX SCIENCE MASCOT Search Results

Protein View: XP_035036110.1

parvalbumin beta-2-like [*Hippoglossus stenolepis*]

Database: NCBI-Pleuronectiformes
Score: 78
Expect: 0.0046
Monoisotopic mass (M_r): 12185
Calculated pI: 4.81

Sequence similarity is available as [an NCBI BLAST search of XP_035036110.1 against nr.](#)

Search parameters

Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: [Carbamidomethyl \(C\)](#)
Variable modifications: [Oxidation \(M\)](#)

Protein sequence coverage: 28%

Matched peptides shown in **bold red**.

1 MAFKNILEDA KIAAALVECK DAGTFCHK**KF FTTCGLAGKS** AVDIKKAFNI
 51 IDQDKSCYIE EDELK**LFLQN FKDSARALTD AETKAFLKAG DTDNDGKIGD**
 101 DEFVVMVNA