

TITLE OF THE PROJECT (ACRONYM): DEVELOPMENT OF A VACCINE BASED ON MEMBRANE PROTEASES AND SURFACE VARIABLE proteinS TO PREVENT SCUTICOCILIATOSIS IN TURBOT (*Scophthalmus maximus*) (VALESPRO)

Objectives

General objective

The main objective of this project was to develop a vaccine made up of plasma membrane-associated proteins, which are involved in both infection and evasion of the host's immune response, and which could be key targets in the development of a vaccine capable of inducing protection against scuticociliates. Likewise, it will also seek to improve the knowledge of the humoral immune response induced by the vaccine, when administered intraperitoneally.

Specific objectives

Objective 1.- To complete the identification of genes encoding variant-specific surface proteins (VSPs) and leishmanolins in *P. dicentrarchi* and to evaluate the expression of these gen in virulent and avirulent ciliates.

1. Characterization of the leishmanolysins of *Philasterides dicentrarchi*

Originally, leishmanolins (LSFs) were identified as plasma membrane-bound metalloproteases that were expressed on the surface of flagellate promastigotes of several species of the genus *Leishmania*. Several studies showed that this protein was very abundant in the membrane and that it had a size of around 63 kDa, which is why it was called p63 or gp63. Proteomic analyses revealed that these proteins were structurally related and conserved among the most common *Leishmania* species. Experimental studies carried out on this parasite revealed that leishmanolysin is a glycosylated membrane protein of the zinc-dependent metalloproteinase type. Likewise, leishmanolins have also been described as important virulence factors of the parasite due to their role in promastigote infection and resistance to the host immune response. The results of the sequencing of the genes related to the LSF family and the biochemical characteristics are presented in Tables 1-4.

<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF1) Genee, complete cds</p> <p>GENE: 1784 bp. Exons:1-40; 100-256; 324-385; 446-736; 802-1034; 1093-1130; 1200-1288; 1361-1515; 1592- 1784. CDS: 1257 bp protein: 418 aa</p>	<p>Gene</p> <p>ATGATTTTAGGATCACCTGAACCTATTAACCTTACAAGAGGTATAGTCATTTAAATTGAGAAGTATAAATATTTAAACA AATAAATCTTATTTTAGATCATTTTGGATGTGAAAGTGCCTAAGGAGCATAAATTAGAAATTAGGATGGAGATGGAG ATACAGGATCTCACTGGGAAAGAACCTATTTTATAATGAAGTTATGAACCAAGTAGTATATAATCAGATTCTGTAAC TTGGTTATTTACATTAGGTAAATATAAAAAATATATGCCATATTAATTAGATCTGAGTATTTAATATATTTTATAAACTCAA CAGCATTCTCAAAGATTCTAAGTATGCGACACATCGATTTTCTTATAGCGAATCTATGTAGGTAAAGAAATCAATTA AACTTATTGAAAGTTGGAATAAGCCATATTTTATTTAATTTTAGTGGGATTAAAGAGAGGATGGTGTATTTAGATTCT ATGCGACAAATTAACCTGAAATTTAAAAAGAGGTACCAAAATATGTTCTTTTATAAATTTGGTTATGGAACAGCTG AAGTGGACAGTCATACCGTACTTGCCTGCTAGGTTCTATAGATACCAATAAGACTGTACAAATCCTAACATATATC TACCTATGCCTCCTAACTAATGAAAAAATTCAGTCGATAGTAAATGCTTTCTTCTAATTAGTCAATTCCTGGCTCAG CTAGTTAAGCAGATTATGTACATAGAGTTTAAAAATTAATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTT AGATGCTACTAATATGCTGCAATGCCCAAGGATAAGTAGAAATTAATTAATTTTATTTTATTTTATTTTATTTTATTTTATTTT ACCTGTACCAAGTGTGCTAGGTTTATAAGCTCCTGCAAGGATGACTGGAACATTAACTGTCCAAGAAAACTATCT GAATTTTGTGATTTTCTATTTCCATGCGAAAAATTTAGTGTGAAAAAGGATAGTGTGAGATAAAAGGAGCAAGGT AATTAATTTCTAAATGAAGAACTTATTTATTTCTATATCTTAATATTTTATTTAAAGTAATGGCATTGTATTTCTGGTTATG CAGGAAAAAGATTGTAAATTTATTAATCTATATATTTATGATATTTAACTAAAAAATTAATTTATTTATCTCCTTAAATAG GTTCCGTTAAATGTGATGGATTATAAATTCATCAAAATCAGTGTGTGAATCTTGTGGAGTAGGTTATTTATGACTAA GGAAGCAAGTAAAGATTTTATTTAATAATATTAGGCTACTTAAATATTTAATTTTACATATTTCTCTATGATTTCTACAG GTGTGCGGAAAGTGAATGCTACTTGTACAAATTTATGACCTTTGTCTTCTAATGACTATTTGCATATAAGGGA AGTTATCATATAGTGGATGTGTAGATTCTTGCCTTAAAGGAACATATCATAAAAAAGAGATAATTTCTGCTAGTA AATTATAATTTATATAAAAAAATCAATCTTTTGAATAAATTTTATTTAATTTTATTAATTAATTTTATTAATTTTATTA CTAATCATGTACCAATGATGTTCTAGATTAGCTAATAATTTTGTGATTAACCTTTTGTGATTAATTAATTAATTAATTAATTA TTGCTTAAGAAATAGGAATTAAGAAAAAGCAGCAATTTCTCAATTTGAATATTTTCTATTAATTAATTAATTAATTAATTA TTATTCATATATCTTATTTGA</p> <p>CDS</p> <p>AATTAGAAAAATTAGGATGGAGATGGAGATCAGGATCTCACTGGGAAAGAACCTATTTTATAATGAAGTTATGAACC CAAGTAGATATAATCAGATTCTGTAACTTCGTTATTTACATTAGCATTTCTCAAGATTCTCACTGGTATGCGACATC GATTTTCTTATAGCGAATCTATGTAGTGGGATTAAAGAGAGGATGTGGTTTTTATAGATTATGCGACAATTAATACC</p>
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	<p>CTGAATTTAAAAAAGAGGTACCAAAATATGTTCTTTTATAATTTTGGTTATGGAACAGCTGAAGTGGACAGTCATAC CGATACTTGGCCGTAGGTTCTATAGATAACCAAATAAGACTGTACAAATCCTAAACATATATCTAGCTTAGCCCTCTAA ACTAATGAAAAAAATTCAGTCGATAGTAATGCTTTTCTTCTAATTTAGTCAATTCCTGGCTCAGGTAGTTAAGCAGATTA TAGATGCTACTAATAATGCGCTGCAATGCCAAGGATAAGTAGAAATTAATAATCAGAGTGGGAGGTTAAGAAAAAGTATT AACCTGTACCAAGTATGCGCTAGGTTTTATAAGCTCCTCGAGGAATGACTGGAACATTAACCTGTCCCAAGAAAACATATC TGAATTTTGTGATTTCCTATTCCATGCGGAAAATTTATTGATGAAAAAGGATACGTGTTTGAGATAGTAAAGGAGCAAGT AATTTGCCATTGATTTTCTGGTTATGCAGGAAAAAGATTGTTCCGTTAAATGTGATGGATTATATAATTCATCAAAATCAGTG TGTGAATTTCTGTGGGTAGCTTATATTATGCACTAAGGAAGTGTGGGAAAGTGAATGCTAGCTTGTACAAAT TGTAATGSAACCTTGTCTTCTGTAATATTGCTATTAAGCAAGTTATCATATAGTGCATAGTACTGATCATCTCTTG CCCTAAGGAACATATCATAAAAAGACATAATCTTGTCTAAATTTGACTAATCATGTACCAATGGATGTTCTAGA TTAGCTAATAATTTTGGTATAACCTTTGCTTTATAAAGACAATAAGCACCTTGTCTTAAGAAATTAGAAATTAAAGAAA AAGCAGCAATTCCTCAATTGAATATTTTTTCTATTATATAACCGTTTTATAATTATCAATATATCCTATTTTGA</p> <p>Protein</p> <p>MILGPELLKLRDHFHCESAQGAQLENQDGDGDTGSHWERTLFYNEVMNPSSIQSDSVTSLFTLFLKDSNMYADIFDS YSESMQWLKRGCGFLDSCDNQYPEFKKEGTQKCSFYNFYGYGTAEVDSHTDCAVGSIDTKQDCTNPKHISTYASOTNE KNSVDSKCFSSNLVNSGSASQADYRCYQYACNAQGQVEIKITVGGQEKVLCTSDAQVLQAPAGMTGLTLCPRKLSEFC DFPIPCENYCSSEKGYCLROKGAASNCHCISGYAGKDCSVKCDGFINSNQCVNSCGVYGYDQGSKYVCCKNATCTNCN GPLSSOCTIQIGKLSYSGQCVSDCPKGTYHKKEDNSCQNCQDQSCNTGCSRLANNCLVQPFVYKDNKHLAQELGIKEKSS NSQLNFSIILT VFIIQYILI</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF2) Genee, complete cds</p> <p>GENE: 3536 bp. Exons: 1-267; 321-502; 560- 1003; 1068-1490; 1556-1898; 1967-2523; 2624-2765; 2826- 3536 CDS: 3069 bp protein: 1022 aa</p>	<p>Gene</p> <p>ATGGAACAACCTCTTCATGAACACTGCCATGCTTTGGTTTTCTCATCAGGAGCTCTGATGGTAGATTTTAGAGATACAG AAGGAAATAAAGAGGATTTTCAGAATGTATTCTATTAAACTAATTAATTAGGGGAAATAATTAACCAACATATTGATC ACTCCAGAGGTAAGAAAATTCAGTCTGAATTTTTCGACTGCGATTAGTGTTAGACGACGCTTAAACCGACCCAGCT TATAGAGGAATGTAATTAGAAAATTAAGGTGTGCATTTTCATTATAACCCATATGAGTAGTATTGTATGAATTTAAT CAGGGTTAAGGAAGTGC CGGAAGTCATTGGGAAAGAACTATTATGTATGACGAAGTAATGACTGCTCCAGACTGGG ATTTACTAAAGCTTTTCGGGCATAACATTTGCGATATTTAAGGATTCAAGTGGTATAAAGTAATGATATTGAACCAT CTAAAAATCTATGGGGATATAAGAAAGGGTATAAAAATTATATAGTTTTTTTGTGGATAAAATTAATTTATTGTTCTATT TTAGTTGCATATTTTAAATATGGTTGCACACATCCCACCTGCTCAAATTTGAAGAGTTTTGCGTAATTTGATGAATAACCT TTTAATTAAAGCATTCAATGCTCAGGTTCCAGAGACAGTTTAGGAGAGTGTTTAATTTCCACAAGTCATTTTGGCCTTTA TGGATGCTAATTTGGAATTTGCAATCCTTTTTCAAATGGTAGATGTTTTGAACCCAAAGATAGCGTTTTGATCAAAG TTATGTCGGCAGTGGATAAAATATAGGTATGCGGATTTCAAATGTTTTACAGACTATTGGAACAGTCCGGTAGGG AACTAATTAATAATGCTATTAAATACAAGTGTGGGAAGATTTGACTTTGGAATAACATTAACATAATTCGGAAGAGGAG TTTTGTTCTATGTTATTAAAGGATAGAAACATAGTGATTGTTGAATTTGTAAAAAAAACCAAGGATTTATT TTTAGTTATTATTAAATTAATAATAGTTAGATCTGGATTTTTATCATGTCTGATCCAGAAGCTTTTTGCAAAATTA GAAAAAAATTCGCCCCAAATTTGGTGTAAAGAAATGGTTATTGTATGGATGGAATTTTGTGCGAATGCGAACAGGATGG GGAGGCCTAGATTGCGAGTTTAAAGGAATAACATTTCCCTCGTGAATAAAGATGGAACACATAAGACAGCGCTATTITT TAAATGAAAAATAAAATGAGTGTTATTCTGGGATTGGTCCGACAAATGTTGCAACCCGCTTTTACCTGTATAAATTTGT AATAAATATGAAAAATATATTTAATAGAGTGTGTGAAGCCCTGCCATATATCTTTGTAAAACGCTGTTATGGGGAGGTTCTGT ATAATTTGCGCAAGTTGTACATAAGAGCTTTATTATTATTTAAAGCTAGTGCATAATTTGTTATTAATTTGATCAATTTGTA TGTCAATTTATGATATAATTTTCAGTATTTTTATTCTAAATAGCCCTTAAGGTTATTTTCCAATGCGCAAAATATTG CTTAAAGTGTAAACCACTACTGCGCAAACTACTTAAATACAACTTCATCTCAATGTCTTTCATGTGTTTGACAATGGGCA AGTGCTCCGAGTGAAGACTTGAGATATTTGGATGATTTGGGAAGGTGTCCTTTTGCTTCAGAATGGACATAAATTT CCATCCCAAAAGACAGCAATTACTCTAGAAGATGCTATGCTTAGTGCCAAATACTAGAATGCAAAAAGGAAACATTAATGT GAAAGATGTGAAGAGGTTAAATATAGAATATTTCTATTAAATATGTGTGGTAAAAACCAATGTCCGGTAATAATTTCT AAATGAAATATTATAAATAAATTTAATTTTTATCACTTTTATTATTCTAAATTTAGAATGAAACTACCCAAATTAATAAG AAAGAGTTTGTATGGATTGTCTATAAAACTGCGTTACCTGCTACGGTCCAACTTAAATGAGTCTACTATCTGTGCTAA TAATTACTTCTTTTACGGAATAGATGTGTATCTAAATATGACTGCCGGTGGGAAAGTTATCCAGAACTCAATATAAAAA AATGCAAGAAATGTGATTCAAGTGTCTAAATGTAAATGGAGATCTTATAATAACTGCAGCAATTTGTGCTATAAAATTT CTATCTTTTTCGAAAAATTCGTATATCAGAATGCAACTAATCTGGATTTTATGCTGCAAAAATCGACAAGAAAGTGTAAAA AATGCCATGAATCTTTGAAGAGTGTTTTAATCAATCAAAATATGGGTGCTTAAAAATGCAAGTGAATTAATATTATTTAA TCAAGGAAATGGAATAATTTATTGTAATTTATCTTGCCTGTTAGGATTTTATAAGATTTTAAACAGAGTATAAAATGCC ATCCAAATTCGAAATATGCAAGTTAAAAACAGCTGACAATTTGTATAAAATGCAAGTGAATTAATTAATTTTAA ATTTCTATATTTATATATTTTTAATAATAAACTAACTTTTAAATTTATTGAAAAAAATTAATATTAATAGTAATAGAC TCTTCTAATTAGAATATTTTCCCTGGATAGTTGTCTCCGAAATGAAATGCTATAAATCTTGTCCATAAAACTATTAC AGGGTTTAAAAAAATATTAAAGTGGAGAAGAAACAATATTAATAATGAATATGTATATTTTTATGTTTGTATTTTAT ATTTAAATTTCTAATTTCCATTTGTTCTATAAGCGTAAATTTAATCAGATGGTGAATGAAGACAACTGCAACATTTACGA GTATTATTGTAGCCCTGCCATATATCATGTAAATTTTGTGAAGGACCACTCAGTACTTAATGTTAAAAGTGAACCAATTTAC TGCTTTTTTAGAAAAATAATAGTGATTATAATGTTCTAATAATAAACTGCAGATTTTACATTAAGAATTTTGTTTTG CTTTAAGTCTTATTAAAAATTAATTTTATTATACTAATTTCTTTGATTATATACTAATTTGCTTTGATTGCAATGATTTTATG ATATATTAACTATACATATATTTCTAATATTATAAATATTATTATAAAAAATGACCATAAAATATAAAAAATAAATTTCT TAAATCTATTTTTTTAATACCTTTTCTTAAATAAAGATATAATAAACATAAATAAATACTACATTAATAGATAATTTATGCT TAATATCAAAATTATACATATTATTACGTATATATTCTATAATTTCTAAAAATAAGTAAATTTCTCTTTTTTTTATGGAAAA AATATTAAATGTAATTTATCAAAATTTCTATTTTACTGTTGCAATTTCTCTCTAATAATTAATAAATAGTAAATTTTAA AAAAATAATATAAGTATTGTTTCACCTTGCTGAATAAAATATACATATACATTTTACCCAGTAAATTAAGTTATAGTATGTT CTAATTGA</p> <p>CDS</p> <p>AATTAGAAAAATTAGGATGGAGATGGAGATACAGGATCTCACTGGGAAAGAACCCCTATTTTATAATGAAGTTATGAACC ATGGAACAACCTCTTCATGAACACTGCCATGCTTTGGTTTTCTCATCAGGAGCTCTGATGGTAGATTTTAGAGATACAG AAGGAAATAAAGAGGATTTTCAGAATGTATTCTATTAAACTAATTAATTAGGGGAAATAATTAACCAACATATTGATC ACTCCAGAGGTAAGAAAATTCAGTCTGAATTTTTCGACTGCGATTAGTGTTAGACGACGCTTAAACCGACCCAGCT TATAGAGGAATGTAATTAGAAAATTAAGGTGGTTAAGGAAGTGC CGGAAGTCATTGGGAAAGAACTATTATGTATGAC GAAGTAATGACTGCTTCCAGACTGGGATTTACTAAAAGCTTTTCGGCACTAACATTTGCGATTTTAAGGATTCAAGTT GGTATAAAGTAATGATATTGAACCATCTAAAAATCTATGGGGATATAAGAAAGGTGTCATATTTTAAATATGGTTGC ACACATCCCACCTGCTCAAATTTGAAGAGTTTTGCGTAATTTGATGAATAACCTTTTAAATTAAGCAATTTCAATGCTCAGGTT CAGAGACAGTTTTAGGAGAGTGTTTAATTTCCACAAGTCATTTGGCCTTTATGGATGCTAATTTGGAATTTGCTCAATCCT TTTTCAAATGGTAGATGTTTTGAACCCAAAGATAGCGTTTTTGATCAAAGTTATGCTGCGAGTGGATAAATATAGGTT ATGCGGATTCTAAATGTTTTACAGACTATTGGAACAGTCCGGTAGGGAACTAATTAAGAAATGCTATTATAACAAGTG TTTTGAAGATTTTGGTGTGAAATAACATTAACATAATTCGGGAAAGGAGTTTTCTGTTCTATGTTATTATGAAGTATAGA AACATAGTGATTTGTTGAATTTATCTGGATTTTATCATGTCTGATCCAGAAGCTTTTTGCAAATGCAAAAAAATTTTC TGCCCAAAATGGTGTGGAATAATGGTTATTGTATGGATGGAAATTTGAATTCGCAAAAGGATGGGAGGCGCTAGAT TGCAAGTTTAAAGGAATAACTATTCCCTCGTGAATAAAGATGGAACACATAAGACACGTTATTTTTAAATGAAAAATA AAATGAGTGTTATTCTGGGATTGGTCCGACAAATGTTGCAACCCAGTTTTACCTGATAAATTTGATAAATATGAATAA ATATATTTAATAGAGTGTGTGAAGCCTGCCATATATCTTGTAAAACGCTGTTATGGGGGAGGTTCTGATAATTTGCGCAAG TTGTGATGAAGTCTTTTATTATTAAAAAGCTAGTGCAATAAATAATGCCCTAAGGTATTTTTCCAATGCGCCACAAAA TATGCTTAAAGTGTTAACCACTACTGCCAAAACCTACTTAAAAACAACTTCATCTCAAGTGTCTTTGATGTGTTGAATAG GGCAAGTGTCCCGAGTGTGAAGACTTTGAGATATTGGATGATTTGGGAAGGTGTCTTTTGTCTTCAGAATGTGACAAAT AATTTCCATCCCAAGAGACGATTAATCTAGAAAGATGCTATGCTTAGTGTCAAATAGTGAATTCGAAAAAGGAAACCT TATTGCAAAAGATGTGAAGAGGTTAAATATAGCAATTTTCTATTAAATATGTGTGGGTGAAAAACCAATGTCGAAATGA AATTAACCAAAATTAATAAGAGAGTTTATGTAATTTGCTATAAAACCTGCGTACCTGCTACGCTCAAGCTCAATTAAT GAGTGTCTATCTTGTGCTAATAATTAATCTTTTACGGAATAAGATGTGTATCTAATATGACTGTCCGGTGGGAAGTT ATCCGAATCAATATAAAAAAATGCAGAAATGTGATTCAAGGTGCTTAAAAATGTAATGGAGATTTCTATAAATGCT CAGCGATTTGTGATAAAATTTCTACTCTTTCCGAAATTCGTGTATATCAGAATGCAACTAATCTGGATTTTATGCTGAAA AATCGCAAGAAAGTGTAAAAATGCCATGAATCTTGAAGAGTGTTTTAATCAATCAAAATAGGAGTGTCTTAAATGCT CTAACTGGGCAGGTGCTGATTCAAGGAAATGGAATAATTTATTGTAATTTATCTTGGCCGTTAGGATTTTATAAGATTT TAAACAGATGTATAAAATGCCATCAAATTTGCAAAATATGCAAGTAAAAACAGCTGCAAAATGTATAAAGTCAATAA AGACTCTTCTAATTAGAATATTTTCCCCTGGATAGTTGTCTCCGAAATGAAATGCTATAGCAATCTTGTCCATAAAACCTA TTACAGGGTTTAAAAAATTTTAAAGTGGAGAAGAAACAATATTAAAAATGAATAATGCGATAATTCAGATGGTG AATAAGACCACTCGCAACTTTACGAGTATTATTGTAAGCCCTGCCATATATCATGTAATTTTGTGAAGGACCACTGAC TACTTAATGTAAAGTTGCAACGAATTCCTTTTTTAAAAATAATTAGTGATTTAATGTTCTCAATGTATAAACTGCG AGATTTTACATTTAAGAATTTGTTTTGCTTTAAGCTCTATTAAAAATTAATTTTATTAATACTAATTTCTTGTATTATACTA ATTTCTTTGATTGCAATTTTATGATATATTAACATACATATATTCTAATATTATAAATATTATTTATAAATAGTAC CATAAAAATTAATCAAAATATAATTTCTAATCTCAATTTTTTAACTACTTTTCTAAATAAAGATATAATAAACATAATAGACT ATACATATATGATAATTTTATGCTTAATATACAATATACATATTATTACTATATATTATTTACTAATAATTTATTAAT ATCTCTTTTTTTTTTATGGAATAATCAATATTAATGTAATTTATCAAAATTAATTTTACTGTGCTTCTCTCTCTAAT GTCTAAAAATAAATTAGTAATTTTAAAAATAATTAAAGTATTGTTTACCTTGCTGAATAAAATATACTACATATTATAC CCAGTACTTTAAGTTATAGTTAGTTCTAATTGA</p> <p>Protein</p> <p>METTLHELHAHLVFSSGALMVDRDTEGNKRGLFQNVILLNQLIRGNQNTNLTPEVRKFTAEFFDCDLVDDAQTDPAYRG METTLHGQGGQSGAGSHWERTIMYDEVMTASRLGFTKFSALTFAIFKDSGWYKVNIDIEPSKILWYKYGKGFILKYGCTHP VKFEFEFCVIDEQFPNQSIQCSRDSLGECLISTSHLAFMDANCGIVNPFNSNGRCFEPKDSVFDQSYAGSGKIYRYADSKC FHSTGTGVGGGTQKQYQKGLCLEDLLEITLQFGKGVSVLICYEGQKHSDLNLNSGLFSCPPEAFCKQEKKFCPNWVC ENGYQMDGNMNCNTKNGVYKGLGITIPSCNKGDNITDLFLNEKQNECYSGIGSDKNCPNPDYCNKYNIFNRVRC EACHISKCTCYGGGSDNCASCHESLYLFQSKCNKQCPQGYFSNADKICLKQPPATAKTQNTSSQCLSCVEQGGKCEC KDLRYLDLGRCLLSSECDNINFPKRDYFSRRYACDPCNTRIEKNLNCERCEGKYMINSISQVGNKCNCPNETYPNQNE RVMCDCHKNCVTCYGTQNECYCANNYFYCNVSKYKDCPQSYSPESIQKKCRKCDKRCQKCNCPNENYCNACGN FYLFCNSCISECNSGIFYAEKSTRKQKCHESCKEFCNQSKYGLKCKQTGQVLIQNGKIFYCNLSCLPLGYFKILNRKICKH</p>

	<p>PNCKICSQKTADNCIKCNNRLFQLEYFSPGQLSPKLKCYKSPQNYRVRQKNIQSGEETILKMNNAIQSDQGEQDQNSNIYEYCKPCHISCKFCEGPLSTQCKSCNEFLFLENNQCIQQCSNIQTADFLLRIFCALSIIQNQILFILIISLIYTNFFDCNDFMIYQLYIFYQYKYLFILKMYHKINQIISNSIFLILFNKDIIINQILYSYNDNLCLIYNYLLLLYILKIIVKIFFFYGKFNINVIYQITFYCCILLLMSKNKISKFQNNYKYCFETLLNKIYYTFYPLVQIVVSSN</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF3) Genee, complete cds</p> <p>GENE: 3107 bp. Exons: 1-474; 677-736; 796-1652; 1723-2245; 2310-3107 CDS: 2712 bp protein: 903 aa</p>	<p>Gene</p> <p>ATGGAAACAACTCTTCATGAACATGCCCATGCTTTGGTTTTCTCATCAGGAGCTCTGATGGTAGATTTTAGAGATACAGATGAAGTAGATAATAGAAATTTTCCTGATCTTATGCTGCTCTTTTCACTCATCTTTAGAAAGGAAACATAAATGTGTTCA TAATTAACTTAAAGAACAAATCATAAAGACTCGATTAGCCAAGACCTGAACCTAGGGGAAGAAAATGCAAGGATA AAAATAAAATATCAGGGTGACATTGGACTACTCCTCTCTTGAGACTGTTCCCTCGGAATACGATTATGTAAGAAATGAA ATTTATTGAATAGTTAGATTTAGCTTCTAAGTTTTCGGGAGAAATGCTCAAAGTAGAGAGATTAAACATATAATACTACTT TCCTGATTGGGGGTATGAACACAAATGTGTGGATGTTTTCCCTCAITTAAGACGATATATAGTATGGAGTCCCAATTC T GATTGTCATATTTATGTGCACCACTATCATGATCCTAAAGATGGAGTTCTAGCATATGCTGATTCTGTCACTGGAGCTG TAAATTTTTTTTAACTTTTGTGTTTGTATGCTAGCTATATATAAGCAACCAAAATATGAAATTAATCATTTATTAATA TCITTTAAITTTAATTAGAAAAGTAAATATGCTATATAATAATTATAACTTACACAAATATTTTAAATAAATAATTAAIT TTATACCAITTTCAACTTTTCGTTTATAAAAAAAGGATGTCAATAGACCCCTGTTTGGAGGAATACCTTTAATACTGG AGTTTTGAAGCTAGTGTAAAAACAATAATATTTAAAAATTTAATTTTAAATAAAATCAITTTATCTGTAGGAAAAAC ATAGACATGACCAATTTAGGATAAATTTGAAACAATTTTACATGAATCATCCATGTTTATAGTTTTTCTGACGGGAATG TGGGAATATTTTAAATCCTGCTACAGGCTTAGCTTATGGAAAGACAATAAACCAAGCATGATAGTTTAGACCAATAAGG GAAAGAAATCAAATATTTTACCACCTCCCAAAATAACAAAATAGCAAGAGAGCATTTCGGATGTGATAGTCTACAAGG AATCCAAATGGAATAAGGAGGTTGATGGTTCTGCAGGTTCTCATTTGAAAGAGTCATTTAGAAAATGAATATATG ACTGCGAGTGCTATATCTCATGACGCGTCTCTGACTGCCTTTACCATTGCTGTGTTAGAAGATTCTGGGTGGTACGAA TCTACAGCGATGAAATCGATGAGATTGACTGGGGGAAGGGCCAAAGGATGCAATTTTTAACCTCTTGCGATGCTAAC AACCTCAGAGAAATTTGATTTCTTAAACGTAATGCAGTTTTTACCATTCTGGAGTGGGAAGTGCACCAACCGGAATAACG ACAGCTTTAATGATAATTTGCAATATTACCTACATATATAGAGACAGTTTTATGCTTAGATACCGTAATATCAGAAAAAT GATGATATTTATGGAATGTGTATGGTTAGAACAAATAGATGTTTTTACAGTAATATTTTGCACTCTCACAAGGTACCCAC CACCTAAAGCGAAAGTGTCTACCTGGGTTTGTAAACAAAGATGGAACGAAATTTGAGTTAGCTTGGGGAATA GTATCCTCTTGATAAGTTACAAATAAAGTAGATGTTTCAGCTAAGGGGTCCAATCAAAGGTAAATCAATATATACACTTT TTTTCAGCTAAAAAAAATATTTTAAAAATTTTTCTTTAAAAAATAGACTAGAAGGATATGTCAAAAGGACCGAGAAGA CTGGAATGCTTTCTGCAATACTACCCCTTGACCTGTCTCAACTCTGTTCTCTCCACGGGTATTGTTTAGGAAGACAG TGTCACCTGGGATTAGATTACGACGGGTCTGACTGCAATCTAAATGCGAAAAATATGGTTACATCTTATAAGGAAT GCTTAAATCTTGAAGATGCTGATGGTACTACCTCAACTGGCGCTCCAAAGAGTGTCTACCCAATGAAATATAGCG CTTCCTGTAATCCTGGGGATTTTAAATCAACTAAGAAATGTTTTCTTCTGCTGAGGATGCTCAGAGGAAGGAGA TTCATGCCAAGCATGCAATTTATCTTGCAACTCCTGCACCACATACTAATTCTGTGAAACTGCAAGGATGGATTTTAT CTTTATGATAATAATGGCCTCAATAGTGTGTTAATAAATGTCCTCAATCTACTATCCAAACAAAAATTAAGAGTGCAAT AAACCTAGGCAATTTGTGATGTACAGCAATTTATTTATTTTAAATAATAAACCAATAACAAAAATTTTATTTTTTTC CAAATCTGCTGCAATATCTGCTCAATTTAATAAGTGTGCTGCTTTCAGTGAAGGCGCAATTTAATCAATAAGTAACT TGCTGCACCTGTATGCTGTTTGTAAACCTTGAAACCTCCAATACCTCTGTACTCTGCGACGCAATTTGTTTTCCTA CTAAGCAATATGCTGGGAAATGCTCTTGGGTATGTTGCCAATTAGAAAAATGATGTGCGAAGCATGTGCTACTGA ATTTTGTGCACAGTGTCTGCTGCAACTCTGTACGAATGCTATCTGGATACTACTAGAAATAAGCAACTAAACC TGTTGTAAGTGTTCTTAAACATGTTTAACTGTACATCTTAAAGAAAAATGCGTGACTGTGCTCAGGATCAGAAAGAG TAACTAATGGTGATGGAAGAAATGTATGTGCATTAATAATTTTGAAGGCTATTTCTAAGTGGTGATAATACATG CAATAAATGTCACAAATAATTTGCTTTAGTGTACATCATCTTAAGTATGCACCAAAATGCTAAGAAAAACACCTTTTGTATT AAAAATAAGGAATATGCCATCTTTGTAAACAGACATTTGAAAACCTGTTCCGATACTAAGTATTTATCTGTGGAAGAT GAGTTACTTGAGCTTAATATAATGGGGAATCTTAGTTGTGTGAAGAAATGTCTACAAAAATTAATAAAGAAAAAACCG AATGTAAAAAACTTCAAATCTGGAAGTCCCATTTTATGGCTGCTTTCAATATTTTGAATATTTATCAATCAAT CTGA</p> <p>CDS</p> <p>ATGAAGTAGATAATAGAAATTTTCTGATCTTATGCTGCTTTTCATCCTATCTTTAGAAAGGAAACATAAATGTGTTCA TAATTAACTTAAAGAACAAATCATAAAGACTCGATTAGCCAAGACCTGAACCTAGGGGAAGAAAATGCAAGGATA AAAATAAAATATCAGGGTGACATTGGACTACTCCTCTCTTGAGACTGTTCCCTCGGAATACGATTATGTAAGAAATGAA ATTTATTGAATAGTTAGATTTAGCTTCTAAGTTTTCGGGAGAAATGCTCAAAGTAGAGAGATTAAACATATAATACTACTT TCCTGATTGGGGGTATGAACACAAATGTGTGGATGTTTTCCCTCAITTAAGACGATATATAGTATGGAGTCCCAATTC T GATTGTCATATTTATGTGCACCACTATCATGATCCTAAAGATGGAGTTCTAGCATATGCTGATTCTGTCACTGGAGCTG ATGTCAATGACCCCTGTTTGGAGGAATTACCTTTAATACTGGAGTTTTTGAAGCTAGTGAAAAAATACAGACATGACCA ATTTGAGGATAATTTGAAACAATTTTACATGAAATCATCCATGTTTTAGGTTTTCTGACGGGAATGGGAAATATTTTA TAAATCCTGCTACAGGCTTACGTATGGAAGAGACAAATAACCAAGTGATAGTTTAGACCATAGAAGGAAGAACAA ATATCTTACCACCTCCCAAAATAACAAAATAGCAAGAGAGCATTTCGGATGTGATAGTCTACAGGAATCCAAATGGGA AATGAAGGAGGTTGATGGTTCTGCAGGTTCTCATTTGAAAGAGTCATTTAGAAAAATGAATATGACTGAGGATGCT ATATCTCATGACGCGTCTCTGACTGCCCTTACCATTGCTGTGTTAGAAGATTCTGGGTGGTACGAATCTACAGGCATG AAGATCGATGAGATTGACTGGGGGAAGGGCCAAAGGATGCAATTTTTAACCTCTTGCGATGCTTAAACATCTAGAGAA TTTGATTTCTTTAAACGTAAATGCAAGTTTTTACCATTCTGGAAGTGGCAATGCAAAACCGGAATGACGACGCTTAATG ATAAATGCAATATAACTATATACCTTTATGCTCTTATAGATACCGCAATATCAGAAAAATGATGATTTTAT GGAATGCTGATGTTGTTAGCAACAAATAGATGTTTACAGTAATTTTTCAGCTCTCACAAGTACCAAGCTTAAAGCG CAAGATGTCTACCTTGGGTTGTAAACAAAGATGGAAGTGAATTTCAAGTTATGCTAGCTGGGGAAGATTCCTCTTGA TAAAGTTACAAAAATAGATAGTGTTCAGCTAAGGGGTCCAATCAAGACTAGAAGATATGCAAGGACCGAGAAGA CTGGAATGCTTTCTGCAATACTACCCCTTGACCTGTCTCAACTCTGTTCTCTCCACGGGTATTGTTAGGAAGACAG TGTCACCTGGGATTAGATTACGACGGGTCTGACTGCAATACTAAATGCGAAAAATATGGTTACATCTTATAAAGGAAAT GCTTAAATCTTGAAGATGCTGATGGTACTACCTCAACTGGCGCTCCAAAGAGTGTCTACCCAATGAAATATAGCG CTTCCTGTAATCCTGGGGATTTTAAATCAACTAAGAAATGTTTTCTTCTGCTGAGGATGCTCAGAGGAAGGAGA TTCATGCCAAGCATGCAATTTATCTTGCAACTCCTGCACCACATACTAATTCTGTGAAACTGCAAGGATGGATTTTAT CTTTATGATAATAATGGCCTCAATAGTGTGTTAATAAATGTCCTCAATCTACTACTACCAACAAAAATTAAGAGTGCAAT AAACCTAGGCAATTTGTCAATAATCGTGCAAATACTGTGCAGTTTAAATGAATGTTCTCTTGCAGTGAAGGCGAGAT T TAAATGAAAAATAAACTTCTCCACTGTATGCTGTTTGTAAACCTTGAAAACTCCAATACCTGTGATTACTTGTGCA GGCAAATTTGTTTTCTACTAAAGCAAAATGTCTGGGAAAAATGCTCTTGGGTATGTTGCCAATTAGAAAAATGATGTG GAAGCATGTGCTACTGAAATTTTGTGCACAGTGTCTGCTGTCAACTCTTGCTACGAATGCTATACTGACTACTTAG AATAAGCAACTAAACCTGTGTAAAGTGTCTTAAACCTGTTTAACTGTACATCTTAAAGATGCAACCAATGCTCTGTGCT TCAGGATCAGAAAGAGTAACTATGGTGATGGAAGGTTGATGTGCATTAAAATGTTTAGAAGGCTATTTTCCATAAGTG GTGATAATACTACATGCAATAAATGTCCAAATAATTTGTCTTTAGTGTACATCATCTTAAAGTATGCGCAAAATGCTCAAGAA AACACCTGTTTTTGTATAAAAATAAGGAATATGCCATCTTTGTAAACAGACATTTGAAAACTTGTGCGGATTAATAGCCTG TTTATCTTGTGAAGATGAGTTACTTGAGCTTAATATAATGGGGAATCTTAGTTGTGTGTAAGGATGCTCTCAAAATTTATA AAAAGAAAAAACGAATGTAAAAAACTTCAAATCTGGAAGTCCCATTTTATGGCTGCTTTCAATATTTTGAATTTTGAATTT TATTTTATCATAAATCTGA</p> <p>Protein</p> <p>MKQIEIFLILCCLFILSRKHKVHNQLKRTNHNKRLDQPRPEPRGRKLQGGQKNIRVTFDYSSLETVPLEYDVYKNEIEQL DLASKFFGEMLKVERLTYYNNYFPDWGYEHKCVDFVFPHQDDIQYGVPSNLDHIYVHHYHDPKGLVAYADSHWITVDNRP LFGGITFNTGFEASEKHRHDQFEDNFETILHEIHLVGFSDGMWEYFINPATGLAYGKNKFPVKVITGRKKSNTLTPWKTK LAREHFCDLSLQIQMIENEGGGDAGSHFERVILENEYMTASAIHSDASLTAFTIAVLEDSGWYESTGMKIDEIDWGGKG GKNFLTSDANNFRFESSQTCQSFYHSGVGTAKFEPFDNQTCFIDNDCNITFYRDSLCLDITDNIRKNDIDYGNVYQGNRRCFY SNIFDSSQGTITTSERCLPWCKDGTGEISVIVAEKYPYLDKVNVDYSAKGSKRQLEQYKGGPREDVNAFNTYPLTCP NFCSHHYVCLGROCHCGLDYAGSDNCTKCNENGYIYKKGKCLNSOECPDGTLLNWPSSKECLLPNENASCNRPDGFKNQE CVSSCPAGFYREGDSCQACNYSQCQSTTYQFCETCKDGFYLDNNGLKQCVNKC PKYYPNNKQECINQAIKHSKCYC AVQNECSLCSSEGEYLNENKLCSTCMSVCKTCENSNTCVTCEANLFYFQSKCLGKCLPVYVANNQKNVCEACFAECQACS AVNSCEYCYGTGYLLEISNQTCVKCSQNCFNCTSKENCLTCAAGSERVTNGDGKVVCLAKLEGYFLSDNTTCKNCPQNN CLOQTSLSQVCTKCQENTFFDQKQIGICHLNHRHCKTCSDTNACLSCDELELLELIMGNLSCVQCEPTNYKKKKNECKKTSN SGPILLAAINLIIFIIII</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF4) Genee, complete cds</p> <p>GENE: 2851 bp. Exons: 11-70; 144-213; 264-576; 638-858; 917-1091; 1155-1203; 1274-1387; 1450-1494; 1556-1849; 1918-2016; 2127-2354; 2622-2851 CDS: 1908 bp protein: 636 aa</p>	<p>Gene</p> <p>ATGATTTAAATGAATTTATGACAGCTCTGTGATTTTCAACCGATGCAGTAATGTCCTAAATTTCTTTAGGTAATAAATTTT TTTTGAATATCAATAATTTGTAATAATAATAAATAAATATTTTATATAGATATAAATACAGCTCTCTTGAAGATTTCAAT TGGTATAAAGATGTAAGAGTAGATGATTTCCGAGAAGATTCTCTTATGGTTATTCATTTGTTAAATTAGATATTCTA ACCCATTTGTTATTTCTGAGGGGAAAGGAAAAAGAAAGAAATTTATGAGAAATAACCTTGCACAAAGGCTGATGGAT CACTTTACGATGAATATTTTCCCGGGTGATATGGAAGTGAAGATTAATGTGATTTTTCATCACTTTGGGTTCGGAAGCTGC TACCAACCAACTTTTATTAGCCAATGTAAAGTCATTTCTATACAGTAATGCAGACTGCACCAACAAATGAAAGATGAC GATAATGCCGAAAGTTATGTTGAAAAACATTTGAAAAAAGGAATTAATAGCAAAATGTTTATGAGTACTATTAACAACAC T AGTTTTAGATCTTACGTAATAATATATAATATTTAAAAATTTTGCAAAATATTTTAAATAATTTAAATTTAAGAAATG CTATCAATACTATTGCGACAGCAATTAATAATCTTGATGATAATTTATGTAAAAAGGAAGTGAATATGAAGTTGGAAGAAC TTTATTCGCACTAAAGTAACCAAGGTCAAGAAATTAATGCCCCACGGAATGACAGAGAAATTAATAGCCCTAATA GTATTGCAACTTTTTCGCGATTGCCAGTTCCATGCCCTAACTATTGTTCTGGAAAAAGGTACTATTAGAAATTTTACAAA GTTTAACAATAATTTTAAATATAATATATTTAATAGATATTGCTTAAAGAAATATGAAGGATGAAGTAAAGGAAGCACT GTCACTGTGTAGATAAATGAAGGAGTTATGATTGCAGTATTTAATGCGCTCTCGGGGAATATGTTCTGGAACATAAAAA TACATGTACCGCTGTTTCTCTGCTGGATATACCAAAAAATGATTATAACAGAGTAATTTTAAATAAATTTTCAATCA TCGAATAATAAATCATTTTTTAAATTTTATTAAGGTGTGTGATTAAATTAATGTCATGGAAACTGTAAATATACTTGCA CAGGTAATAAATCAATTTCTAACAGCAATTAACAAGTAATCTTTATATAAATAATTTTTTAAATAAATAGGTCCTAAT GAAAGTGATTGTACAGATTGCCCAATAGGAAAAATCTTTCATAACAAATAATGTGTTTATAGTGTCTCTCACTGATTTT TACGTATTGAAGTGAAGAAATTTGCAAAAGGTATTTTCTTTTATTAACATCTTTTATTAATGATTAATGATTTTAT TTAGAGCTTGTACCAATAATGCAAAATCCGGAAGAAAGATGTATAGAAATGTGTAAAACCTTATAATTTAGTATATTTAAT GTATATATAACAAAAATAAATTTTATATAGAAACTGTAGACTACAGAAATATACCTGTAGTGAATATGATGGAG</p>

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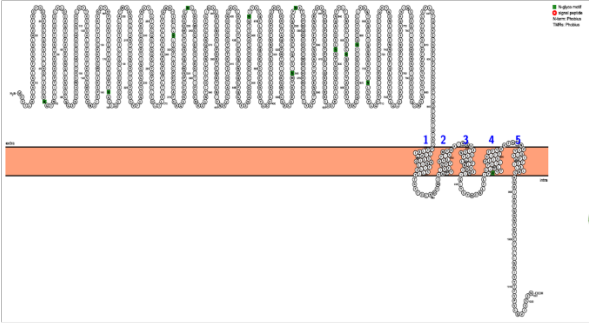
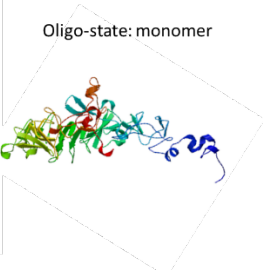


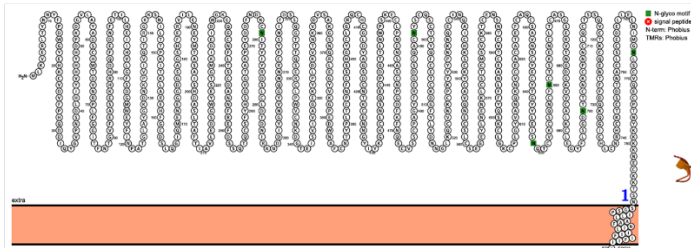
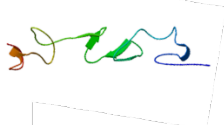

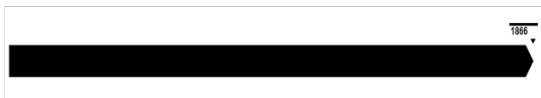
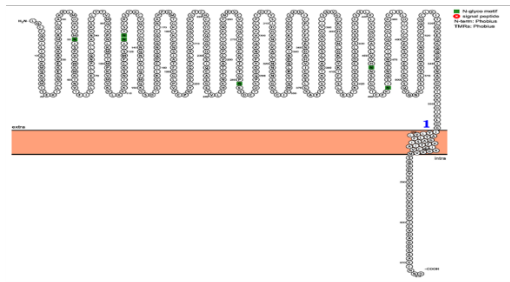
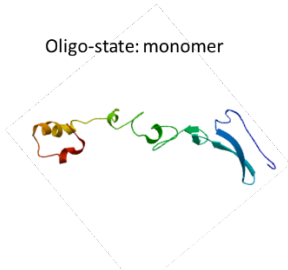
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<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF6) Genes, complete cds</p> <p>GENE: 2834 bp. Exons: 1-255; 322-1178; 1247- 2072; 2139-2834 CDS: 2634 bp protein: 878 aa</p>	<p>Gene</p> <p>ATGCTTCTCGTAGAAAAGATTGACTGAAAAATAATTACTTCCAGAAATGGGGTAGATAAGATAAATGCATGGAACATACC CTCTGAAAAAGATATAAAGAAAGGAGTCGCTAATTCAGATCTCCACATTTATGTTTCTCATTATCCAGCACCTTAAAGA TGGATATTAGCCTTTGCTGATTTCATGCCATTGGACCTTCATACAATAGACCAATTTTGGAGGAATTAACCTTTAACAC GGAGGATTTTTCAGCAAGTGTAAAAATTAATTTTAAAAATTAACACTTTAAAGTACCAAAATTAATTTCTATTAAATTTAA GAGGAAAAATACAGAAATGACTATTTTGAAGACAATTTGAAACAGTAGTACATGAAATATGACCGTAGTAGGATTTT CCACCGGAATGTATGAATCTTATTAAACCTGCCACTGGAAGGCTTATGGAAGGATAACCAACCCCTTGATTTTAA AACCATTAGAGGATAAAAACTGGAATTTCTACTACCCCAAAAGTAACAAAAATAGCCAGAGAGCATTTCGGGTGCC CACTTTAGAAAGGAATTCAAATGGAATAAGGAGGAGAGTGCCTCTGCAGGAGCCCACTTCGAAGAGTAGTCTTAG AAAAATGAATTTATGACCGCTGGTGCCATTGCTCATGACGCCCAACTACCAGACTTCACCATGGCTGTTTAGAAGATT CTGGATTTTGAATCGACAGAAATTAATAGATACTATTGACTGGGAAAAAGGACAAGTTTGCAACTTTTGAACCTGA ATGCACAGCAGGAAAAATACAGAGAATTTGACAGTTCGCCGCCCAATGCAGTTTCTATCACTCTGGAGTCGGAAGAG 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CTGCGAATTAGGATTGTATTAACACACAGTTTAGTCCCAATGAACATGCGCT TCAAAATGCCTCGAAAA CTCTACGTTTCTTCAACTTTTATGATGATCGATTAAATCCATTTGGGATAAATCTGCTGAA GTTGCGAAA ACTCTTCACTGTGTCTCTGTAAACCCGATTTTACTCCCAACTCTCAATTTTAAACAAATGGCCC CGTTGGTTTCGGAATAACCAATAAAAACAATGCTAAGTCTGCTCTAATAATGATATGTGAAGAAATGT TCAGATGTA AATACTTTGACCAATGCAAAATCAGGACATTTCTTAGACTCTAGCAAGGAACTTTGTGCA AATGTTTCCAATTTGCGA AAGAATGCACCTTCTTCACTAAATGCTTATCTTGAAGTGTGAATCTGTAAA AGTTGAAATGCAGGAAGCGGAACCTG CTAAGTGGCTTTAAAAATTTGAAGGAAGGAAATTCCTGGATGAA AAATCTGCTACCTGTGAAAAATGCCCAAAATG GTCTTCTTGTAAAAATGCCAACTCTTGACCAATTT GTGGACAAATACTTTCTTAGACAAGGAAAAATGTGTGAATG CAATCAACTTTGCAAAACCTGCTCTCC TGATAATTTCTGTCTTACCTGTGAAGACTAAGCATTTGAATTTGATTCGAA AAAAAATCTGTTGTGA GATAAATGCCCTGCTGAACATAAATGGAAGGAAGTAAATGTGTTAAATCATTCTCTGGAA TTTGGAATTTGCTTATATTTTGGTTCTCACAATTAATAGTTATTTTGAATTTGA</p> <p>Protein</p> <p>MLLVERLTENNYFPEWGRQDKMETYPPEKDIKEGVANSDLHIYVSHYHDPKDGYLAFADSCHWTSYNRPFGGITTNTG GFLASGKYRNDYFDNFETVYHEIMHYFNSGMYEYFNAPATGKAYKDNPLIFKTRGQKTLGTPKVKTLAREHFCG PTLEGIOMENEGDASAGAHFERVLENEFMTAGAIAHDQLTDFMAYLEDSGFYSTGIKLDTIQWKGQGNFLTEC TAGKYREFDSSAAQCSFYHSGVGRAPELDYYNDNCNITAIYDLSLCFDNINRIPLDPVKNGNVIYNINRCFYSNIDIEGQP TDLQRCLPWVNCEDGSGVYIIVGGEKYAINKATNKADIKQGNTRLEGYVNGPADWQGFNTYPLQCPNFSAHYCYCL GRQCHCGVGYAGSDCSIQCGNNYLNHGTCLNSQVCPDGTANKYTKCEIKNPDPAPCSEGGFKHAGCQVSSCKPDFF KEGNSQCACNYQCTSGGYQFCDTCREGFYLFQANGKKECITKCLKGTTLDQKNECINDNLLPCPNONEFRLQKGVH QCPSKLFRLLNNSCVNQCPKEYYSAQLCQKCDSDSKTCSNGNACDTCELGLYQHTVQSNKQCLSKCPENFYGSSNFQCI DQSIDKSCQSCENSNSCVSKPDFYSHNSQCLTKCPVFGNNQKQCOQVCSNNDICEECSDVNTCTKCKSGHFLDSK GTCVCKSNLCKECTSSTKCLCEVSVKVENAEDGTAKCVKNCKEGEFLDEKSATCEKCPKNCLCKNANSCTNGCQGS FLDKEKCEVQNLCKCTCPSDNCLTCEQDAFEFYIQKQLVCVDKCPAEHLKSGSKVKFSFSGILNFAIYLVLTIVILI</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF7) Genes, complete cds</p> <p>GENE: 2870 bp. Exons: 1-50; 116-263; 345-524; 604-666; 737-956; 1018-1384; 1442-1693; 1761-2145; 2208- 2870 CDS: 2328 bp</p>	<p>Gene</p> <p>ATGACCCATGATCACTATATTGAACCGAGCAATGATATGAACCTCCAGAAGGTATATATATATTGTTTAAAACTATATCTA ATATGAGTTTGTATCAATAAATTAATAATTTAGAGAGTTACAAGTACTGAATACGAACCTTTAAGAGTAACCTTTTGA CTTTTCTAGTTTAGATTCTTTAGCATCATCAAGGCCTGCAGTTATTGATTACATTAAAAAGATAGGTATATATGCGAGCTT AGTTTTCGGCCTAAAACTTAAGAGTAATTAATAATTTGAGTATTTTATTAITACTGACATGAAGTATTTTATATAAAA CTTAAACACTTTTTTTTTCAAGGTTAGTAAGAGTTTAAAGAAATAACAAATTCCTGCTTTTACTCTAAATGTTAGGGA TCTGCTCCTCCTTAAAGATGATAAGATGTTGGTATTCTCTAATTCGACTTGACACTATATATTCTCCAGTACGAAGATT TGAATCCTCCACTCTGCTTTTGTCTGGAGCTTGTGCTTTGACCGTAAGAAGTTTATTTTATAAATACTAAAAAGTGAAC CATAATATAGAAATGTTAATTAATATAATTAATTAATTAAGGATACAATAAGGAAGCACTTCTCGGTAGAGCTACTTA CAATCTTGCTAAGATTTCATAAAATGAAGTTTATTAATTTTCATATAATATATATTGTTTACCTTTTACATATATTTCT AATCAACTATAGCCTTCTCATGAAGACTTTGAAGATGATTAGAAACAACCTCTTCAGCAAAATTTACTCAGTTTAAAGGT TTGGAAGTTGGATGTATTAATACTTCATTAACCCCGCTACTGGAAGAGCCTATGGAGGAAGTAAATGAACCCATTTGAC CGAAACTATTAGAGATCATGACTCCTAAATATTGCTACCCCTAGAGTTTAAAGAGTTACAAGAGATGATACCTGGAAT TTTTAATTTTAACTTTTAAATATTTATTTATAATATTTTCACTCTTAGGATGTTCTACCGCTAAAGGAATGCAAA TTAGAAAACTAACTGACTTCAAAAAATCTCACTGGGAAGCTACTGTTTATATAACGAATTTATGTACGCTAGAGCTGT</p>

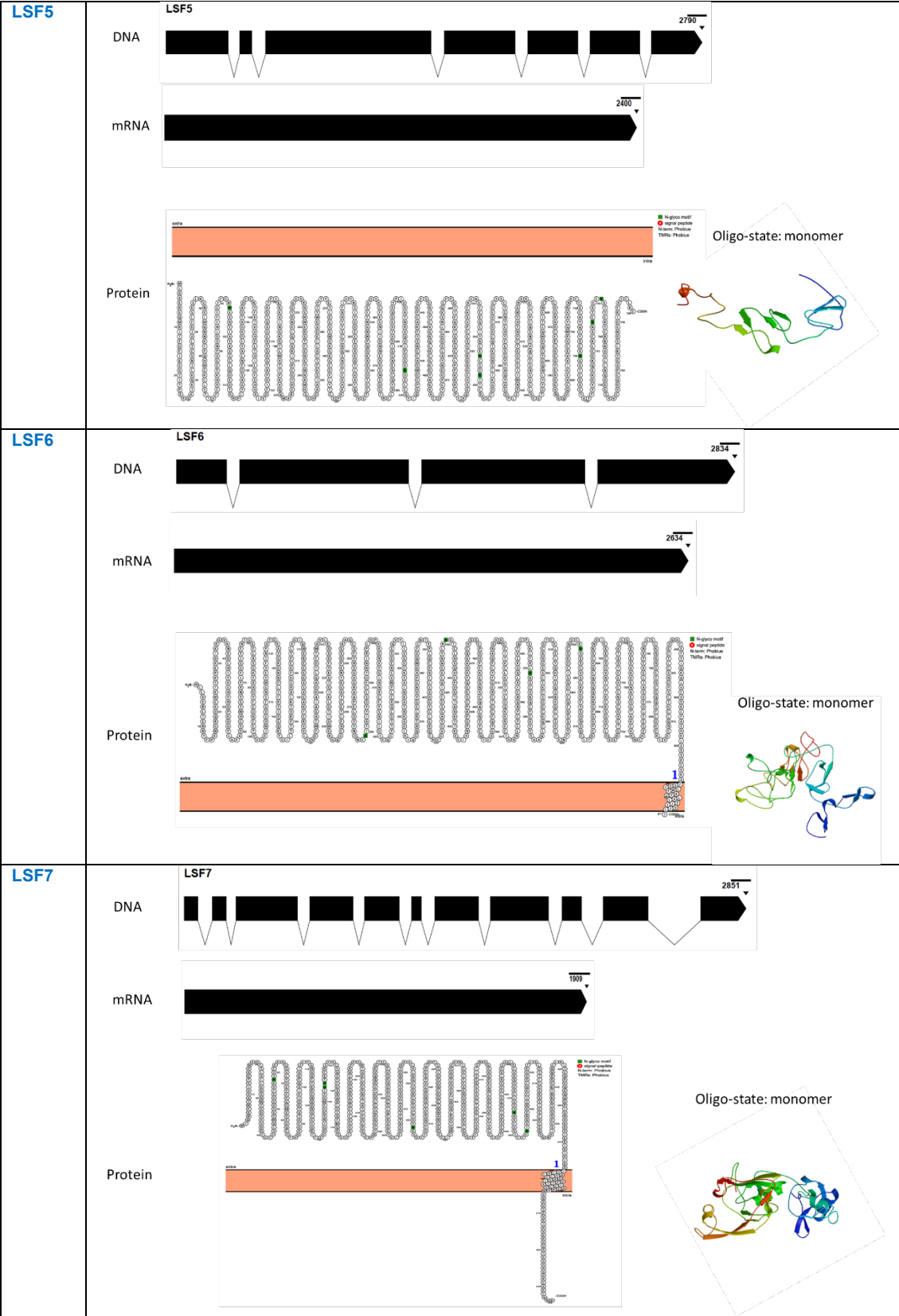
<p>protein: 776 aa</p>	<p>TAGTGGAGATGGAGCTTATTACGCTTTTCACTTTTGCTTTATTAGAAGACTCTGGATGGTACAAAGCTGTTACGAAAAAT GCTGATGATTACAATGGGAAAAAATTAAGGATGTGATTTCTTAACCTCTTGATCTCAACAAATTCAGAGAATTCAG GAAATAGCAGCAGCCAAATGTGATTTCTTTAACAAGGATAGGATACTCAACTGTTGAAACAATACACTGATACATGTAG TGTTCTTAAGATATATAAATAACAGGTATATTAATAATTTTAAACAAAATTTAGAAAATATTATTTTCAATTATTAATAGA CACTGTTGCAGATACATAGAAGCTGGTGGAAATGATTAACTACTCGGATTCCATTGGGGAACAAGAGAGTAGATGTTT GAAAGTAATATCGTAGACAGAAGAATTACCAGCCACTCTTAATTCGATTAAAGATGTATTGAATATGTAATGATTTCT AAGGAGGAATTTGATGTTTACGTACAAGGAGTTAAATATACTATTGTAGTAGAAGCTACTAACAAAGGCAAGCGTATCCCCAAA AGATGCTATAACAATAGTATTAATTAATTAATAAATGAACCTCAACAAATCTTAATTTCAATTTTCAATTTTAA TATACCGGAGAAGTGCCTGCTTAAGCTGAGGATGATTTCTGTAATAACCACTCTCTCAATTTCTGTAATTTCTG AGCTCTCAAGGATATTGTTAAGATAAAAATGTAATTGTATATTGAAGCTTTGCTGGAGAAGATTGTAGTATTAGTTGTCC CAATGGTAGCTATTGCCAATGGATAATGTTTGAACAATAAAAGCTGTCCCTCTGTTTCTAAGTCAACCAAGGAAT AAAGTTTGTGAAGTATCCTCTCTCTTAATAATGGAAATGAAGGAAACAATGGAATAATGGAACACCAATGCCAATG GATGTTTATTGACTTAAGTCTCCACGAAGGAACTGCATTAGTGTGTTGCCAGTGGATACATGCCGTAAAGATATT TAAACATAAAAAATAATCAATAATCAAAATTAATTTAAATATTAAATATAGGATTCTGATAAAAACTGCCAAGATGTAAC GCCACTTGCTTAACCTGCAACGACAATCTACTTGTTCTAGTTGTACCGAAGGATCTTTCTTAAACTCTAGTAAATATG TACTGTTTGTCAATAGGGATGCCAACAATGCAATAACGGAACCTCATGTTAATCCTGTACCGCTTCTTCAACAATCATTA ATTGATTCTAATGGATAAATTATTATGTTTGAACATAATGTCCTCCACTCACTTCCTTTAATAAGGCAATGTTTAGCTTGT AATTGAGGATGTTCCAGCTGTTCTGATAGTTTGTACTGTTTGACATGTTCAAGTTCCTATTACAAATATGAAACAATG AAAAATACAATGTTGTTGTTCTTGTCTGAAGGTTATTAAAGCTGACGGAACAGAGTTTGCCAATAACAAACTACCTAA CCTACTACTTGCAAAAGCAATTAATATTAAATGAACCTGGAGAATGATTGACTGCTCACTATAGTTGTAAGACTGTG GAGATTGAGCATATAGAGTTTAAATTAATGACTTCTGTTTGTGATGGATTCTATTAACTTTGGAGAAGTGGAAAA GGAAGATGTTATAACAGATGTTACTCAGGATACACCTTAATTGGAGACAAATGCTTAGACCTGATTAAATAGTGA</p> <p>CDS</p> <p>ATGACCCATGATCACTATATTGAACCGAGCAATGATGAACCTCCAGAAGAGAGTTACAAAGTACTGAATACGAACCT TTAAGAGTAACCTTTGACTTTTCTAGTTTGAATCTTTAGCATCATCAAGGCCTGCAGTTATTGATTACATTGCGGTAAAGATATT GTGTAATTGGCAGCTTAGTTTTTGGCCTAAAACCTTAAGAGTAGTAAGAGTTTAAAGGAAATAACAAATTCCTCGCTTT TACTCTAAATGTTAGGGATCTGCTCCTCCTTAAAATGATAAAGATGTTGGTATTCCATAATCTGACTTGCACATCTATAT TTCCCACTACGAAGATTGTAATCCTCCACTCTTGCTTTTGTGGAGCTTGTGCTTTGACCGGATACAATGGAAGACC TACTTTCGGTAGAGCTACTTACAATCTTGCTAAGATTTCATAAAAATCCTTCTCATGAAGACTTGAAGATGATTAGAAA CAACTCTTCAAGAAATTACTCACGTTTAGGGTTTGGAAAGTTGGATGTATTAACTCATTAACCCCGCTGAAAA AGGCTATTGGAGAAGGTAAATGAACCCATTGTTACCGAACTATTAGAGGTATCAGTACCTAAATATTGTCACCCCTAGA GTTTAAAGAGTTACAAGAAGATACTTCGGATGTTCTACCGCTAAAGGAATGCAATTTAGAAAACTAAACTGACTCTAAAA ATTCTCACTGGGAAGCTACTGTTTATATAACGAATTTATGTCAAGCTAGAGCTGTAGTGAGATGGAGCTTATTACAGC TTTCACTTTTGCTTTATTAGAAAGCTCTGATGCTGACAAAGCTGTTCCAGAAATCTGATGATTTCATGTCGCGAAAA AATTAAAGGATGCTATTCTTAAGCTCTTGCTGATCCTAACAAATTCAGCAATTCAGAAATGACGACGCGCAATGTGATT TCTTTAACAAGGTATAGGATACTCAACTGTTGAACAATACACTGATACTGTAGTGTCTCAAGATATATAATACAGA CACTGTTCCAGATACATACAAGCTGGTGGAAATGATTAACTTCGGAATCCATTGGGGAAGAGATGATTGTTT GAAAGTAATATCGTAGACAGAAGAATTACCAGCCACTCTTATTCTGATTAAAGATGTATGAATAATGATGTACTGCTC AAGGAGGAATTGATGTTTACGTACAAGGAGTTAAATATACTATTGATAGAAGCTACTCAAGCAAGCAAGCATGCCAA AGATGCTAACAATAATATACAGCGGAAGAAGTCAATGGTCTCTGCTAAGTGGGATGATTCTGCTAATAACACTCCTCAAT TGTAATAATTTGCGAGCTCTCAAGGATATTGTTAAGATAAAAATGTAATTGATATTGGATTTGCTGGAGAAGATTG TAGTATTAGTTTGTCCCAATGGTAGCTATTGCCAATGGATAATGTTGAACAATAAAGAGTGTCCCTCTGGTTTCTAA GTCAACCAAGGAACATAAGTTTGTGAAGTATCCTCTTCTTAATAATGGAATGAAGGAACCAATGGAATAATGGAA ACACCAATGCCAATGGATGTTTATTGACTTAAGTTCTCCACGAAGGAACTGCATTAGTGTGTCGAGTTGGATACTA TGCCGATTCTGATAAAAACTGCCAAGATGTAACGCCACTTGCTTAACCTTGAACGCAATCTACTCTTGTAGTTGT ACCGAAGGATCTTTCTTAACTCTAGTAATAATGACTGTTTGTCAATAGGGATGCCAACAATGCAATAACGGAACCT CATGTTAATCTGTACCGCTCTTCAACAATCATTAAATGATTCTAATGGATAATTATTATTGTTGAACATGTCCTTCCA CTCACTTCTTTAATAAGGCAATGTTTAGCTTGAATTCAGGATGTTCCAGCTGTTCTGATAGTTTGTACTTGTGACA TGTTCAAGTTCTCATTACAAATATGAACAATGGAATAATACAATGTTTGGTTTCTGTGCTGAGTATTATTAGCTGA CGGAAACAGAGTTTGCCAAATAACAACACTACCTAACCTACTACTTGCAAAAGCAATTAATATTAAATGAACTTGGAGAA TGATTAGCTGTCACTATAGTTTGAAGACTTGGAGATTGAGCATATAGAGTTTATAATTAAATGACTTCTGTTTGTGA TGGATTCTATTAACTGTTTGGAGAAGTGGAAAGGAGAGATGTTAACAAGATGTTACTCAGGATACACCTTAATTGGGA GACAAATGCTTAGACCTGATTAAATAGTGA TTGCTTATATTGTTTCTACAATTATAGTATTATTGATTGA</p> <p>Protein</p> <p>MTHDHYIEPSNDMNSRRELQSTYEYELRVTFDFSSLDLASSRPAVIDYIKRQCNMMAQFLAQLNLRVVRVGNKNKFPAY SKQSGSAPPNQDKDVGIPNSDLHIYISHYEDSESSLAFAGACALTYNGRPTFGRAATYLNKISQNPSHEDFDDLETTL HEITHVLGFGSWMWYQYFNPATGKAYGEGNEPIVETIRIGSTQIFATPRVQEVTRRYFGCSTAKGMQLENOTDSKNSHWE ATVLYNEFMSARAVSGDGAYSATFTALLEDGSGWYKAVHENADDLQWGNKQGCDFLTSCDPNKFREFRNSGQDFNKF GIGYSTVEQYTDNCSVPKYNPNRSCSDTITDAGNDQYFHFHWWQESRQFESNIVDRRTISYSGDORCMKYECTAQGGI DYYVQGVKYIDRPNKWDYFKNMIDYSEVNVNPDYKNNPDYKNNPDYKNNPDYKNNPDYKNNPDYKNNPDYKNNPDYK PNCYVYHNGQKLDNNKSCPSGFGVNOCTKVCEVSSSSNNGNEGNNGNNTNANGCLLTQVHLEGNCISVPCPVYVADS DKNCQRNATCLTNDNSTCSSCTEGSLNSSKQCTVCOQQCQTCNNGTSCQSCSTAYKSLDINSNGOLLCLNQCPSHFH LQQSQCLACNSGCCSDSLYLCTCSSSHYKYETNGKIQLVSCPEGYQADGNRVCCQQTPTTCKSNQYQNELGECI DCHYSCKTGDSAYRVNQCTSCDFGYLTVWRSGKGRYCNRCYSYGTUGDKCLRPDQQ</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF8) Genee, complete cds</p> <p>GENE: 1773 bp. Exons: 1-359; 440-694; 769-1087; 1156-1338; 1411-1773 CDS: 1479 bp protein: 493 aa</p>	<p>Gene</p> <p>ATGCAACTTGAAGAAATGAGGTAAGTACTGGATCAGCTGGTTCTCAATTGGGAGCGAGTTATTCTTGAAGAAATGAATACATG ACAGCAAGCGCCATTCTCACGATGCTGTATACTTAAATTTACTTTTGCCCTGCTAGAAGACTCTGGAATGGTACGAG CCTTCAATGAAGAGTGTAGATGAATTCGATTGGGGGAAGGAAGAGGATGTGAATTTCTAGAAGTTGGCATAATAAA TTTCTGAAATTTACGAGTTCCGAATACCAATTGTAATTTCTATCACTCCACTGGATTAATAAATTTCTTCAATAAAGA TGTTTTGCTGACAACTGCAGCATGGGAATTTAATCACAACCTAGTAACCTAATTTATTATTATTGTTATGAATAATG CTTGTTTTTTATATAATTTTAAATTTTAAATTTGTTACCAAAAGTAATTCGATTAATCCATCTCAAGCCGGGAACAATGA TAAATCACTGGAAATTTCTTGACTGTAGTAAGCAGATGTTTTGACAGTAATCTTATTTCACCAAGTATTATCATA ATACTTTTAAAGATGTTTCGATGCTTCAAGTATGAATGCACCTAGTGTGAGGATTTGATGATTTTTGGCTGGGGAAAA ATATTCAATTTGATAAATAAAGCAACAATAAGATGTGCTTCTCAAGGATCAAACTAATTTGATAATTATAAAATTTAT AATAAATAAATTTATCAGGTGTATAGTTATTTCATAATTTTACCTTATAGTCTCAAGGATACGTAATGGACCCCA GATTGGAATGGATTTTGCAAACTTACCCTACTCCTTGCAAAACTATTGCAAGTTAGAATGATTTGTTGTTGCTAAAT ATGCCACTGCAGTGAAGGATTGTCAGGTTCTGATTGCTCCATTGCATGTGCCAATAACAACATATGTTTAAAGATGGAAC ATGTTTGAACAGTAAAAATTTGCTCCTGGATCAATTTTGTAGCCTGCATTTAAAGATGATTATACAATGCATCTCTTCTC AAGTTTATTACAATGGATAATGTGACAGAAAGTTGCTCAACAATAATTTATCCTGTAATAAATATTAAATTTACTGCTTT AGTATATATAATAGTTACATTTTATACAATATCATTTTAGGATGGCTAAAAATATGTTAACCTGCAAAAGCTGGATGTG GTACTTGTGAAAAATGCAATTAGTTGTGCTGACTTGTGAAGTTGGAAGTTTAAATGATGAGAAGTAATCACTATTATCTAA TGTTTGGACAATTGCCAGCTAAAAATGTTTTCAGGTTCTTAATAAGTTTGTCAAAATGGCAGCTAAGCAATTTGTTTAT TCATTTATTGATTGACGAAAGAAATAAATATAATTTAAAAATTTATATTTCATAGTCTTCTTGTGTTAAACATGCTCTAATG CGGTCTTGTTAGCACATGTGAAGAAGGAAAAATACAAACAGGTTCTTCTCTTGCGAAAAATGCAATGTGCTGATGTTGG TTGCTGTAATAATGGAAGTACTGTGAAGTAATGTGAATAAGGAAAAAGCTAAATATGAAATGATTAAAAATAAACAAGTT GTGTTGATTCTTGCTCTGGAACCCATGAAAAATAAAATGGAATTTGTTATTTAAAGATAAATCATCTGACAACTCTGGGA AACTCAAAACCCGCGAAAGAAATACAACAAAATTAGTTTCAAAAAATTTAAATTTTGGATTGATAAATAGTATTGCTTT AATATAATCTTGTCTGA</p> <p>CDS</p> <p>ATGCAACTTGAAGAAATGAGGTAAGTACTGGATCAGCTGGTTCTCAATTGGGAGCGAGTTATTCTTGAAGAAATGAATACATG ACAGCAAGCGCCATTCTCACGATGCTGTATACTTAAATTTACTTTTGCCCTGCTAGAAGACTCTGGAATGGTACGAG CCTTCAATGAAGAGTGTAGATGAATTCGATTGGGGGAAGGAAGAGGATGTGAATTTCTAGAAGTTGGCATAATAAA TTTCTGAAATTTACGAGTTCCGAATACCAATTGTAATTTCTATCACTCCACTGGATTAATAAATTTCTTCAATAAAGA TGTTTTGCTGACAACTGCAGCATGGGAATTTAATCACAACCTAGTAACCTAATTTATTATTATTGTTATGAATAATG GATTAAATACACTGGAATTTCTTGACTGTAGTAAGCAGATGTTTGTGACAGTAATCTTATTTCACCAAGTATTATCATA ACATAGTTTAAAGATGTTTCGATGCTTCAAGTATGAATGCACCTAGTGTGAGGATTTGATGATTTTTGGCTGGGGAA AAATATTCAATTTGATAAATAAAGCAACAATAAGATGTGCTTCTCAAGGATCAAACTAATTTCTCAAGGATACGTAATA TGGACCCCAAGATTGGAATGGATTTTGCAAACTTACCCTACTCCTTGCAAAACTATTGCAATGATAAGATGATATTG TTTGCTAATAATGCCACTGCAGTGAAGGATTGTCAGGTTCTGATTGCTCCATTGCATGTGCCAATAACAACATGATGTTA AAGATGGAACATGTTTGAACAGTAAAAATTTGCTCCTGGATCAATTTTGTAGCCTGCATTTAAGAAATGATATACAATG CACTTCTTCTCAAGTTTATTACAATGGATAATGTGACAGCAAGTTGCTCAACAATAATTTATCCTGAGGCTAAAAATAT GTTAACCCGCAAGCTGGATGTGGTACTTGTGAAAAATGGAATAGTTGTGCTGACTTGTGAAGTTGGAAGTTTAAATG ATGAGAAAGTAACTACTTATCTAATGTTTGGACAATGCCCAGCTAAAAATGTTTTCAGGTTCTTAAATAGTTTGTCTAA AATTGCGACTTCTTGTGTTTAACTGTTCTAATGCGGTTTCTGTAGCACATGTGAAGAAGGAAAAATACAAACAGGTT CTTCTCTTGGCAAAATGCATGTGCGGATGTGGTTCGTGTAATAATGGAAGTACTTGTGAAGTAATGTGAATAAGGAAAA AGCTAAATATGAAATGATTAAAAATAACAAGTTGTGTTGATTCTTGTCTGGAACCCATGGAAGTAAATGGAATTTGA GTTATTTAAAGATAAATCATCTGACAACTCTGGAACCTCAACCCGCGAAAGAAATACAACAAAATTAGTTTCAAA AATTTTAAATTTTGGATTGATAAATAGTATTGCTTTAATATAATTTCTTGTCTGA</p> <p>Protein</p> <p>MLQEEYGGTGSAGSHWERVILENEYMTASAISHDAVYSKFTFALLEDSGWYQPSYESVEIDWVGKRGCFLESCDNKF PEFNDSEYHCNFYHSDGLKIPTNKDFVADNCSMGILITNYNCINPSNAGNNDQYTGNNFDLQSGRCFDSNLSTLKSHFT KDVRCFYECTSDGGIDVFLAGEKYSIDKQSNKIDVLPKGSNQFLKYGVNPGQDWNGFKCTYPTPCNKYCSQNGYCFAC QHCSEFGAGSDCSIAICANNYVKDGTCLNSKNCPGGSILQPAFKECICQCTSSQVYVYNGQQTSCPNKYYPDGQKICQP CKAGCGTCENGISCLTCEVGKFKYKESNTIIQCLDNCPAKMFGSGSQVQCQNCSSCLTCSNAGSCSTCEEGKYTTGSSSLC</p>

	<p>TGGAATGTAATAGATAATGCAACAAAGAATCAAATAAAGAACACGAATTATTAGTGGAAATGGAAGACCTTCTATCGAA CTAAATCAACTGAAGTCGCTAAAGTAGCTTAAGAATATTCAAATGTAATACTATTACAGGAGCTTTGATGGAAAAAT AAGGTGATTAAAGGTAGTAAAGATGACCCTGGGAGAGATCATTAAATGTACACGAATTTATGACAGCTTCCCGAAATTT AAAGTGACGGTGTATTCTAAGTTTACGTTAGCTTTCTTCAAAGACTCAAATTGGTATAAAGAAAGTCAACATGGATT GGCAGAGAATATGTAATGGGGATTGTAATAAGGTTGTGATTTTACAATTAATTTGCAATGGAGTCAACACGATACCCC GAATTTACTAAAGAAACCACTGATACATGCTCTTTCCATCAGTTTGGAAATAGGAAAGGGTACCGCTGAAACATTTCACTG ATGGATGCAGAGTTGTTTCAATTTATAAAAACTACACTGTACTGACACAGGCCAACTTTAAAGAAAACCCACATAATTTAT ACAGAGAAAAATTTGATATCCAAAGTAAAGTCTTTTGAAGCTACTTTAATAAAATCAAGGTTTATCTGATATGCTCTTTA GTAGAGTGTCTATAATTACAATGCCATAAATGTTGTTTATATCTAAGTTGCAAGATTCGAATTTCAATTTGAAAGATTAACTTA GACAGCGAAGTATTAAGGCCCTCTTGATTTAATAGGATAGTTAACAATGTCTTAAAAATTTAAATACATTTTGCAACTT CCCCGTACCTTGCAAAAAATTGTCAGTACAAGGGATATTGCTTAGGAAAGGAGGAATCACTAAATGCAGTGCAT TGATGGATATTAAAGGTTTGAATGCACTGTTTGTGCCCCAGCGCTGTATGTACATAAAGTGCAGACTGCAAAACCTGG GAAACTTTATGTCAGGAAGTACAACCTGTAATAAATGTCATGATCTTGCCCTGATTCTATGCACTGGTCCATCTCAA AGTGAATGCACAGTATGCCCTCCGGAAAGGCTTGGCAGGAAATAGCTGCGTTGATAGTTGTCTAAAGGTAGTCA AGTTGTGGATAAATTTGTGAAGTATGTGATACCTGCTTGACGGAAATAAACCAATACAGGAGACTTCAATTTCTA AGCAATTCAATACCTCTTATTATGTCATTAAATGTTATTTTATAAAGCTTTAATAATGTGA</p> <p>Protein</p> <p>MLARIFIAILLLLCITIGVISHKGCKHYENLPQHWKDHIEYRKNVIEQYKIEREKTGYRSADQVPRRLNATPOPLRVTFDYSR IQNLPSEQSKDAITGALEISKYIADLLKVEPLTSNIVSDNAGPADLGGCVAKLDTQYYSINADKTTGIANSDLHYVWNWNA NDQSLAYAQACQYKNYRAIVGOINFNYKWLDEYNPKSGASFEYNLETSLHELHVLVFGSSKEIDHFLDNAGNVIDNATKN QIKNTNYQWNGKTSIELKSTEVAKVAQEFYKCNITGALMENEGDQGSKDDHWERSLMYNEFMATSGIQSDGVISKFLTAF FKDSNWYKEVNMMDAEDMQWFGEGQCDFYNQICNGVNYQPEFTKETDTCSEHFHFGKGTAEFTFDGKQIVSYKNLH CTDTANFKENPHNYTEKFDIQSKCFEGTLIKSLSSYALQQRRCYNYKLNNVVYIQVGEFNKCTGKDEVLKAPLDYQGG LTCPNKLSFCNFPVPCNKYCSNKGYLRKGGISKCKIDGYQGLDCSVQCPSAVCTGCDNKGTDYAGTRCTCNCHK DSCPDSCCTGPSQSECTVCPSGKVLAGNSCVDSCPKGSQVVGQICEVCDTCLDGNKPNTGDSSILSNSIPLIALIVLQTLIM</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF11) Genee, complete cds</p> <p>GENE: 2286 bp. Exons: 1-199; 258-410; 471- 658; 714-800; 857-976; 1031- 1206; 1263-1454; 1512-1587; 1643-1858; 1923-2286 CDS: 1773 bp protein: 591 aa</p>	<p>Gene</p> <p>GGTATTAGATGACCTTGTTTCTGTTTGGACAGAAGAAGAAATTGAGGCAATGAATTACAGTAGAGATAAAATCATTCAAA CATAAATGTGTTACAGATTCTTATTGTTGATAAAAAATGAAAAATACGAGAAAAAACATCCAAAAATCATAAATAAGTTAAA TTCAGAAAAAGTATAGAAACATAAGGAAACATCGTCTGGGTAAATTTAGTTAAATGTTTAAATATTTTATATAAAAACT TAAAAATAATTTTATAGTAGCCTTATAGATTCATGTTAACTTTGAAAAATAGAAAAAGGTGACATCCCTTAAAGGAT GTAGATTTTATAAAAAATAAGCTTATCCCGCAGCAAAAGCCTTATTTGAAACCACTTTTAAATGTTTAAAGATTAACTTA GCCAATTATCGTATTTTATTAATTAATTATTAGTTATTATTGATTTTGATTTTGTATTTTATTTTAAAGCCTAGTAGAATATT ATACTTAATAGGAAAAATTGTAAATGGAGAAACCTCTGTACCTAAAAGTCTCTATAGTGGAGTAGATAATGGCGATT GGTTATGATGATTACAGCTAAATATGCTCCTAATGAGACATACTTACCTACGCTTCAAAATGCTATCAAGGAGAAATAG GAATTAATAATTTTAAAGTAAATTTCTTTCTTAAAAATTTTACCTATTAAACATTATTAAATCTTTTAAAGCCTACAGGTG GGATTTTTCTTAATAAATGACAGCTTATTAGAAAAATCACTCAATGCCAATAGATGGTTTACGTGGATTGAGACTTCGCTA ATATAAATTTTATAAAAAAGAAAAAACAATAATGTATAAATATATATAGAAATTCATGAAGTAGTACATGCTATGGGA TTTTCAAAGGATAATTTCTAAAACTTTTAAAAATGGAAAAATTTGGATTAAATCCCGCGGTTTAACTGCTTTTATGGGACC TAAACAGCTTAAGTACCAATTTTACTTATAAATAATAATTAAGTATGATTTTAAATTTGCTAGGATTGCTGAATATTAT TATAAATGTATAATGTAATGAGTCCCTATAGATAAATAATGAACTAGTGGGCTGTCGACGATTCTGATTTGGGAAAGA GTAGCTCTTGGGAATGAGGGAATGACTGCCTGTGATTTCCGAGATGCTGTTTTAGTGAGTTTACTTTAAACTTTTTCG AAGGTACTTACTAATTACATATGAACATAATACCCATACTTAATTTTATAAAATAGACTCAGGATGGTATACCTATAA TGAAGGTAATGCTTAGTAATTTGGTTTGGGAAAAAGGAGCGGGATGTAATACAATGCAAGGAATTTTGTGATAAAGCTTC TTTGCACACAGCTGCCTGAAGGAATCAAGGGATGCAATTTTGACTATTCTGCCAGAGGAATTTTGTGTTCTGGAGA TACCTTTGCTCCTAGTACTCAATTTGTAATTTTAAATTTTATTAATTAATTTTATTTTATTAAGTTCAAAGGATGCCCTAT TATGCTGGATAGACAATGGACATTGTGAGTATGATACTTTTAAATGCCAATTAATCAACAATTTGTAATAATATAATTTTA AACAGTCTCGCACAGTTAAATAAACAAATTTCTTAAATAGAGGAGGTGAAGTATCGACTGATGCTAGATGCTGGGTAGT AAGTAAATTAATAATTAATTTAGGGGAAGTTAGATCAGCATGCTATAAAAGTAGATGTTTCAGGAGAAAACTTAAAGTAT TTCTTTTTCAATAAGGTGATACCTTTGGAATAAAATGGATAAATATATCTCAAAATGGTATTTCTATAAGATGTCCCCC AAAAGTGATATTTTGTCTGATATATTTTTCGTATCTATTATAGAAATAATAATTTTATTTCCATTTGTATAATATAG AGTAGAATCTGACTGTCCCAATTCATGTACAGCTCAGGGAAACCTGTGTCGATGGAAAAATGCAATTCGTATAAATGGTTA CGCAGGTACAGATTGTAAAGGAGATCTGGATAAAAAAATTAAAAAAAACCTCATCTGAAACAAATTTAGGGAATGGT ACAAGACCAACAAGTAGCGGATCTTAAACTTATTGCTTGACTAGCAGAGGAGATTACTGGGTAATACCAAAAAATAG TATTATTGCTGTAAACAAGAAGAAGAAAAATGTTGGTATTATTAATATCCAAAATATGAGATTGTATTGTCTCTCC TGGATATGTTGTCATAGCTTATTAATGTATGTGAGCTTAAACACTGA</p> <p>CDS</p> <p>ATGGTATTAGATGACCTTGTTTCTGTTTGGACAGAAGAAGAAATTGAGGCAATGAATTACAGTAGAGATAAAATCATTCA AACATAAATGTGTTACAGATTCTTATTGTTGATAAAAAATGAAAAATACGAGAAAAAACATCCAAAAATCATAAATAAGTTAA AATTACAGAAAAAGTATAGAAACATAAGGAAACATCGTCTGGTAGCCTATTAGATTCATGTAACCTTTGAAAAATTAG AAAAAGGTGACATCCCTTAAAGGATGTAGATTTTATAAAAAATAAGCTTATTCGCCGACGAAAGGCTTATTTTGAAC CACTTTTAAATGTTTAAAGATTAACTTAGCCAATTATCCCTAGTAAGTATTATACTTAATTTAGGAATGATTAATGGAGA AACTCCTGTACCTTAAAGCTCTCTATAGTGGAGTAGATAATGCCGATATGGTTATGATGATTACAGCTAAATATGCTCCT AATGAGACATACTTACCTTACGCTTCAAAATGCTATCAAGGAGAAATAGGAATTAATAATCTTAAAGCCTAGCATGGGAT TTTTCTTAATAAATGCGAGCTTATTAGAAAAATCACTCAATGCCAATAGATGGTTTACGTGGATTGAGACTTCAATTCAT GAAATGATGCAATGCTTGGGATTTTCAAAAGGATTAATCAAACTTTTAAATGGAAAAATTTGATTAATTTCCGGC GTTAATACTCTTTTATGGAGCTAAAGCAAGTAACTATGTAATTTATATTAATTAATTAATTAATTAATTAATTAATTAATTAAT ATAGAATAAAATGGAAGTACTGGGTCTGCGAGTCTCATTTGGGAAAGAGTACTCTTGGGAATGAGGGAATGACTGCC CTCTGATTTCCGAGATGCTGTTTTAGTGAGTTTACTTTAAAACTTTTCAAGACTCAGGATGATGCTACATAATGAA GGTAATGCTTAGTAATTTGGTTTGGGAAAAAGGAGCGGGATGTAATACAATGCAAGGAATTTTGTGATAAAGCTCTTTTC GAACACAGCTGCACCTGAAGGAATCAAGGGATGCAATTTTGACTATTCTGCCAGAGGAATTTGTGTTCTGGAGATACC TTTGCTCCTAATTTGCCCTATTATGCTGGATATAGCAATGGACATTGTGAGTATGATACCTTTAATGCCAACTTATCTAA CAATTTAGGAGGTGAAGTATCGACTGATGCTAGATGCTGGGTAGTAAGTATTAATAATTAATTTACAGGGAAGTATG ATCAGCATGCTATAAAAGTAGATGTTCCAGGAGAAAAAATGGAAGTTTCTTTTCAATAAGTGGTATAGTTGATAAATAA ATGGATAAAATATATCTCAAAATGGTATTTCTATAAGATGTCCCCAAAAAGTGATATTTTAAATGATGCTAGCTACT CCCAATTCATGTACAGCTCAGGGAACCTGTGTCGATGAAAAATGCAATTCGTATAATGGTTACGACAGGTACAGATTGT TAAAGGAGTACGTGGATAAAAAAATTAAAAAAAACCTTCATCTGAAACAAATTTAGGGAATGGTACAGCAACAAGATA CGGGATCTTAAACTTATTGCTTGACTAGCAGAGGAGATTACTGGGTAATACCAAAAAATAGTATTTTGTCTGTAAACA AGAAGAAGGAAAAATGTTGGTATTATTAATATCCAAAATATGGATATTGTTATTGTTCTTCCGTGATTTGTGCTCAATA GCTTATTAATGATGTGAGCTTAAACACTGA</p> <p>Protein</p> <p>MVLDDLVSVVTEEEIEAMNYSRDKSFKHKCVHDSIVDKNEKYEKKHHSKIINKLNSEKVKQHKETSSWQPIRFHVNFENIEKG DIPQKDVDFIKNKLIPAAKAYEFFTFNQRLLTQPIIPSKYYTQYGLKCNGETPVPKSLYSVGDNDQMVMIMTAKYAPNETTYLA DIQKCYQGEQLKSLRPTMGFFLINAAYLEKSLNANRWFTWIETSIHEMMHAMGFSKQGFQNFQNGKIGLNSAGQVFFY GPKTVNYGKLYKCNVNVGPIEQNGSSGSAGSHWERVALGNEGMTASDFGADVSEFTLLKLFEDSGWVYTYNEGNAQQ LVWEKGAGCNTMQGFCDKASFHSECTEG IKGCFNFDYSARGICVSGDTFAPNCPYYAGYSNGHCEYDTFANLNSNLLGGEVSTDARCWVVTSONQYSGEVRSACYKSR CSGEKLEVFFNWKYTCEQNGQIISONGISIRCPKVIFCSVESDCPNSTCAQGTCTVDGKNCNNGYAGTDCQRRSSGQKN QKKTSSSETNLGNTRPTSSGSQTYCLTSRGDYLGYQNKYYCCNKEEGKCCGIQYPKYGYCYCLPGVYVNSLLNVCEL KH</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF12) Genee, complete cds</p> <p>GENE: 2517 bp. Exons: 1-137; 203-512; 623- 682; 744-1603; 1664-2018; 2079-2378; 2461-2547 CDS: 2079 bp protein: 693 aa</p>	<p>Gene</p> <p>ATGAAATAGATAATTGCACACTGTTGTAGTTCTTACTTTATTTGCCAGTCAAATTCGCCTATATCATTTATTGCTAGCACAG TGCTAGAGAAAAATACGGCTTTTATAATAGAACCTAGATAATTAGCTATACTGAGTTGTAATAATTTTAGTTTGTGTT TTTTTCTAAATATAAAACATTTAAATAATTAATATTTAAAGAGATCCACACCCTCTTAAAGATAATCTTCGATTACTCT TCTATCCCTGATAATTTTGCCCAAAGAGATTATATTTCGCTTGTGTAATTAAGCTCTTCTATTATTCGAATAGAAATCTA TAAGTAGTTACTGAAACATGCAGAGCAGAATAATTAATTTCCAAATGTTTAGGGAGTATTTTGTGGAACATAATAAATCC AGAAATAGATAAAAGTGTGGAAATCAATAATATGATCTGATCTACATATTATTTGTGGAACATTTCACTCAGGATAGACAAT CCTCTTGTGCTACTCTGTGTTTGGCTTTTGGTATATTATTTACAATTTAATTCCTATTATTTTGTTTTGTATTGATATTAA TTATTACTTACTTTATTAATAATTAATGATTCTAATCTTATATTATTAATATTAGGAGTAGACCAAAAAAGACCATC ATTTGGATATTAAATATTAACTTTCACTTAAGTGAATAATAAAAGTATAGAAACCTTTTATAAATCGAAAAATTTCA TTATATTATAAAAGACAATTGGAGATACAGGAAAGGAAAGGATTATGAAGATTATTATGAAGGAATTTATCCATGAAG AGTTCCATGATTAGGATTTCCTAATCTCTGTTCCCCCTATTAAAAATAGCTTGACTTTTGAACACGAATTTGACATTA AGGTAGCCTACAACCTTGGGTAAACATCTCCAAAAGTTAAGGAAGTGGCTCGCAAGCACTTTAACTGACTGATGCTAT TGAATGTGAATTAGAAAAATTATAATGGAGACAACAATGAAACCAACATTTGGAAGAGCTTTTACGTAATAGAACCT ATGACTGGAAATTACCAAGAGCCCTTTTGGATTGGTATCTATTTTACATGGGCGCTTGTTAGAAGATTCTGGGTGGTACT TTCTTCTCATTTAATTTATGGATTGAATGGAATGGGGAAGAGATAGAGGCTGCAGTTTTTATAGATTGTTGATCCGAA TAAGTTTAGAGAAATTTTACGCTTACAATGGAAAGAACCATGTATATTATTGAATTTTATTATTCTCGCAATTACTCGTTT CTATTAGCTTGGAGAGACTTAGGGAGAAATGCTAAATGGTACTGATTACTTAGATTGGAATCCAGGAAGTGGATA ATAGTATGATCATTGACTTTAAATATATATTATAGCAAAAGTAGATGCTTTAACACGCGACATAATTAAACACCAACTTGC TACCCGAAAAAGAAAAATGATTAACTATTACATTTTCTTCAAGATGCTATTAAATGAATGCACAGATACGGGGATA GATGTGATAATTAGTAACAAAAGATTTCAGATAAAAAATGGAGAAACATAAGTATATATTAAATCATCATCCCCGACGG AAGAGTATACATTTATTATTACCTTTGAATTTATTTATATTATATAATTAATAAATTTCTTTAGAATTGCGAGAACTATATA TGTCCGAGAAAAATTTGATGCAATTTTGAATCGAATCCCAATACCCCTGTTTAAATTAAGTGTAGTGAAGAAATGGGTACTGC TAGTGAGGGAACATGTCACTCGGATACAGACTATGCAAGTGAAGACTGTTCTTTAATGTGCCAATGGAATTAACGT TACAATGGGGTGTGTTAAACGAAAAAGAGTTGTGCTGAGAACACTTTATTAACTCTCAACATAAATAGTATATAG GACAACCTTGTCCAGGAAGGATAAGTTAAATTCGGACATAACTGTGTTTTCAGAAATGTCCCTAATAGTATTTTAAAGGTGG</p>


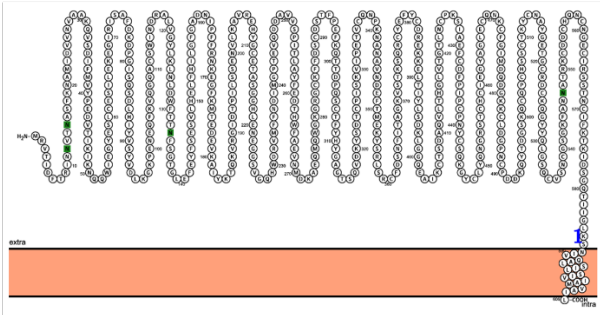
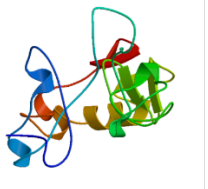


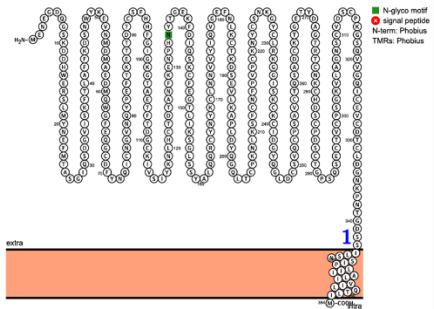



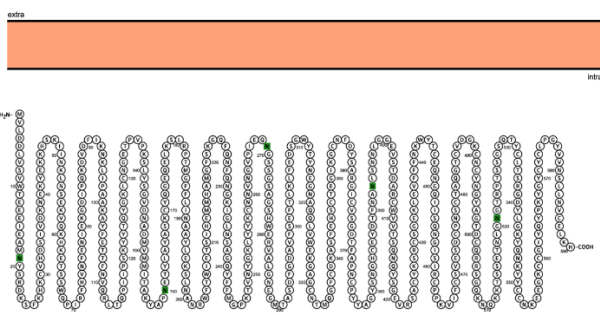

	<p>TCAATCATGTGTACCCTGCTTGGTAATTTAAATCATAATTAAAAAATCATTAAAACTACTATATTTTATTTATTATTAATCTAG CCTGGTTGTGAATTTTGTCTCACAAAAACACATGTCATATTTGTTCTGTGGGATATGTTCCCGACTATTATCATAAAAAAC CTGCTCAAGATGCTGGAAAGGTTGTGCATAGTGTGATGGTAACAACCTGTTAAAGTGTGAGCCCTGATTATTTTCCCTAT GATTCAAATCAATCACTACTAGTTGATGCTGAAAGTAAAAAACTATTAGTTACTATACAGCAATAATGTGCTAAATCCTTG TCCTGCTGATTCCATTTTATTTGAACAGTAATAAAGTGTAAATAGATGTGATTCCAAATTTGAAAGCCCTCTTAATTTAT CTATATAATGAATTAATAATATATTATCTTACTATTTAATTATTTTAAATTCAAGTATAAAGGATGTAAAGAT GCAGTGAACCTGAATGTTTTGGGGATAGGGACCATGA</p> <p>CDS</p> <p>ATGAAATAGATAATTGCACCTAGTTGTAGTTCTTACTTTATTTGCCAGTCAAATTCCTATATCATTAATTGCTAGCAGACAG TGCTAGAGAAAAACGGCTTTTATAATAGAACCTAGATAATTTAGCTTACTGAGTTAGATCCACACCCTCTTTAAGA ATAACCTTCGATTACTCTTCTATCCCTGATAATTTGCCCAAAGAGATTATATTGCGTTGTGTAAATTAAGCTTCCT ATTTTTCAATAGAAATCTATAAGTACTGAACTGCAGAGCAGCAATAATTAATTTCCAAATGTTTAGCGAGTATTTT GTGGAACATAATAAATCCAGAAATAAGATAAAGTGTTGGAATCAAAATATCTGATCTACATATTTATGTTGGAACATTC ACTCAGGATAGAAACATGCTCTTGCTACTTGTGGTGTTCGCTTTTGGGTAGAGCAACAAAAAGACATCATTTGGGA TATTTAAATATTAAACCTTTCACTTAAGTACAATTTGGAGATACAGGAAAGGAAAAGGATTATGAAGATTATTAGAAGG AATTATCCATGAAATGTTCCATGTATTAGGATTTTCTAATCTCTGTCCCCCATTTTAAAAATAGCTTGACATTTGAAAA CAAAATTTGAAGAGGTTAGCCTCAACCTTGGGTAACATCTCCAAAGTTAAGGAAGTGGCTCGCAAGCAGCTTTAACTG TACTGATGCTATTGGAATGTAAATAGAAAATTAATGGAGACAACAATGAAACCACATTTGGAAAAGAGTTTAACTG AATAATGAACCTTATGACTGGAATTAACCAAGGCCCTTTTGGATTGGTATCTATATTTACATGGGCCCTGTTAGAAAGATT CTGGGTGGTACTTCTTCTCTAATTAATTTATGGATGAAATCGAATGGGGAAGAGATAGAGGCTGCAGTTTGTGAATAG TTGTGATCCGAATAAGTTTAGAGAATTTAGCCCTACAATGGGAAGAACCTGATATATTGTAAATTTTATTATTCTG CAATTACTCGTTCTCATTAGCTTGGAGAGACTTAGGGAGAATGCTAAATTTGGTACTGATTACTTACGATTGTAAGATCC AGGAAGTGGATAATAGTATGATCATTGACTTTAAATATATATTCATAGCAAGTAGATGCTTTAACAGCGACATAATTA ACACCACTTGTCTTACCGGAAATGAAAAATGATTAATCTATTACATTTTCTTCAAGATTTCAAAATGTAAGTACACA GATACGGGGATAGATGTGATAATTAGTAACAAAAAGATTTCAGATAAAAAATGGAGAAACATAAGTATATTAATACAT CATCCCCGACGGAAGAAATTCAGGAACATATATGCTCCAGAAAAATTTGATGCAATTTGTAATCGAATCCCATAAACC CTGTTTAAATTTACTGTAGTAGAAATGGGTACTGCTATGGAGAACATGTGCTGCGATACAGACTATGCAGGTGAAGA CTGTTCTATTTAATGTGCCAATGGAATTTACGCTACAATGGGGTGTGTTAAAAACGAAAAAGAGTTGTCTCGAGAACCT TTATTTAACTCTCAAACTAAATAATGCTATATAGGACAACCTTGTCCAGAAAGGATAAGTTAAATTCGGACATCACTGCTG TTCGAATGTGCTCATAAATGATTTTAAAGGTGGTCAATCATGTGTACCTGCTTGCCTGGTTGTGAATTTTGTCTGCA CAAAACACATGTCATATTTGTTCTGTGGGATATGTTCCCGCACTTATCATAAAAACCTGCTCAAGATGCTGGAAAGGTT GTGCATAGTGTGATGTAAGTAAACCACTGTTAATAGTGTGAGCCTGATTATTTTCTTATGATTCAAAATCACTACTGTT GATGCTGAAAGTAAAAAGCTATTAGTTACTATACAGCAATAATGTGCTAATCCCTGTCTGCTGTAATGCATTTTATGGA ACAGTAATAAAGTGTAAAGATGATTGCCAATTTATAAAGGATGTAAAGATGCAGTGAACCTGAATGTTTTTTGG GGATAGGACCATGA</p> <p>Protein</p> <p>MKQIIALVVLFLFCOSNCLYHYCOHSARENTAFIEPROFSYTELDPHPLRITFDYSSIPDNFAQRDYIIRLNQASLFFNR NLQVYTEHAEQNNQFPNVGVFCGTKEQPEQDKSVGIKQSDLIHYVITFTHGQNNALATSVCSVLGRATKRPSPFYLNINT FHLSTIGDTGKEKDYEDYEGIIHEMFHVLFGSOLFPHFKNSLTFENKIVRGQPTTWWTSPKVKYVARKHFNCTDAIGMQL ENYNGDNNENGHWKRVLNNELMTGNYQRPFLVSIWTFWALLEDSCWYFYSYQFMDEIHWKDRGCSFLDSCDPNKF EFOPYNNGRNVHYVCNFYYSATRSQHLGETQGEQIGTVDLDCQDPGSGQQQYDHLTLNYSQQRSRNSDIINTLTMNE NMNILLHFSRCYQYECTDGDIVISNKRFIQKNGETQVYIKSSSPTEIAGTIYAPENIDAGCNRIPOCLNVCRSNGCYG GTCHCDTDYAGEDCSIQCANGNYVYVNGQNEKSCPENTLFSNQTKQCYIGQPCPEQGVKFGHNCVSECPHYFQGGQ SCVPLPGCEFCSSQNTCHICSVGYPDYHYKTCRSRWKGCACQCDGNNCQCEPDYFYDSKSIILVDAESKTIISYYTR QCANPCPADSFIEQQQKCNRCDFQIYKGCKRCSETECFWGGQP</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF13) Genee, complete cds</p> <p>GENE: 1744 bp. Exons: 1-803; 884-1238; 1299- 1595; 1688-1744 CDS: 1512 bp protein: 504 aa</p>	<p>Gene</p> <p>ATGTTTCACGTATTGGGATTTTCCCTAATCTCTTTTCCCATTTTAAGAATAGCTTGACATTTGAAAAATAAATCAATATT GAAAGTGGAAAGCCAACCTACTTGGGTAAACATCTCCTAAAGTCAAAGCCGTGGCTAGAAACCCTTTAGTTGTAAACA GCTATAGGAATGTAGTTAGAAAAATACAACGGAGACAACAAAGAAACCAGCATTGGGAACGAGTACTATTAAATAGT GAGCTCATGACAGGAAATTATGAACGAACCTTTTGGAAATGTTTCTATTTTACATGGGCAGCTGCTAGAGGATTCTGGAT GGTATATACCAAAATATGAATTTATGGATGACATTCAATGGGGAAGGCAAGGGCTGCCTTTTATAGATTGTGCAA TCCTACTTATTTAGAGAAATTTAACCAAAATGGGGATATTCTAAATAATATTTCTGTAGCTTTTATTTCTGCAGTTAC TAGTCTTCACCGACGAGGATATACTACTGATAAATGTCCAATCGGCACGGAGGTAATAGACTGCTAAGATCCAGGAAG TAAGGGAGGGTTTTACGAACATTTGTTTCCAAATGTTTATTCTATTAAAGTATGTGCTTTGAAAGTAAATGTTACAGATT ATAAAAAATTCGAAGGAACGAAAAATTAATTTAGTACCAGGGTTTTCTTCAAGGTGCTTTTAAATATGAATGCACATA ACTGGAATTAGATGTGATAGTAAAAACTAAAGGTATACCTTTTCAGTAAGGTGTATCCATTAAAGTTAATATAACATCTGA AACAGAAAAAGTAATAATCAATTAATTTGAATTTTACAAATTAATATGTATTCAATTAATTTTATTTTATCTATTACTCT ATTTGACGATTACAGGAATAATATAACCTCCTGAAAAATATTAGTGCCTTTGTAAATCAATTCCTAAGCCCTGCTTAAAT TATTGCGATAGAAATGCATACCTGCTATGCAGGAACATGTCACTGCGATCCAGATTATGCAGGAAGAAAGCTGTCTGTT TACTGCGCCAATGGAACATATGATATAATGGTGTGTTGCGAAATACAAGAGTTTCCGCAAAAAATCACTCTCTCAACT CTTAAACCAAGAATGTTACTGGGTAAACCTTGTCTGGAAGGATTGGTTAAGTTAGGCATTAATTCGCTTCAAAAGT TCCAAATAGATATTTAAAGGTGGCCAATCATGTGTACCTTGCAATGGTAATTTCAATATTAATATATATTTGGTTTAATAA TAATTTTATTTAAAAATTTTAAAGCCTGGATGTGATTATGCAAAATCATAATTTTATGTGATTTTGTGTCAAAGGGATT ATTCTGATTACTATCACAAAACCTTGCTCAAGATGCTGGAAGGGCTGTGCAGAGTGTAGAGAAATGAATGAATGATTATTT GTGAACCGAGTTATTTCCATTACCTTGGCCAAATATACAACTGATTGATCCTATTATAAGTATGAATATAATTTCTTA TGCAATGCGCAATCCATGCGCTGTACATTTCTATTCAATAAATTAATTAAGTTAGTGTATAGATGTGATTGTATTA TGAAAAAATTTCTTTAAATTAATTTAGAATAATTTTGTTTAATTATATATGCAAAAAATTTGTTAATTTTATTTGTTATTATTA TATTTAAGCATTTGGGGTGAATAGATGCAATGAACCTGAATGTTTGGGATACAAGTCTATGA</p> <p>CDS</p> <p>ATGTTTCACGTATTGGGATTTTCCCTAATCTCTTTTCCCATTTTAAGAATAGCTTGACATTTGAAAAATAAATCAATATT GAAAGTGGAAAGCCAACCTACTTGGGTAAACATCTCCTAAAGTCAAAGCCGTGGCTAGAAACCCTTTAGTTGTAAACA GCTATAGGAATGTAGTTAGAAAAATACAACGGAGACAACAAAGAAACCAGCATTGGGAACGAGTACTATTAAATAGT GAGCTCATGACAGGAAATTATGAACGAACCTTTTGGAAATGTTTCTATTTTACATGGGCAGCTGCTAGAGGATTCTGGAT GGTATATACCAAAATATGAATTTATGGATGACATTCAATGGGGAAGGCAAGGGCTGCCTTTTATAGATTGTGCAA TCCTACTTATTTAGAGAAATTTAACCAAAATGGGGATATTCTAAATAATATTTCTGTAGCTTTTATTTCTGCAGTTAC TAGTCTTCACCGACGAGGATATACTACTGATAAATGTCCAATCGGCACGGAGGTAATAGACTGCTAAGATCCAGGAAG TAAGGGAGGGTTTTACGAACATTTGTTTCCAAATGTTTATTCTATTAAAGTATGTGCTTTGAAAGTAAATGTTACAGATT ATAAAAAATTCGAAGGAACGAAAAATTAATTTAGTACCAGGGTTTTCTTCAAGGTGCTTTTAAATATGAATGCACATA ACTGGAATTAGATGTGATAGTAAAACTAAAGGTATACCTTTTCAGTAAGGTGTATCCATTAAAGTTAATATAACATCTGA AACAGAAAAATTCAGGAATAATATAACCTCCTGAAAAATTTAGTGCCCTTTGTAATCAATTCCTAAGCCCTGCTCTAA ATTATTGCTAGTAAGATGGATACTGCTATGCAGGAACATGTCACTGCGATCCAGATTATGCAGGAAGAGAGTGTCTGT TTTACTGCGCCAATGGAACATATGATATAATGGTGTGTTGCGAAATACAAGAGTTAGCAGAAAAATACTCTCTTCAA CTCTTAAACCAAGAATGTTTATCTGGGTAACTTGTCTGGAAGGATTGGTTAAGTTAGCAGATGCTGGAGGGCTGTGCA TGCCAATAAGATATTTTAAAGGTGGCCAATCATGTGTACCTTGCAATGCGCTGGATGTGATTATGCAAAATCATAATTTTA TTGTGATTTTGTGTCAAAGGGATTGAATTCCTGATTACTATCACAAAACCTTGCTCAAGATGCTGGAGGGCTGTGCAAG TGTAGAAATAATGAATGATTTATTTGTGAACCAAGTATTTCATACGTGTCGCAAAATATACAAGTCATTTGATCCTAT TATAATACTGAATATAATTTTCTTATGCAATGCGCAAAATCCATGCCCTGTAGATTCTATATTCAATTAATAATTAATTA GTGTTAAGATGTATTTGTAAATACATTTGGGGTGAATAGATGCAATGAACCTGAATGTTTTGGGATACAAGTCTACTA TGA</p> <p>Protein</p> <p>MFHVLGFSQSLFPHFKNSLTFENKINIESGKPTTWWTSPKVKAVARNHFSQQTAIMQLENYNGDNKENQHWERVLNSE LMTGNVERTFGIVSIFTWALLEDSGWYIPNYEFMDDIQWKGKGLFLDICNPYTFREFQPKWGYSKQYFCSFYSAVTSL HQQGYTITDKCPIGTEVIDCQDPGSKGGFYEHLFPNVYSYQSMCFESNVTDYKLLQNGEKLVLVPGFSSRRQFYQECTNGLG DVIYNQRYTFQQGVSVKVNITSEMITGIQPPENISAFNCQPKPCLNVCRSRNGCYAGTCHDPTDAGEDCSVYANG NYVYNQVORNKYSCPENTLFSNQTKECYTGQPCPEGLVKLGHNSFDPPIITEYNFLMQCANPCPVDSIFDIQINQCLRCDFVI HLGNCNRNETECFWDLSL</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF14) Genee, complete cds</p> <p>GENE: 1502 bp. Exons: 1-70; 144-205; 276-572; 627-868; 937-1227; 1316-1502 CDS: 1149 bp protein: 383 aa</p>	<p>Gene</p> <p>ATGTTTTATAATGAATTAATGAATCCTGCAGGTTTAAATCAGACTCTATAACTTCTAAATTCACATTAGGTATACTAGC ATATAATTAATACTTAACAAAGATATATCAAAATTAATTCATAAATGCATATATAGCTTACTTAAAGATAGACA ACTGTGACCGAGATGTACACTTCTTTGCTGAAAGTTTCTAAATTAATTTAGTATTATTAACCTTTTAAATATAAAA AAGCAAAATTAATAAAAAATTAATCATATTAGTGGGGAAGAAATAAAGGGTGTGATTTTCTTTCAGTGTGCTCAAGTTC CTATCCCGAATTACAGACAATCTCTATAAAATAAACCTGTACATTTCTATCATTTCGGAATTTGGATTTGGAAGACAGAT GACTCTCTGACGATTGTCCATTGGCACTCTTTATACAAACACTGACTGTACGAATCCTCTCATCTGCACCAACCAGC CACCGAACATACGAATCATTTTCTTCTCAAAAGCAAAATGTTTGGATTCCACATTTATTTAAATGGATTTTCTCTCTCT ATACTTCAAGTAATAAGTTAATATAGAAATTAATAATTAATAAAAAATTTAAATCCAAATTTTACAGATGCTACAAATTA ATGTGTAATAATGAAGAAATTTCTATAGAGATCACTCTGTAAATGGAGGAACCTGAATATAAAATACATGTACACAACAT GGAGAGGTTTATATAACCAACGCAATTTATCAGGATTGGTAGGGTAACCTCACATGCTCTCAAGGATTAAGTGAATTTTGT CATTCCCAGCTCCTTGGCAAAATATTGTTCTTCCAATGGATATTGTACAGAGTAAGTGGAAAAAGTACGAATTAAT AATTTATTATTATTATTAACCAAAATAAAATTTATATTTTAAATTTATTTAAGTTCTGTTATTGCTTAAACGCTTATAC AGGAAGTATTGTTCTACCAAGTGTGATAATTCGTAAACATAAAGTGGTTAATGTGTAACATAGTGATGTTTCAAAACGA TATTATCATTTGGGAAATAAAATTTGCTCGTAGTGTCATTCAAAATGTTAGTAATATTGTTCAGGGCTCACTTAAAGTA TTGCAGAAAAATGCCCTAACCTTAATCAATGACAACGGAACCTGTGTCCAACAGTGTCCCAAGGAAAAATATGCAAGAT AAAAATCTGGAGATTGCTTAAATGGTATAAAAAATTTATTATTATAAATCAAAATGAGATGATTATTATTAATTAATTA</p>

	<p>TAAC TACACGAAATTGCTCATCCAATTGAGCTCATCCAATTGAGTATTGGTGCATAAGGGAAAGTGATAATTGTTGATTTTATCTAAAGAAATCAAAATAATGACGCTGTAAAAAAGACACAAACTCGAATAGTAAGGGAGAAATTGGTTAGTCTTTTATTATTATGTTGTTTACTATATTTTATAGTTTCTACTTTACTTTATTGA</p> <p>CDS</p> <p>ATGTTTATAATGAATTATGAATCCTGCAGGTTTAAATCAGACTCTATAAATTCTAAATTCACATTAGCTTACCTAAAA GATAGCAACTGGTAGCGAGATGTAGACTTATCTTTTGGCTGAAAGTTTCTAATGGGGAAAAAATAAGGGGTGTGATTTTC TTTCAAGTTGCTCAAGTTCCTATCCGAAATTCACAGAACTTCTATAAAAAAACCCTGTACCTTCTCATCTCCGGAATT GGATATGGAAAGACAGATGACTTCTCTGACGATTGTCCCATGCCACTTTATACAAACACTGACTGTACGAATCCCT CTCATCTGCCAACCCAGCACCACGAACATAGGAATCATTTCTTCCTAAAGCAAATGTTTGTATTCACATTTATTAA AATGGATTTTCTTCTTATACCTTCTTAAAGACTGTACAAACATTAAATGTGTAATAATAGGAAATTCATAGAGATGAC TCTGTAAATGGAGGAAGTGAATATAAAATAGAGTGAACAACTGGAGAGGTTATATAACCAACCAATTTATCAGGA TTGTTAGCGTAAATCAGATGTCCTCAAAATATAAGTGAATTTTGTTCATCCAGATCCCTTCCGAAATTAATGTGCTC CAATGCATATTGTACAGAGTAAATGGAAAAATTCTGTATTAGCTTAAACGGTTATACAGGAAGTGAATTTCTACGC AAGTGTGATAATTCGTAACCTAAAGTGGTAAATGTGTAACCTAATGTGATTCCAAACAATATTATCTTGGGAAATAA AATTTGCTCGTAGTGCTTCAAATTTGTAGTAATATTGTTCAAGGCTACTATAAATGATTGCAGAAAAATGCCCTAAC TTTAATCAATGACAACGGAACTGTGTCCAACAGTGTCCCAAGGAAAAATATGCAGATAAAAAATCTCGAGATTGCTT AAATGTCTCATCCAATTGTAGTATTGGTGCATAAGGGAAAGTGATAATTGTTGATTTTATCTAAAGAATATCAAAATA ATGACGCTGTAAAAAAGACACAACTCGAATAGTAAGGGAGAAATGGTGTAGTCTTTTATTATTATGTTGTTTATTA CTATATTTATAGTTTCTACTTTACTTTATTGA</p> <p>Protein</p> <p>MFYNELMNPAGIQSDSITSKFTLAYLKDSNWWYADVLSFAESFOWGKNKGCDLSSCSSSYPEFTLSIKQCTFYHFHIG YKGTDDFSDDCPIATLYNTDCTNPSHLPHAPTRYESFSQSQKCFDSTFIQNGFSSSYTSQRQCYKHQCVNNGNSIEITLK NGGTKEYIKCTNNNGEVIQPNLSGLVQLTCPQISEFCFPAPCENYCSSNGYCHRVNKNSCYCLNVTGSDCSTKCG DNFVNQSGQCQVNCQDSKQYYHLGNKICQCHSNCOQYVCSGPTQNDCKRCPQLQFNDNGTGVQQCPQGYADKNSGD CLNCSSNCSIGCIRESNDCLILSKYQNNDAVK KDTNSNKGIGQSFYLFMVVLLYFIVSTLLY</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF15) Genee, complete cds</p> <p>CDS: 2319 bp protein: 773 aa</p>	<p>CDS</p> <p>ATGATAATTAAAGAAAAGTTAATTAATGTCTGCACCTTTAGCTATAGACTTTCTTTCTAAAAACTTAAAGGTGATAGACT CTAAAGAAATAATATATCCCGGACTATGAAAAGTGCTTATACACTACTGTTCTCGAAAAATACAAACGTGAAGAGGAT GCTGATTCCGATTTACACATTTTTATCGGATTTTTGCACAACCCCTGAATCTAGTACCATACGATCGCATCTTGTAT TTAAGATGGAGATACCAAAAGACCACCTTTTGGAAGCCTCTGACTATAACTTGCTTTATGAGTGCCTGATGCTGATGCT TTCGACTTCGAAATTAATTCGAAACAGTCTGTCACGAACTAACTCACATTTCTGGATTTTCTCGGTATGTTGATGAT AATTTATCGACGCTTAACTGGGAAGACCTTTGCTTGAAGGTAATTTGGAACCTCAGATCTCCCGTAGAAAAGGAAGTCA TTAATGGAAAGGACACCTTTGGCTCACCACCTCCCATCGCTACGCGAATGGCAAGAGAACACTACGGATCGCTCTCTT TGAAGGACTCCAATTAGAAAACGATTACGGAGGAGGATCTAGAAAAATCTCACTGGGAGCAATCTACCTTCGAAAACG AGTTTATGAATGCACTCGTCTGTCATGATGCTGTCTACAGTAAATTTACTTTCCGCCATTTTGAAGATTTCCGGTTG GTACAAGCCTACCTATAAATACCTAGACGAAGTTACTTGGGGAAGGGGTAAGGATGTGATTTTTGTATCTTTGTGA CCCTAATAAGCATAAAGAAATTTACCAATGAAACCTAGTCTGTAGTTTCTATCACAACTACCTCGGAATATGATGTGC CTGAAACGGAAGAGATAGAAGAGCCTAAGTATGTCATGTTTCTAATCTGTTTCTGCTGATTGCACAGATATAAAGAG AAACAATTAGGATTTGGAGCGGAACACCTTCAGTGACAAAAGTAAATGCTTTGATAGCAACCTTTCAGATGGTCCGG AAGAAGTTCTTACGGAGATCATAGATGTCTGGAGTACGAGTGTACTAAAAAGGGAGGAAATAAATGTTTAGTAAATGG ATAAAATACAAATTAACAGAGAACTATAGAGTCTGTGTAAAGCGGAAGGAAAAATGATTTTACGAAGAGGATTT ATTTAAGGACCTGAAGATTTGGAATGCTTTGTGTAATTTTCCCAAGCTTCTGCGCAAACTCTGCGACCTCATGAGCT ACTGTGTCGATACTAATGTCACTGCTTACCTAATTTTGCAAGGATAGGTTGTACAGTATATTGTCTGACGAAAAATTA TGATTTCCAAGCGAAATGTATATATACCAAAAGATGCTGTAAATACAAACCTCCAAGAACCTCCAAGAAATGTTTA GAAGAAAAAGAGAACTATCCTGTAACACGCGACAAGTTACTCGTAGTAATATGTGTGAATGAATGCCCCGCTCC TACTCAAAGGAGATGAATATTGTGAATACTGCATCGCTCAATGCGAAGTTTGCATGATGAACACACCTCGCAATGA TGTAACACAGGCTTGATCCTCTCTAGTGACAAAACCTAGTGCAGAGAGTGTGAAGAAGGATGTTCATCTTGTGTTCT AGAGGAACTGCCACTCTTGTGAACCAAGGATATATTACAGAACTCCACGATGATCCTTTGAGTAGAGATTGGATTATG AAATGTGTGAAGAGTGCTCAGATTCTCACTACAAATAATAAAGATATTGTCTTCTTGCAATGGCTTAAACCTGTGCTA AATGTTACAGCTGCTTATGAATGTGAGGTATGCGCGAAGGATATCAAGAAATTACTAAGTACCTGTGAATGCTATA GTGTTATATTCTCAATTGTGATGTTTGCGAAAAATAACTAACTGTGTCGAATGCAAGAAGGATACACACTGAAAAAG GAAAAACGGAGCAGATTAAATGTAGAAAAATAAGCTGTTGAATGTAAGCCCTGGCAATATTTCAAACCAACGAATGT CAAGAATGTTAGTACCAATGCTATAGATGTTCTGACGGTAATCTTGTGATTCTTGTACTCTGGAATACTACCAGATG CTGCCCTTAGAGATTGCGTTAAATGTACCGGTTTGTGCTTCTTGCAACAGTGCCTCTAATGTGCAGAACTATGTAAGA AAATGCCTATCTTTTATAACAAGATGATGAAAAATCAACTTTGTGTCGAAAGATGCCCTCCCGGATACAAAGCTATTATA AACAGATCAACTGGGAAGGGGAATGTCAAAGAGAATGA</p> <p>Protein</p> <p>MIIEKLVNCTLAIDFLSKNLKVORLQKNIFPDYKCLYTTVPEKYKREGVADSLHIFIGFFDNPESSIAYASSCIQDGD KRPLFGASDYSFMSADADADGFENYFETVVELHILHLSRGMFDQFIDAQTRPFEVNEGLRSPVEKEVINGKDTLW LTPVQRMAREHYGSSSLKQLGLENDYGGGSRNSHWQSTFENEFMAMHSGHDVAVYSKFTFALEDSGWYKPYTKY LDEVTWKGQGCDFLYTCDPNKHKEFTNETQFCFYHNYIGNHYVPENEEDRRAQVCHVFGQVSDADTDIRNNQDWS GNTFSDKSKCFDSNLSDGSGRSSYGDHRCLEYECTKKGGINVLVNGQYKIDRETNRYVVRPRKNTSLFEGIFGPEEDV NAFCNIFPKACPKPFCSSNGYCVNDQCHCLPNFAGVGDVYCPDGNVYFQGKCIYTKRCPVNTTQETSKELKEKEELSC KHGQVILDEQCYNCPASYKGYDEYCEYCIAQCEVNDENTCEQCKTLGLLSDKTQRCRECEGGSSCGNRCNHCPEPG YITELHDDPLSRDWIMKCVKECSDSHYKQORYCLPCMAQNCAKYSAYECEVCAEYGRNYQGYCEMLQCYGNGSDC ENNQICSQCKEYTLKRENGADQVEIQAVECKPWQYIQNNEQCQCYQCYRCSDGNSDCSISYGYHDAALRDCVKC TGLCASNSASNCRICKENAYLLQDDENNICVERCPPGYKAIINRSTGRGECQRE</p>
<p><i>Philasterides dicentrarchi</i> leishmanolysin family protein (LSF16) Genee, complete cds</p> <p>GENE: 3646 bp. Exons: 1-134; 223-562; 648-710; 781-1634; 1699-3646 CDS: 3339 bp protein: 1113 aa</p>	<p>Gene</p> <p>ATGAAATTGCGCACTAACATCTATCTCTTCTCATTTTAGTAGTCTCTTTTCCAACCTCTCTTTGGATCAGGACACGATTGTAT GCACACGAATTGGGATATTGTCCTTTAAAGAACTAATATAAACGCACTAAAAATTAGTAATTTATCTTTTATAAATAG ATGATTAGAATTTAGAATTTTGTTTTATTTTTGTGTATTTATTAAATATTGATATTAAAGAGGGAGATTGCTTTAAGAAC AAAAAATGGAAACCCATAAGAGTTAATCTTCGGAAGTGGGCTTTGGATATAATTCCCGAAGAGTATGCTCGAGTAAAA AACAGATTGTCACTAGTAGAGCTTATCTGCGCAATTTTCAATGAAATTTGAATGTTTGAAGCTTATAGAGCTTATCAACAC AAATCTCGTATTTGGAAGAAGAGCTGTGAGATGTCAATTGTACCTACCACTCTACTGACAGAAATGCTGGAATGGAAC AATTCAGATTGCGATTTTATGAGATCAATTTACCAATGCTACTAGAAGTACTTTGGCTTATGCTTCTGTGCTTAAAT TAGAGTAATTTGTTTATCAAAATGATAAAATTAATCAAAATTTTAAATCAATTCATAGCTTAACTTTAATATTATATAAA ATTACAGAGCGCAGATAAAAGACCAATTTTCGGAAGAGTTCGCTTCAATTTGGCTAAATTCGATCAAGCAATTAATGA TAAAAATATTAAATTTATCAACAAATGAAAAATACCAATTACCATTTAATATTTTAAATAGAAAATTAACATAACCAAGA AATTTGAAGCAACTTCCAAAGTACTAGAATTTACATGAGTTAACTCAGCTTTTAGGATTCTCCAAAGTATGTTATCTTCTTCA AAAACTCTTTAACTGGAGCTCCCTACGGAGATGATAACAAACCACTGATTACTAAAGTATTAGAAAATTTAGAAACCTTA AATTCGACTACTCCCAAGTACTAGAATTTGACCAGATAATATTACGGATGCCCTACCGCGAAGGAATGTAAATTTGA AAATGAAGGGGGTTTCGGATCTGCTGGTTCTCACTGGGAAAAGAGCTGTAATAGAAAATGAATACATGACTGCCACTG CAATTGATCAGGATCGAGTTTACTGATTTCACTTTTCGCTTTCGCTTGAAGACTCAGTTGGTATGAAAAATCAGCCAT AAAACTGATAAAATGATTGGGGAAGGAAAAAGGATGTAACCTTTTAGAAAGTTGCAATCCTGACAGAAATCAGAGAA TTCAACACCGCTCCCAAAATAATGTAACCTCTATCACTCAGGAATGGGATATGTTAAATCTGACTCGCCAGAGAACTTACA ATGATAAATGCCATATTACCTCAATTTGGAGTAATAGACACTGTAAAGACCCCAATAATGATCAAGTTTGTATCAATAT TCTGCCAACTCCTATGCTGAATAAAATAGATGCTTCAACAGTAACATAGCCTATTATAAACTTCTAGTACTCCTTTGAG ATGTCCTCTTATGAATGCACAAAGGGAGGAGAAGAAATCTCAGTCATTGTAGCAGGAGAAAAATATGCCCTTTGATAA AGAAAGTAATAAGATTACTCTTGTAAAAAGGGAAGAAATGACAAGTAAATAAAAATTTAATTTTATAAAAAA TAAATAAATATTGTTTATTTAAATAGATACGAAGGCTCTATTGAAGCTCCTGAAATTTGGGACGCTTCTGTAAACAG TTATCACAACCCGTGCCCTCATTCTGTTCTTCCAACGGATACTGTTATGCTGAAGAATGTCACTCGCACTTAAACAT GCTGGAAAAGATTGCAGTATTGAATGTGTTGATGAAAACCTACGTTTCTCAAGGTAATGTGGGATCATAAACCTGTG CTGATGGAACTGTAGTGAACAAACCCCAAGAAATGTGTTGTCCTCAGATCAATTTATGCAACACCGGAGCTGTGCT TTTCTCAATGTCTGAAATTCACACTACGGAAGAGGGATCCTGTGTCGAATGTGAATCTTCTGTGCGCACTGTTCGAGA ATCTCAATTTGTTTAACTGTAAAGAGGCTTCAACTCTCTTAACTGTAAGTCAAAAGCAATGTTGCTCAACTGCTG CCGATAATATACAAACTGATGACATTAATCAAAAGCAAGTCACTGCCCACCTTGTCTGATTTGCTTACTGCTTCA CGATTGTACCACTGCGTTAAGGGAATAACTTGAATCTAGCGACAGATACTGCCAGCAATGTTTGAAGAGGATGGA AGATTGCACCGAATAAGAATGCAAGTCTGCTGTCTGATACACAACTCAATGGAGCAATGTGTGAAGATGCTC TAAAAATATTACGTTTTCGGAAGTGACTGCGTTTCTGTGCTGATAACTGCACCGGCTGTTCCGAAGCCTTATATTG CAGCGAATGTTCACTGACTCTCACAAATTTTCAATTTCTCCTCTGACAAATTCGAATTTGACTTGCACCACTGTCCAAA GGCACCTACTTGAATGCTGATAACACACCTGTACAACCTGTAGTGAACACTGTACTGAATGTCTTCCGCCACTGAG TGCACTGAATGTCAATAAGGATATGAAGTCAAGACGGCCAAATGTTCTTACATAATTTGATTAATGTACAACTGTGAGC TTTGCACTACTGAATCTGACTGCCAATCCTGTAAGTGGACATAATCTTCAACCAAGTGAAGGTTGTTTGAATGTGT TGACAAATGCTCTGAAACTGTTTCGGAACCTGTCGCAAACTTGCAACCAATGCCCTGAAACTGCGCACTGTTA ACAAAGTTTGGGATAAGAATGTGAAGTCTGTCAAGACGGATTCCAATGAGTTCTGATAAAAAATGCATAAATGGCA GAAAAATGCAAGTCTTCTGCTCCAGTAATCTGCAACCGCTCTGCAAGACAAAACTCTACAGCTACAACGAGGAGT CAACACTTCTTGTGTGGCTGAATGCTCCTCACTCCACCTATGCAGATGGAGAAAGCAAATCTGTGAAAAATGTTCTGA TTAATGTGAAAAATGTGATCAAAAAGCGAATGCATCACCTGTAGTGAAGTCAATTTCTCAATGCAACAACTCAACT TGTGAAAAATGTGGAATCAATTTGCTTAAATGTAGTAACAGTTAAACCTGTTTAGTCTGTGTGAATGGAACGTAATCA GAAGAAAAAGACCATGTAATTTGTAGATCTCATTGCCCAAAGGTCAATACAGTAATTAACATAGTACTTGTCTCA ATGTCCTTCTTTTGTACCTCATGTTCCGATGAAAATACTTGTGACGAATGTGCTAACTCTTCTTATTTCAACAGCTTAA CTATAAATGTAATTAATCATGAAGACAAATTTGGTCTGTCTTAATGAATGCCCCAAAGGATATACACCTAGTAAATAAAGAAAT</p>

<p>LSF2</p>	<p>LSF2</p> <p>DNA </p> <p>mRNA </p> <p>Protein </p> <p>Oligo-state: monomer </p>
<p>LSF3</p>	<p>LSF3</p> <p>DNA </p> <p>mRNA </p> <p>Protein </p> <p>Oligo-state: monomer </p>
<p>LSF4</p>	<p>LSF4</p> <p>DNA </p> <p>mRNA </p> <p>Protein </p> <p>Oligo-state: monomer </p>



<p>LSF8</p>	<div data-bbox="363 188 1177 293"> <p>DNA</p> </div> <div data-bbox="363 293 1070 405"> <p>mRNA</p> </div> <div data-bbox="363 405 1326 728"> <p>Protein</p> <p>Oligo-state: monomer</p> </div>
<p>LSF9</p>	<div data-bbox="363 728 1209 840"> <p>DNA</p> </div> <div data-bbox="363 840 1114 943"> <p>mRNA</p> </div> <div data-bbox="363 943 1326 1301"> <p>Protein</p> <p>Oligo-state: monomer</p> </div>
<p>LSF10</p>	<div data-bbox="363 1301 1220 1413"> <p>DNA</p> </div> <div data-bbox="363 1413 1007 1525"> <p>mRNA</p> </div> <div data-bbox="363 1525 1326 1877"> <p>Protein</p> <p>Oligo-state: monomer</p> </div>

<p>LSF11</p>	<p>LSF11</p> <p>mRNA</p>  <p>1821</p> <p>Protein</p>  <p>extra</p>  <p>Oligo-state: monomer</p>
<p>LSF12</p>	<p>LSF12</p> <p>DNA</p>  <p>1394</p> <p>mRNA</p>  <p>1095</p> <p>Protein</p>  <p>extra</p>  <p>Oligo-state: monomer</p>
<p>LSF13</p>	<p>LSF13</p> <p>DNA</p>  <p>2286</p> <p>mRNA</p>  <p>1773</p> <p>Protein</p>  <p>extra</p>  <p>Oligo-state: monomer</p>



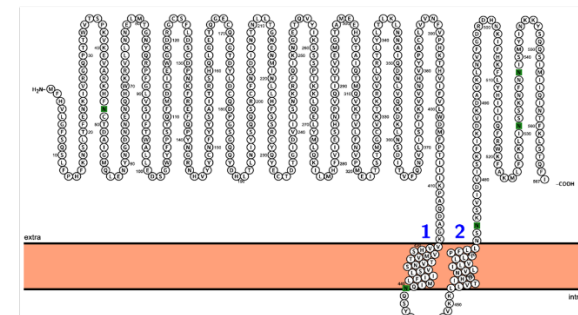
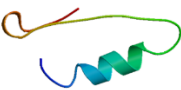


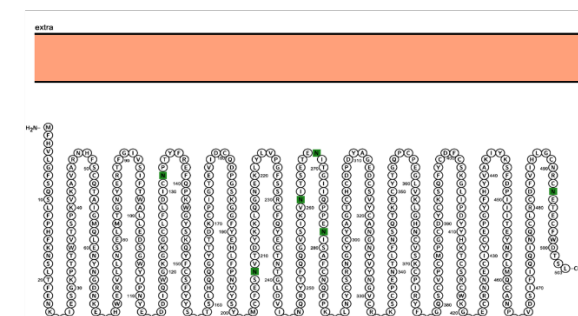
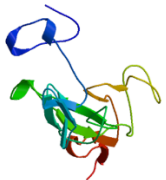


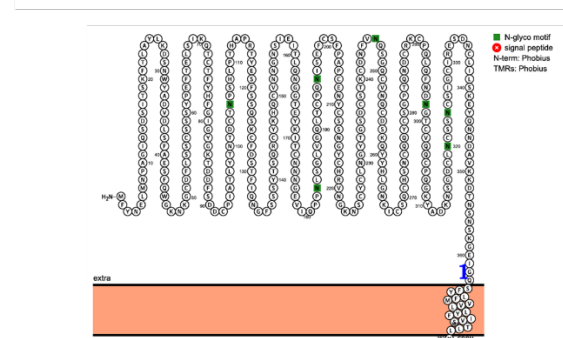
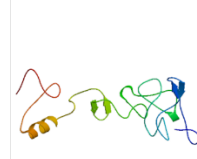
LSF14	<p>LSF14</p> <p>DNA</p>  <p>mRNA</p>  <p>Protein</p>  <p>Oligo-state: monomer</p> 
LSF15	<p>LSF15</p> <p>DNA</p>  <p>mRNA</p>  <p>Protein</p>  <p>Oligo-state: monomer</p> 
LSF16	<p>LSF16</p> <p>DNA</p>  <p>mRNA</p>  <p>Protein</p>  <p>Oligo-state: monomer</p> 

Table 2.- Schematic description of the genes encoding the LSFs, the mRNAs, the proteoforms of the expressed proteins and the modeling of the proteins based on their homology using the [ExPASy web server](#).

1: LSF2	100.00	26.82	28.16	27.34	27.39	28.38	29.29	30.75	31.51	28.39	25.07	24.05	27.75	30.96	31.18	27.27
2: LSF12	<u>26.82</u>	<u>100.00</u>	67.20	35.29	34.04	37.73	39.80	35.05	34.88	35.39	22.09	27.05	29.38	27.97	31.52	28.48
3: LSF13	28.16	<u>67.20</u>	<u>100.00</u>	38.07	36.34	37.28	37.76	38.07	36.84	35.25	22.94	26.58	30.65	30.24	31.79	29.70
4: LSF5	27.34	35.29	<u>38.07</u>	<u>100.00</u>	62.86	38.93	38.54	38.87	40.27	39.94	22.98	24.88	27.73	29.69	27.57	28.53
5: LSF15	27.39	34.04	36.34	<u>62.86</u>	<u>100.00</u>	39.71	40.40	37.80	39.78	35.25	23.46	23.88	27.29	28.97	29.27	28.01
6: LSF7	28.38	37.73	37.28	38.93	<u>39.71</u>	<u>100.00</u>	44.27	40.85	40.03	40.13	22.75	25.78	28.96	32.60	33.95	31.29
7: LSF8	29.29	39.80	37.76	38.54	40.40	<u>44.27</u>	<u>100.00</u>	41.46	48.23	41.29	27.15	29.66	31.58	34.70	32.85	33.84
8: LSF16	30.75	35.05	38.07	38.87	37.80	40.85	<u>41.46</u>	<u>100.00</u>	42.33	43.14	23.48	27.17	29.81	30.66	30.65	28.36
9: LSF3	31.51	34.88	36.84	40.27	39.78	40.03	48.23	<u>42.33</u>	<u>100.00</u>	59.50	24.13	27.44	29.49	33.39	33.80	29.25
10: LSF6	28.39	35.39	35.25	39.94	35.25	40.13	41.29	43.14	<u>59.50</u>	<u>100.00</u>	25.06	27.40	28.52	32.44	30.79	27.11
11: LSF11	25.07	22.09	22.94	22.98	23.46	22.75	27.15	23.48	24.13	<u>25.06</u>	<u>100.00</u>	25.08	22.02	23.45	23.17	23.79
12: LSF4	24.05	27.05	26.58	24.88	23.88	25.78	29.66	27.17	27.44	27.40	<u>25.08</u>	<u>100.00</u>	30.73	40.65	36.19	35.89
13: LSF9	27.75	29.38	30.65	27.73	27.29	28.96	31.58	29.81	29.49	28.52	22.02	<u>30.73</u>	<u>100.00</u>	34.11	41.09	38.04
14: LSF10	30.96	27.97	30.24	29.69	28.97	32.60	34.70	30.66	33.39	32.44	23.45	40.65	<u>34.11</u>	<u>100.00</u>	44.36	41.11
15: LSF1	31.18	31.52	31.79	27.57	29.27	33.95	32.85	30.65	33.80	30.79	23.17	36.19	41.09	<u>44.36</u>	<u>100.00</u>	45.07
16: LSF14	27.27	28.48	29.70	28.53	28.01	31.29	33.84	28.36	29.25	27.11	23.79	35.89	38.04	41.11	45.07	100.00

Table 3. Amino acid identity matrix between the different LSFs of *P. dicentrarchi*.

Standard name	MW	pI	VSP domain	Signal peptide	Transmembrane	O-sites	Genee (bp)	mRNA (bp)	Exons/ introns
LSF1	45806.08	5.34	Yes	None	Yes	Yes	1784	1257	9/8
LSF2	117193.02	7.92	Yes	Yes	Yes	Yes	3536	3069	8/7
LSF3	225018.15	4.94	Yes	None	Yes	Yes	2809	2433	5/4
LSF4	68634.30	8.40	Yes	None	yes	Yes	2632	1866	12/11
LSF5	91197.18	5.31	Yes	None	None	Yes	2790	2400	7/6
LSF6	97237.18	5.42	Yes	None	Yes	Yes	2834	2634	4/3
LSF7	70517.85	5.46	Yes	None	Yes	Yes	2851	1909	12/11
LSF8	86013.61	5.56	Yes	None	None	Yes	2870	2328	9/8
LSF9	54129.34	5.33	Yes	None	Yes	Yes	1773	1479	5/4
LSF10	45140.65	5.00	Yes	None	None	Yes	1854	1118	10/9
LSF11	67863.50	5.34	Yes	None	Yes	Yes	-	1821	-
LSF12	40155.26	5.15	Yes	None	Yes	Yes	1394	1095	6/5
LSF13	66470.53	7.59	None	None	None	Yes	2286	1773	10/9
LSF14	65831.37	8.93	Yes	None	Yes	Yes	2138	1832	5/4
LSF15	57781.41	6.03	Yes	None	None	Yes	1744	1512	4/3
LSF16	42555.05	5.24	Yes	None	Yes	Yes	1502	1149	6/5

Table 4.- Summary of the main biochemical and genomic characteristics of the LSFs of *P. dicentrarchi*

- *Expression of leishmannolysins during infection*

Ciliate: Up-Down regulated membrane-embedded proteases related genes during infection (1, 2 and 4h)			
	Name	Log Fold change	Gene name
1h			
	LSF18	13.0222215	Leishmanolysin-like peptidase
	LSF17	6.66019971	Leishmanolysin-like peptidase
	LSF12	4.63653377	Leishmanolysin-like peptidase
	LSF15	2.78970774	Leishmanolysin-like peptidase
	8301	-3.34146	Leishmanolysin-like peptidase
	LSF13	-7.49960275	Leishmanolysin-like peptidase
2h			
	LSF13	56.7174342	Leishmanolysin-like peptidase
	LSF18	15.9960053	Leishmanolysin-like peptidase
	LSF12	6.39724179	Leishmanolysin-like peptidase
	LSF17	5.93652415	Leishmanolysin-like peptidase
	8301	3.47813417	Leishmanolysin-like peptidase
	LSF15	-5.42524079	Leishmanolysin-like peptidase
	LSF16	-19.3446574	Leishmanolysin-like peptidase
4h			
	LSF16	18.3686417	Leishmanolysin-like peptidase
	LSF18	13.7957243	Leishmanolysin-like peptidase
	LSF12	12.0971403	Leishmanolysin-like peptidase
	LSF17	5.54326522	Leishmanolysin-like peptidase
	LSF13	-29.9741977	Leishmanolysin-like peptidase

Table 5.- Expression of *P. dicentrarchi* LSFs during infection using a transcriptomic analysis of mRNA expression at different times of infection (1-4 h). In green are indicated the LSFs that are overexpressed and in red those that are underexpressed.

By means of a transcriptomic analysis using massive sequencing (NGS) we have verified that most LSFs are overexpressed after infection, indicating that they are probably proteins associated with virulence, in this case, of strain I, which is a strain of high virulence.

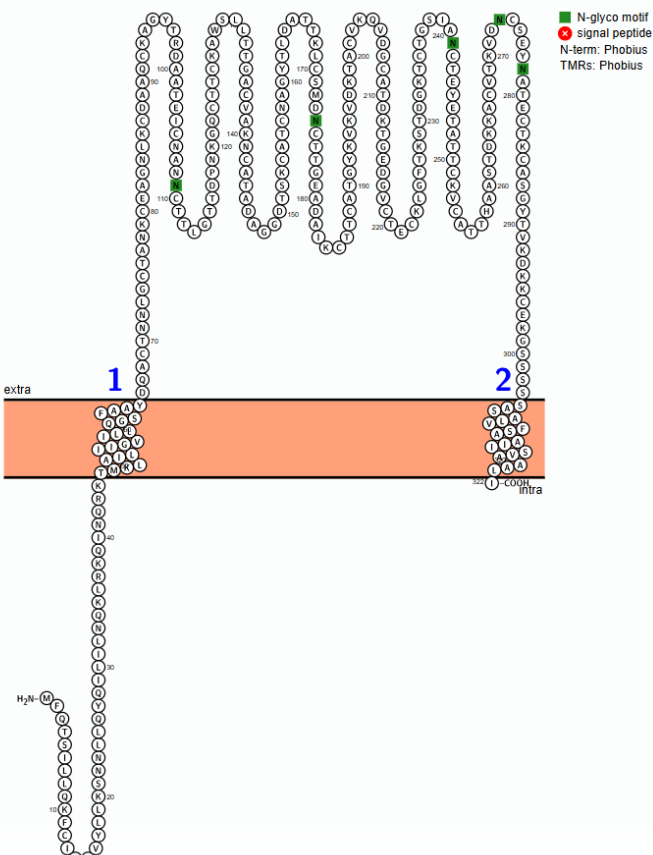
- Characterization of surface variable proteins (VSPs) of *Philasterides dicentrarchi*

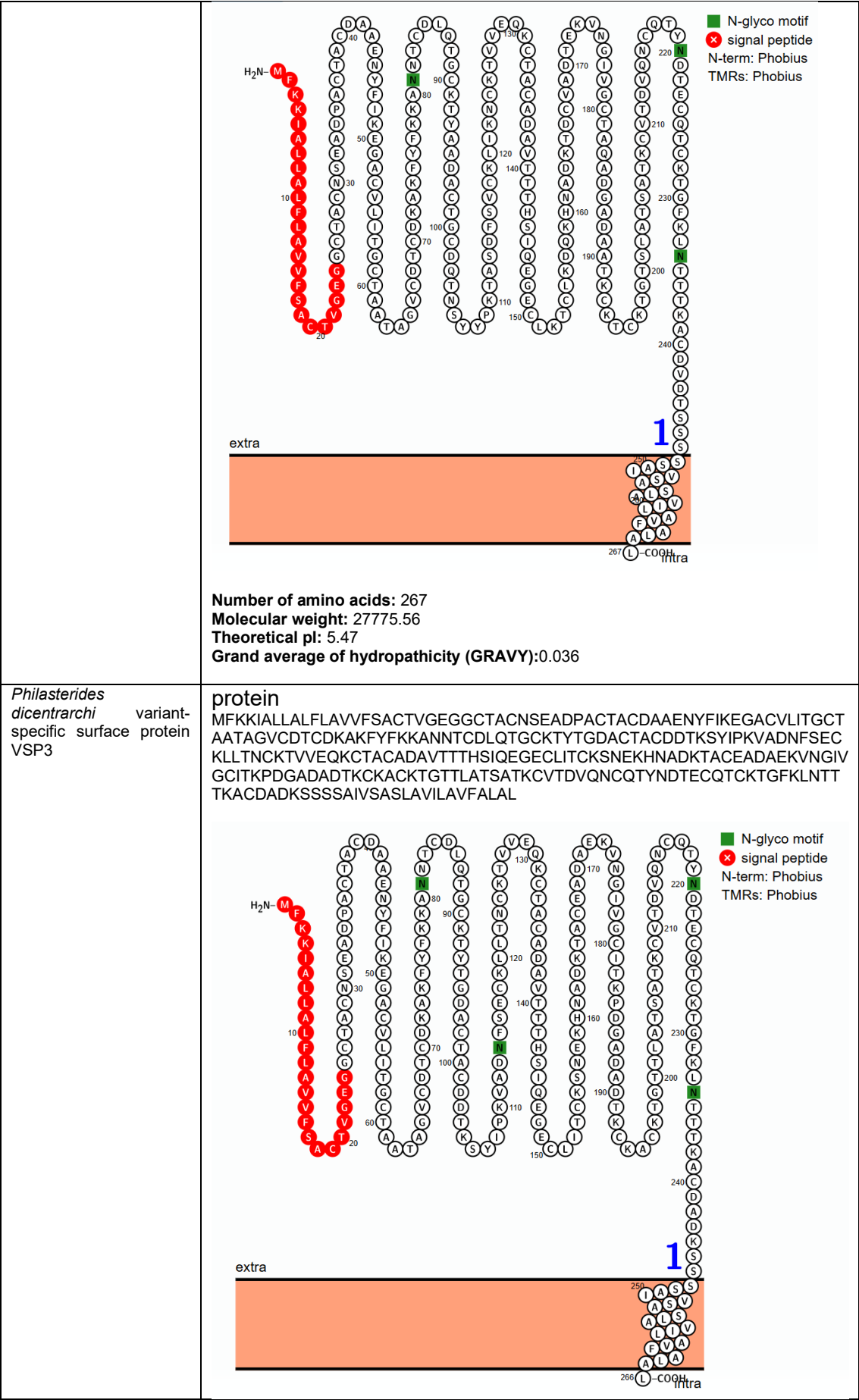
Variable surface glycoproteins (VSPs) are glycoproteins that are densely packed on the cell surface of most protozoa. In some parasitic microorganisms, mutually exclusive changes in the expression of these antigenous organisms have been shown to allow the parasites to evade the host's immune response. It is widely assumed that antigenic variation in protozoan parasites is achieved by spontaneous emergence within the population of cells expressing antigenic variants that escape antibody-mediated cytotoxicity. It has been widely reported that antibodies against VSPs are cytotoxic and may protect hosts from infection. Therefore, it appears that VSP-mediated antigenic variation is a tunable process between the host and the parasite.

The main characteristics of *in vitro* VSPs are:

1. Present in all isolates
2. Only one VSP per trophozoite
3. VSPs change spontaneously *in vitro*
4. The rate of change depends on the VSP and the isolate
5. VSPs differ in their resistance to trypsin and chymotrypsin

In the present project, we have identified 8 VSPs in *P. dicentrarchi* whose characterization is presented in Table 6.

<p><i>Philasterides</i> <i>dicentrarchi</i> variant-specific surface protein VSP1</p>	<p>protein MFQTSILLQKFCIFDVYLLKSNLLQYQILNLQKLRKQINQRKTMKLAILIIGVILLQGSFAAYDQACTNNLGCTANKCEAGNLKCDAAQCKAGYTRDAATEICNANNCTTLGTTDPNKGQCTTCKAWSLLTTGACVAKNCATADAGGDTSKCATCNAGYTLDATTKLCSMDNCTTG EADAICKTTCATGYKVKVDKTACVKQVDGCATDKTGEDGVCTECKLGFTKSTDGKTCTGSIANCTEYETATTCKVCATTHAASTDKKACVTKVDNCSEYNATECTKCASGYTVKDKKCEKGSSSSSASALVFSAAIISVAAALI</p>  <p>Number of amino acids: 322 Molecular weight: 33808.68 Theoretical pI: 8.31 Grand average of hydropathicity (GRAVY): -0.080</p>
<p><i>Philasterides</i> <i>dicentrarchi</i> variant-specific surface protein VSP2</p>	<p>protein MFKKIALLALFLAVFSACTVGEGGCTACNSEADPACTACDAAENYFIKEGACVLITGCTAATAGVCDTCDKAKFYFKKANNTCDLQTGCKTYAADA CTGCDQTNSYYPKTASDFSVCKLIKNC KTVVEQKCTACADAVTTTTHSIQEGECLKTCLKDQKHNADKTD CVADTEKVNGIVGCTAQADGADAATKCKTCKTG TSLATSATKCVTDVQNCQTYNDTECQTCKTGFKLN TTTKACD VDTSSSSSAIVSASLAVILAVFALAL</p>



	<p>Number of amino acids: 266 Molecular weight: 27707.53 Theoretical pI: 5.17 Grand average of hydropathicity (GRAVY):0.018</p>
<p><i>Philasterides dicentrarchi</i> variant-specific surface protein VSP4</p>	<p>protein MFKKIALLLFLAVVFSACKDTAGCICITGTGSTETCTCDKATFYFENSKNVCVQLTGCK SYASDACTACDEVKFYYPKTTTDFSVCKLITNCKTVVNQKCTACADDAPKTHSIQEGEC LITCLKDQKHNAAKTACVADTEKVNIGVGTAKADGADADTKCKTCKTGTSLATSATKC VTDVQNCQTYNDTECEQCNTGFKKNTSTKACDADAAQNVNIGVGCITKAEGTDADTKC KACVDGKTLATSATKC</p> <p>Legend: ■ N-glyco motif ✗ signal peptide N-term: Phobius TMRs: Phobius</p> <p>Number of amino acids: 252 Molecular weight: 26485.08 Theoretical pI: 7.22 Grand average of hydropathicity (GRAVY):-0.189</p>
<p><i>Philasterides dicentrarchi</i> variant-specific surface protein VSP5</p>	<p>protein MLKKIPLIALFLVFVFSKCEDGKNGCTTCEDADNCDVCDQANFYFKKADKNCDLQTGCK TYAADACTACDETKFYFPKIANDFSECTLFSNCKTVVDQKCTACADAVTATHSIQEGEC LKICLDQKHNADKTACEADAQKVNIGVGTAKADGADADTKCKTCKTGTLATSAIKC VTDVQDCKTYNDTGCELCNSGFKLNAAATKACEAEGEATENINNISGCTAQADGADAAT KCKTCKTGTLATSATKCVTNVQECQTYNDTGCEKCKTGFKLNTTTKACEADNSSSSS AIISASLTVFLAVCSLAL</p> <p>Legend: ■ N-glyco motif ✗ signal peptide N-term: Phobius TMRs: Phobius</p> <p>Number of amino acids: 252 Molecular weight: 26485.08 Theoretical pI: 7.22 Grand average of hydropathicity (GRAVY):-0.189</p>

	<p>Number of amino acids: 311 Molecular weight: 32803.99 Theoretical pI: 4.99 Grand average of hydropathicity (GRAVY):-0.210</p>
<p><i>Philasterides dicentrarchi</i> variant-specific surface protein VSP6</p>	<p>protein MFKKIALLLALFLAVFSQCTEGTTGCICIAKEDDHNCLCDTTTTYFIQDSACVLLTGCKTA SKSAVCSACDEEFYYPKTSTDFSVCKLIKNCCTVVNQKCTVCAEAVSTTHSIQEGECLI TCKSYEKNADKTACEADPEMVNGIVGCITKAEGADAETKCKACVDGKTLATSATKCVT DVKDCQIYNDTGCEQCKTGFKLNTTRVCDVDKSSSSAIVSASLAVILAVFALAL</p> <p>■ N-glyco motif ✗ signal peptide N-term: Phobius TMRs: Phobius</p> <p>extra</p> <p>1</p> <p>234(L)-COOH intra</p>
<p><i>Philasterides dicentrarchi</i> variant-specific surface protein VSP7</p>	<p>protein MLKKITLLSLFLAVAFSACTVSGGGCTACNQELVPACTACDETKEYFLKDGACVLLDKCI GAPNGVCNQCDETNFYFLKDDACVFIYGCAFTDDAGACTTCHEDRSYFPKTQNDASV CKLLTNCKTVVDQKCTACADAVNTTHSIQEGECLKTCEKDQKHNAEKTDCVADIENVN NIVGCTAKAEGADADNKCKICKTGTTLAISTTKCVTNVKDCQTYNDTGCDKCKTGFKLN TTTQACDVKSSSSSSAIVSASLAVILAVFALAL</p>

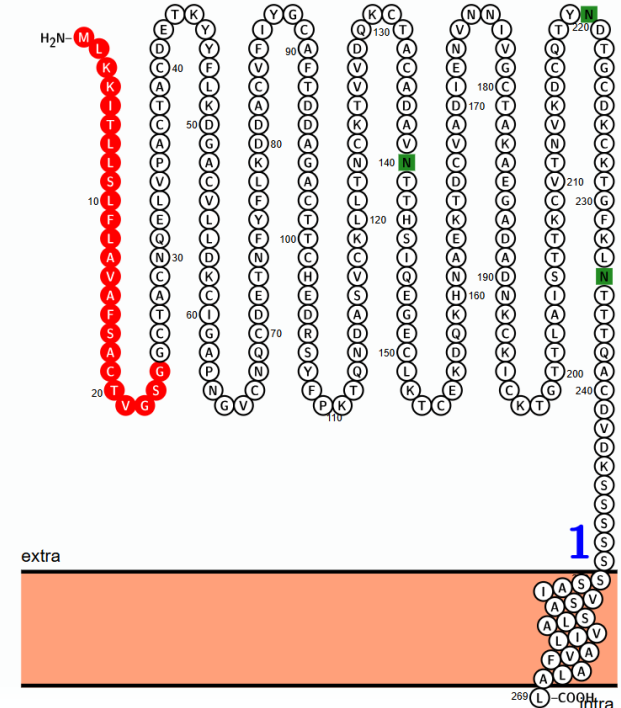
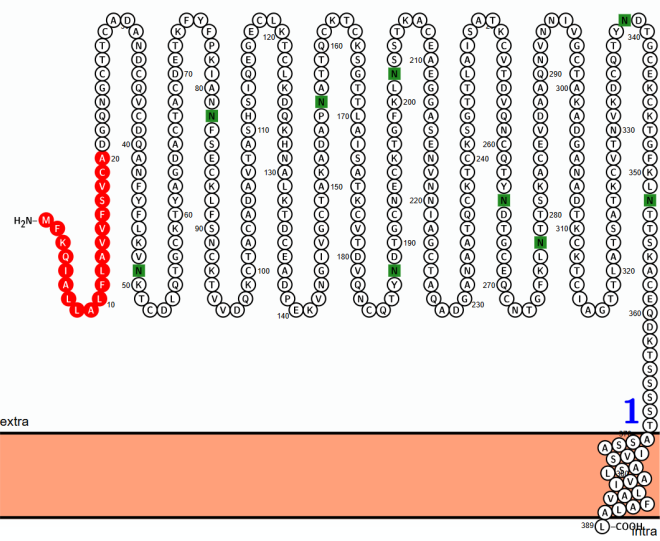
	 <p> ■ N-glyco motif ✱ signal peptide N-term: Phobius TMRs: Phobius </p> <p> Number of amino acids: 269 Molecular weight: 28505.40 Theoretical pI: 5.02 Grand average of hydropathicity (GRAVY): -0.005 </p>
<p><i>Philasterides dicentrarchi</i> variant-specific surface protein VSP8</p>	<p>protein</p> <p> MFKQIALLALFLAVVFSVCADGQNGCTTCADANDCQVCDQANFYFLKVNKTCDLQTGC KTYAGDACTACDETKFYFPKIANNFSECKLFSNCKTVVDQKCTACADAVTASHSIQEGE CLKTKLDQKHNALKTDCEADPEKVNIGVGTAKADAPNATTQCKTKCKSGTTLAISATK CVTDVQNCQTYNDTGCECKTGFKLNSSTKACEAEGGASENVNNIAGCTAQADGANA ATQCKTKCKSGTTLAISATKCVTDVQNCQTYNDTGCEQCNTGFKLNTTSKACEVDAQN VNNIVGCTAKADGANADTKCKTCIAGTTLATSAATKCVTNVKDCQTYNDTGCECKCTGFK LNTTSKACEQDKTSSSSTASSAIVSASLAVILAVFALAL </p>  <p> ■ N-glyco motif ✱ signal peptide N-term: Phobius TMRs: Phobius </p> <p> Number of amino acids: 389 Molecular weight: 40558.64 Theoretical pI: 5.84 Grand average of hydropathicity (GRAVY): -0.178 </p>

Table 6.- Biochemical and genetic characterization of variable surface glycoproteins (VSPs) identified in *Philasterides dicentrarchi*.

As can be seen in Table 6, VSPs are glycoproteins that have some transmembrane domain.

Objective 2.- To design recombinant proteins and peptides of VSPs and leishmanolins, taking into account the intraspecific variations observed in the different strains.

Throughout the project, several plasmid constructs were performed for the expression of leishmanolins and VSPs in yeast and in human cells HEK 293 (Table 6)

<i>Species</i>	<i>Recombinant protein</i>	
<i>Saccharomyces cerevisiae</i>	VSP1 VSP2 Chimeric LSF5-LSF14 / VSP1-VSP3 VSP8 Chimeric VSP1-VSP2-VSP3-VSP4-VSP5-VSP6-VSP7-VSP8	
<i>Kluyveromyces lactis</i>	Chimeric LSF5-LSF14-VSP1-VSP3 VSP8	
<i>Komagataella pastoris</i> (sin. <i>Pichia pastoris</i>)	VSP8 VSP3	Chimeric
<i>HEK 293</i> (human cells)	VSP1 VSP1-VSP8	

Table 7. Expression of recombinant antigens in the yeasts *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Pichia pastoris* and in human embryonic human kidney cells HEK 293.

For the expression of recombinant proteins in *K. lactis* yeast, we have used the secretion strategy (Fig. 1) using the protocol presented in Figure 2 and using the pKLAC2 expression vector (*K. lactis* Protein Expression Kit, New England Biolabs).

Building Recombinant Proteins in Yeast

Kluyveromyces lactis:

As indicated above, in this project we have designed a series of plasmid constructs to be able to express surface proteins in expression vectors in yeasts:

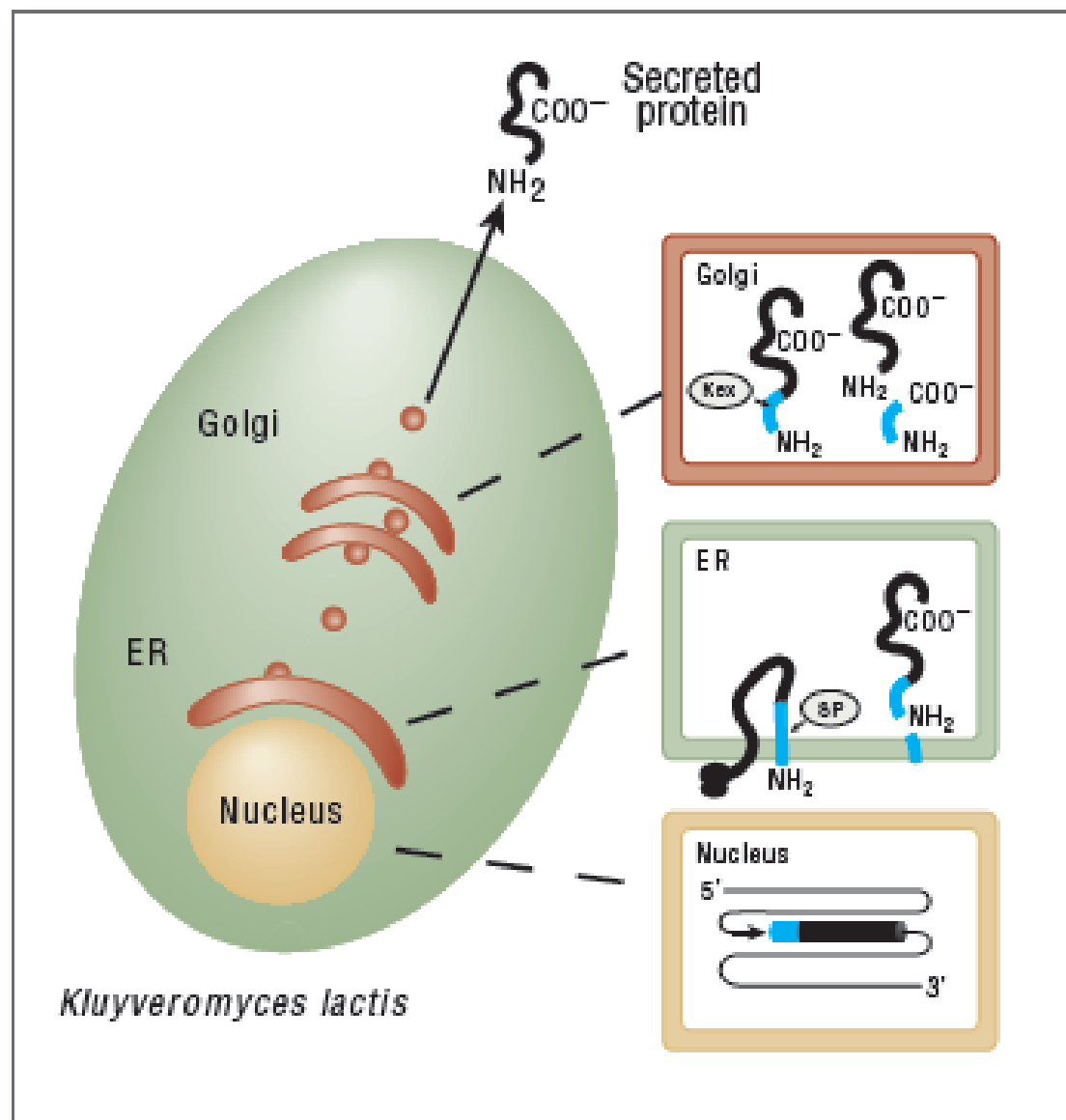


Figure 1. Graphical model representing the processing and expression of recombinant proteins secreted by the yeast *Kluyveromyces lactis*.

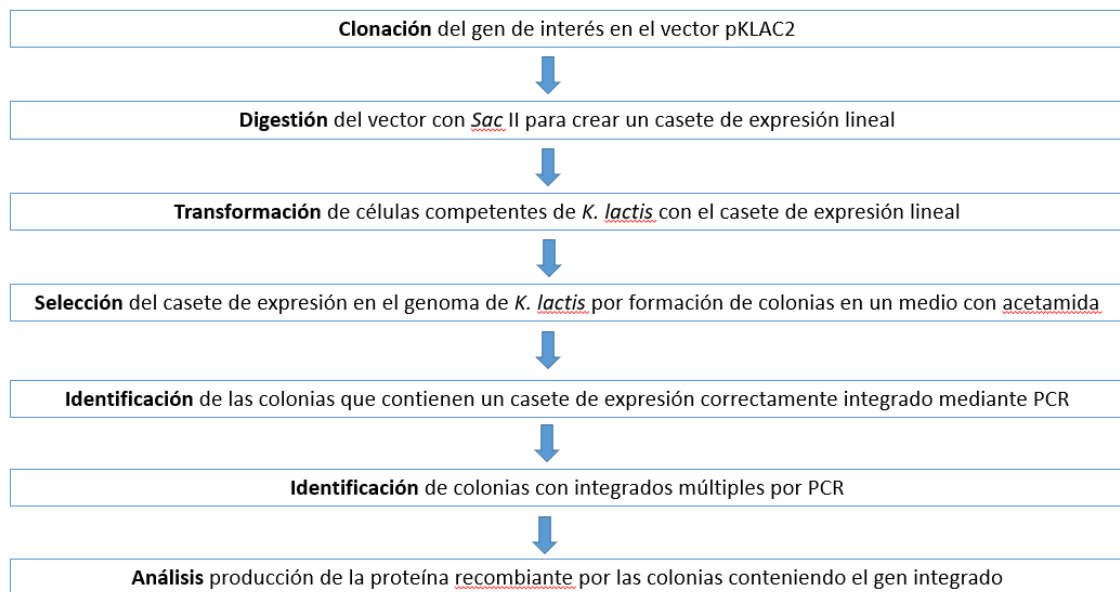


Figure 2. General description of the method of expression of recombinant proteins in *K. lactis*.

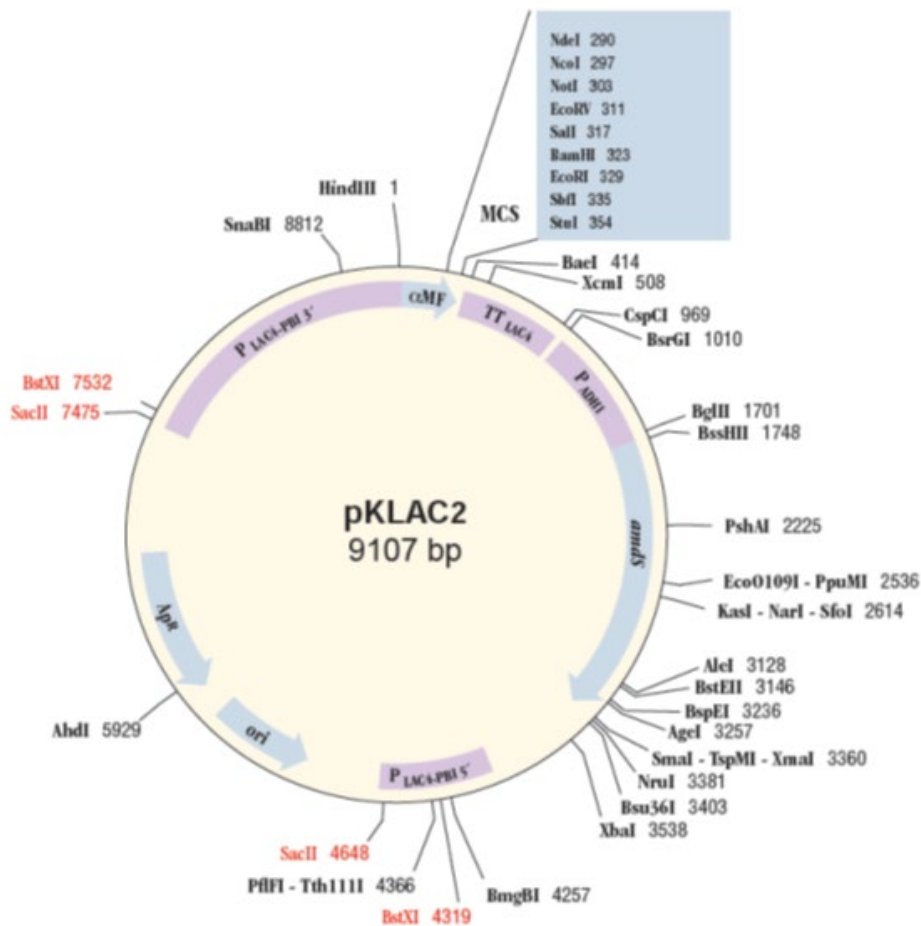


Figure 3. pKLAC2 expression vector used for the production of recombinant proteinS in *K. lactis*.

Chimeric protein production

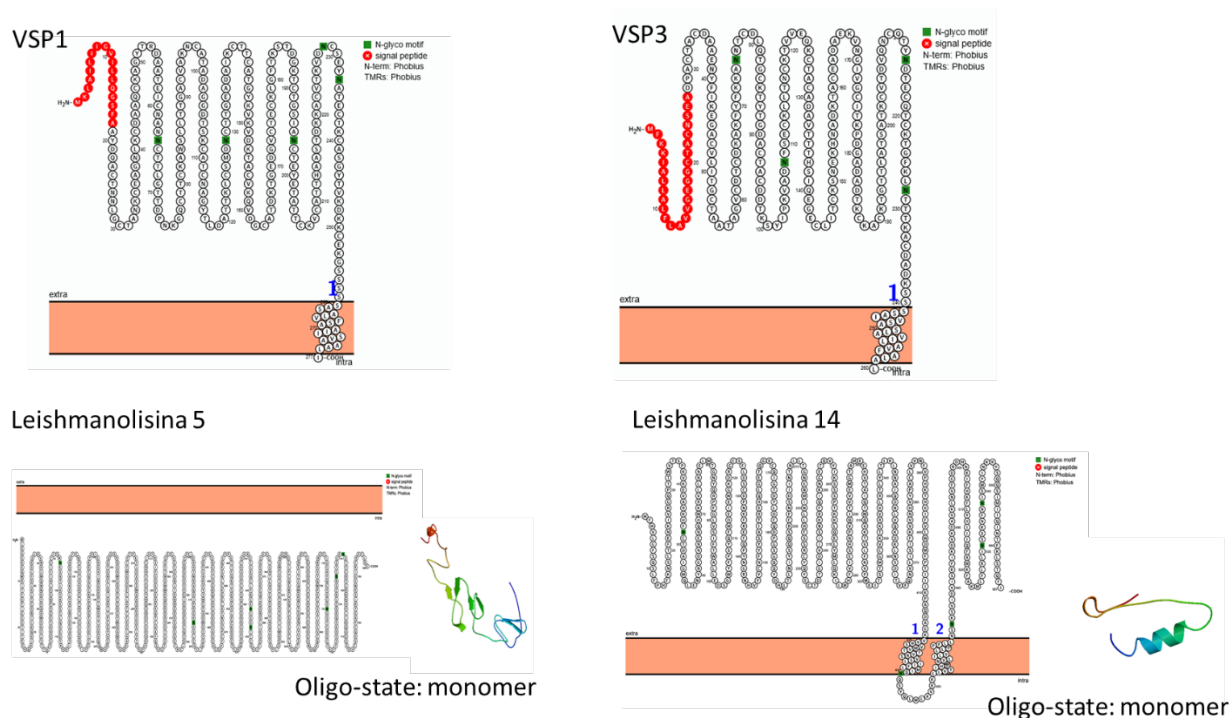


Figure 4. VSP and LSF proteins used in the design of the chimeric recombinant protein *PQLeish2* expressed in *K. lactis* yeast.

We have designed plasmid constructs for the expression of a polycistronic (chimeric) protein containing two LSF fragments (LSF5 and LSF14) and two VSPs (VSP1 and VSP3) that we call *PQLeish2_TRITAG* that we have previously selected based on the prediction of their immunogenicity using antigenic peptide prediction bioinformatics programs - <http://imed.med.ucm.es/Tools/antiGeneic.pl> and an immunogenic epitope database (IEDB) https://www.iedb.org/home_v3.php (Fig. 4). In the protein design, the fragments were separated through the insertion of KK separators (Mol Immunol. 2019;106:1-11. DOI: [10.1016/j.molimm.2018.11.019](https://doi.org/10.1016/j.molimm.2018.11.019)) and a signal peptide corresponding to the protein T2A of the trichocyst matrix was included (sequence ID: AWX67865.1). In the plasmid construction we have also included in the c-terminal region a tag, the peptide TRITAG (MTFSVPIS), which is recognized by a monoclonal antibody that we have developed in our laboratory called US9 (Mol Immunol 2004; 41(4): 421-33. DOI: [10.1016/j.molimm.2004.03.032](https://doi.org/10.1016/j.molimm.2004.03.032)). The amino acid sequences and their proteoforms and the prediction of their 3D structure are schematized in Figures 4-6.

The nucleotide sequence corresponding to the chimeric protein *PQLeish2_TRITAG* was cloned into the pKLAC2 expression vector using a pair of primers shown in Fig. 7 and subsequently amplified in competent *E. coli* strain DH-5a bacteria (Fig. 8). The cloned pKLAC2 vector was digested with the restriction enzyme *SacI* I to linearize the vector (Fig. 9) and insert it into competent yeasts that were selected for their ability to grow in an auxotrophic medium containing acetamide (Fig. 10).

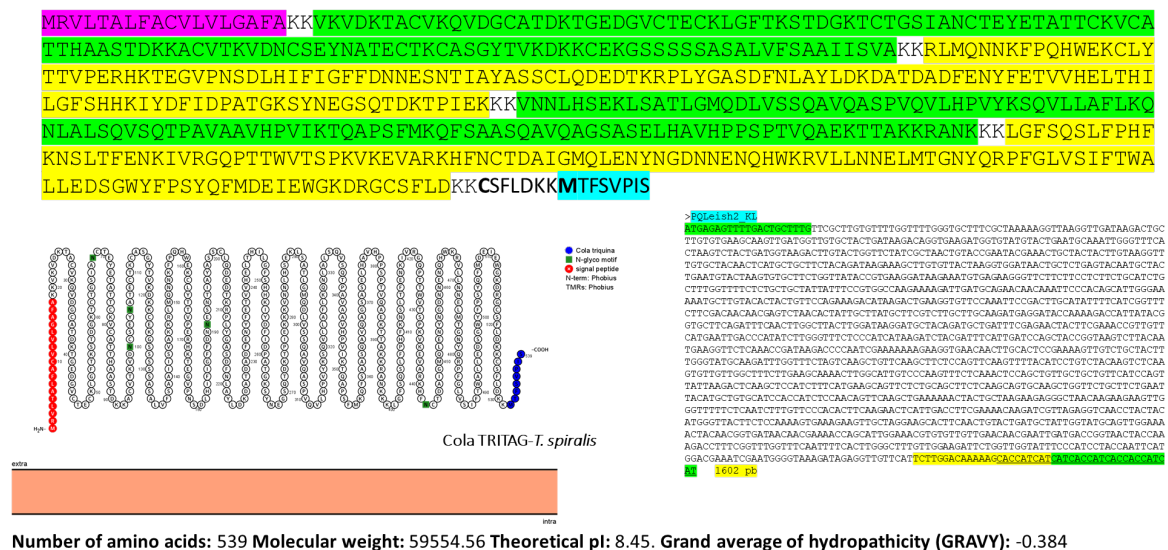


Figure 5. Design of a chimeric protein containing VSPs 1 and 3 / LSFs 5 and 14 and carrying a label at the c-terminal end constituted by a peptide present in the gp53 protein of the nematode *Trichinella spiralis* that is recognized by the monoclonal antibody US9 (TRITAG).

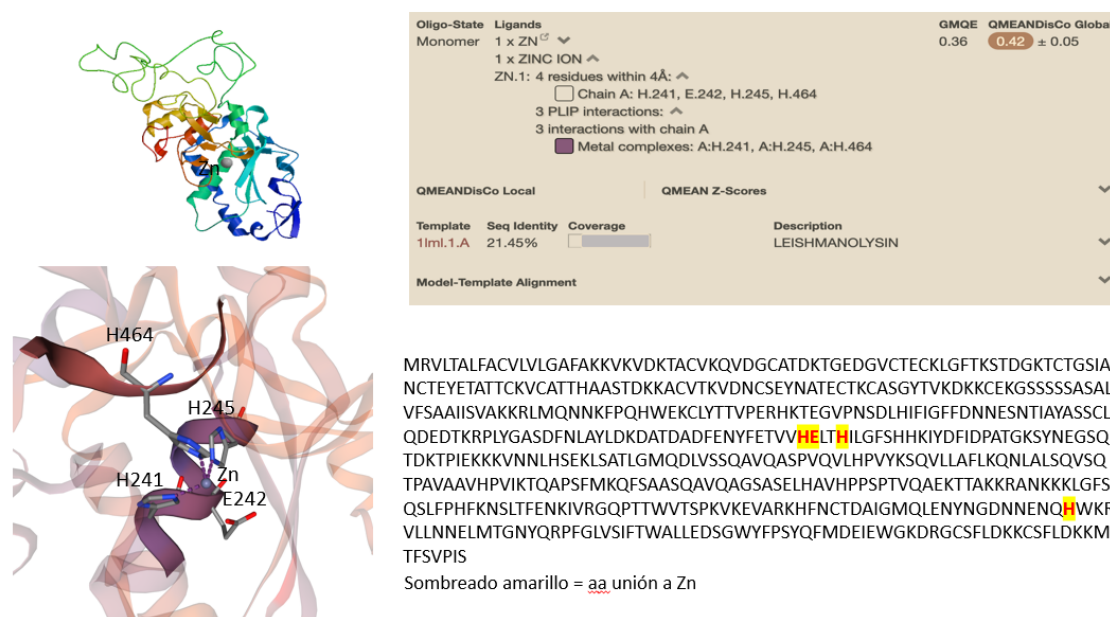
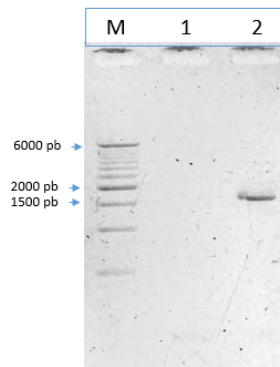


Figure 6. Prediction of the 3-D structure of chimeric recombinant protein PQLeish2_TRITAG

PCR. AMPLIFICACIÓN PQ2Leish_TRITAG. Gel agarosa al 1%



DISEÑO DE Primers clonación en pKlac2:

FLeis2KL: 5' CGC CTC GAG AAA AGA ATG AGA GTT TTG ACT GCT TTG 3'

RLeis2KL-FC: 5'ATA AGA AAT GC GGC CGC TTA AGA AAT TGG CAC CGA AAA TGT CAT TTT TTT GTC CAA GAA TGA ACA 3'

← **PQLeish2_TRITAG (1602 bp)**

PCR. Amplificación secuencia PQLeish2-KL-TRITAG / primers FLeis2KL + RLeis2KL-FC

M: Marcador de tamaño ADN (Ladder 500 bp)

1: Control PCR (sin molde)

2: Amplificación secuencia PQLeish_KL_US9 con primers FLeish2KL/RLeish2KL-FC.

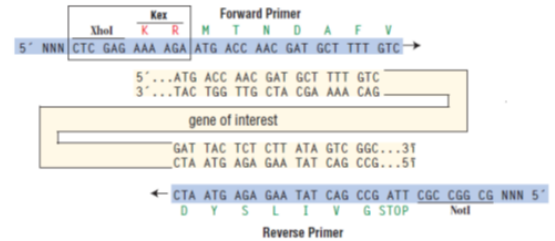
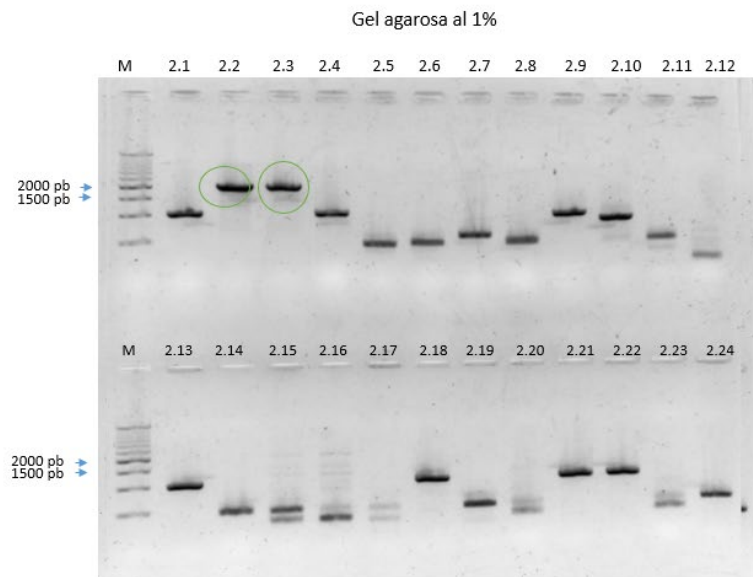


Figure 7. Cloning of chimeric recombinant protein PQLeish2_TRITAG in the expression vector pKLAC2.



M: marcadores de tamaño de DNA

Canales 2.1-2.24: colonias de *E. coli* transformadas con pKLac2_PQLeish2_US9. Los círculos representan las colonias utilizadas para la extracción de los plásmidos

Figure 8. Amplification of the pKLAC2 plasmid containing the recombinant protein gene PQLeish2_TRITAG in *E. coli*.

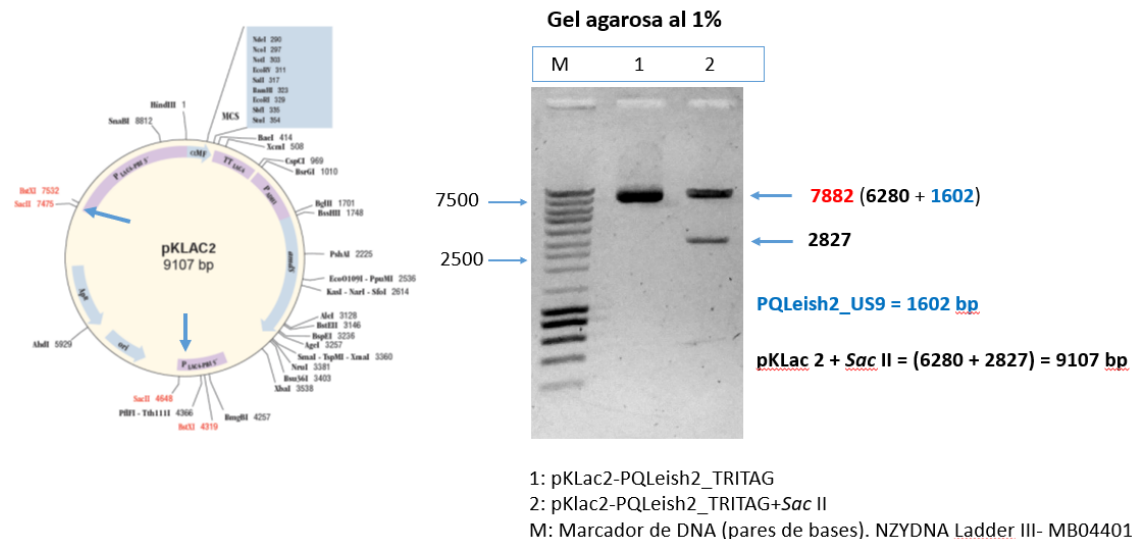
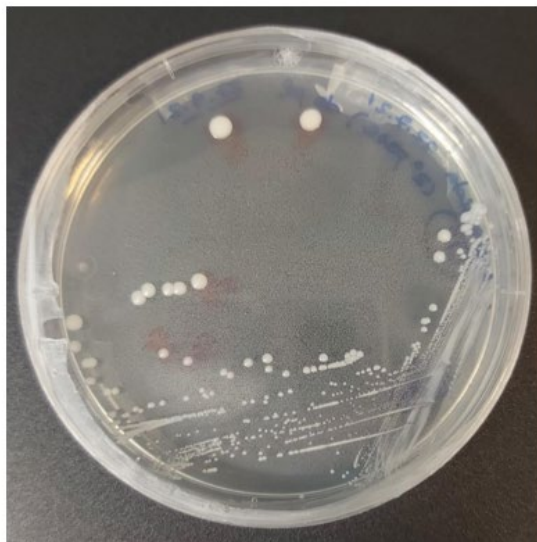


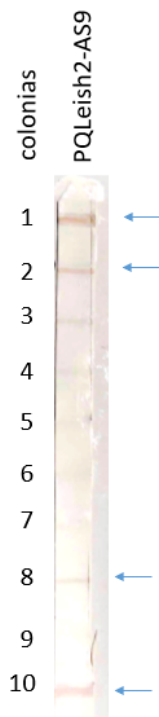
Figure 9. Digestion of the cloned pKLAC2 vector with the sequence PQLeish2_TRITAG and digested with the *SacII* enzyme to create a linear expression cassette inserted into the *K. lactis* genome.



Colonias de *K. lactis* transformadas con el plásmido pKLAC2_PQLeish2_US9 (triquina) + *Sac II*

Figure 10. Transformation of competent *K. lactis* cells with the linear expression cassette and selection of colonies in yeast carbon base medium (YCB) containing acetamide (axotrophy mutant selection method).

After the transformation of *K. lactis* yeasts and incubation in auxotrophic medium containing acetamide (Fig. 10), we selected 10 positive colonies that were incubated in YPGal culture medium at 30°C and in agitation at 250 rpm for 4 days. The culture medium was tested to determine the production of the recombinant protein by the chosen colonies by means of a DOT-BLOT immunoensium using an anti-TRITAG monoclonal antibody (label initially added to the construction). The colonies that react in the nitrocellulose strips were selected as yeasts producing the chimeric protein PQLeish2 (Fig. 11).

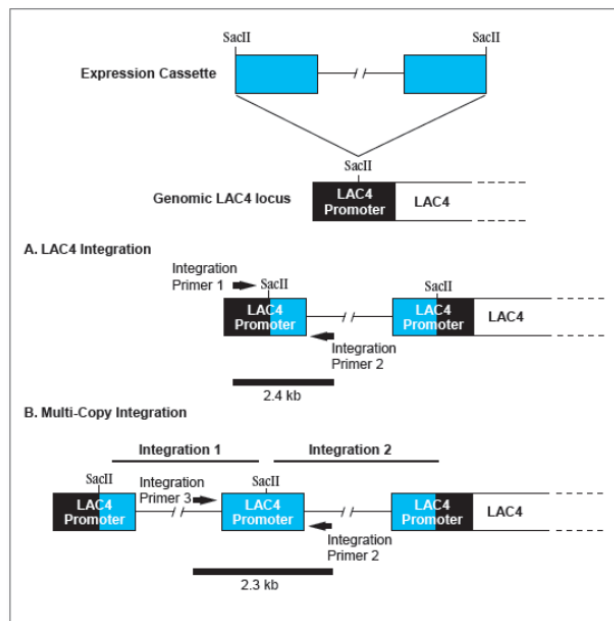


Dot-Blot.

- ☐ Sobrenadante cultivo YPD-Gal (500 µl, 96 h) conteniendo levaduras *K. lactis* transformadas (1-10) con pKlac2-PQLeish2-TRITAG.
- ☐ Se utilizó para el revelado del dot-blot el anticuerpo monoclonal US9 anti-péptido TRITAG

Figure 11. Verification of the heterologous expression of the recombinant protein PQLeish2 by yeast colonies that have formed colonies on the acetamide selection plates.

The selected colonies were tested for the identification of colonies containing an expression cassette correctly integrated into the yeast genome (Fig. 12) by PCR.



INTEGRACIÓN DEL CASSETE DE EXPRESIÓN

1. Tras la transformación de las células de *K. lactis* con el casete de expresión linealizado, el fragmento se inserta en el promotor del locus LAC4.
2. La integración de una sola copia en el locus LAC4 se puede detectar mediante PCR utilizando los cebadores de integración 1 y 2 para amplificar un fragmento de diagnóstico (A) de 2,4 kb, mientras que la integración de copias múltiples se puede detectar utilizando los cebadores de integración 2 y 3 para amplificar un fragmento de 2,3 kb (B).

Primer de integración 1: 5' d(ACACACGTAAACGCGCTCGGT) 3'

Primer de integración 2: 5' d(ATCATCCTTGTCAGCGAAAGC) 3'

Primer de integración 3: 5' d(ACCTGAAGATAGACTTCTAA) 3'

Figura 12. Integración del cassette de expresión

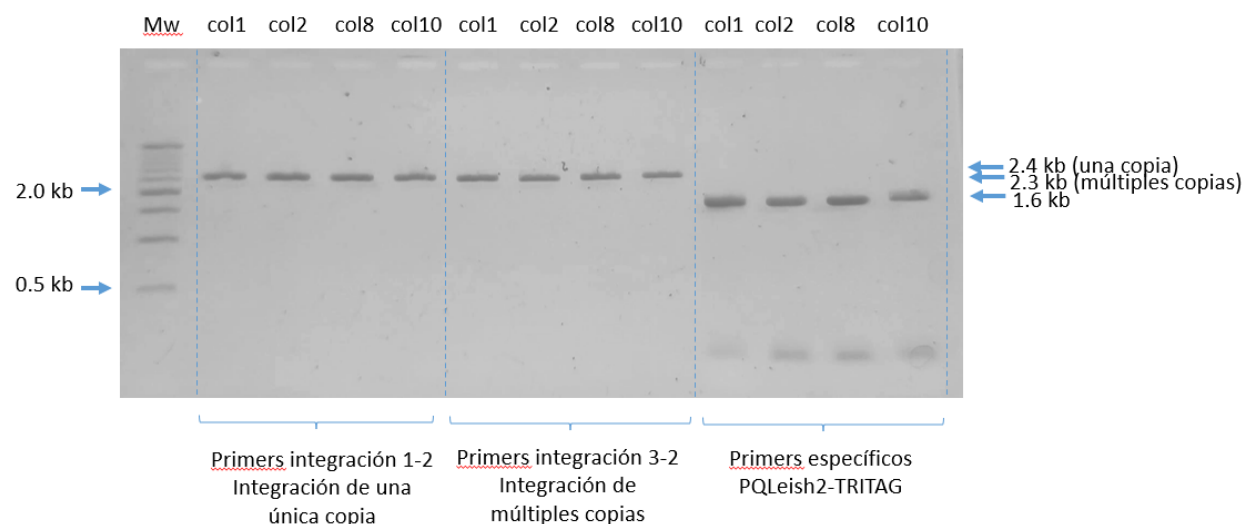


Figure 12. PCR used for identification of transformed colonies in which the PQLeish2 expression cassette has been correctly integrated into the *K. lactis* genome.

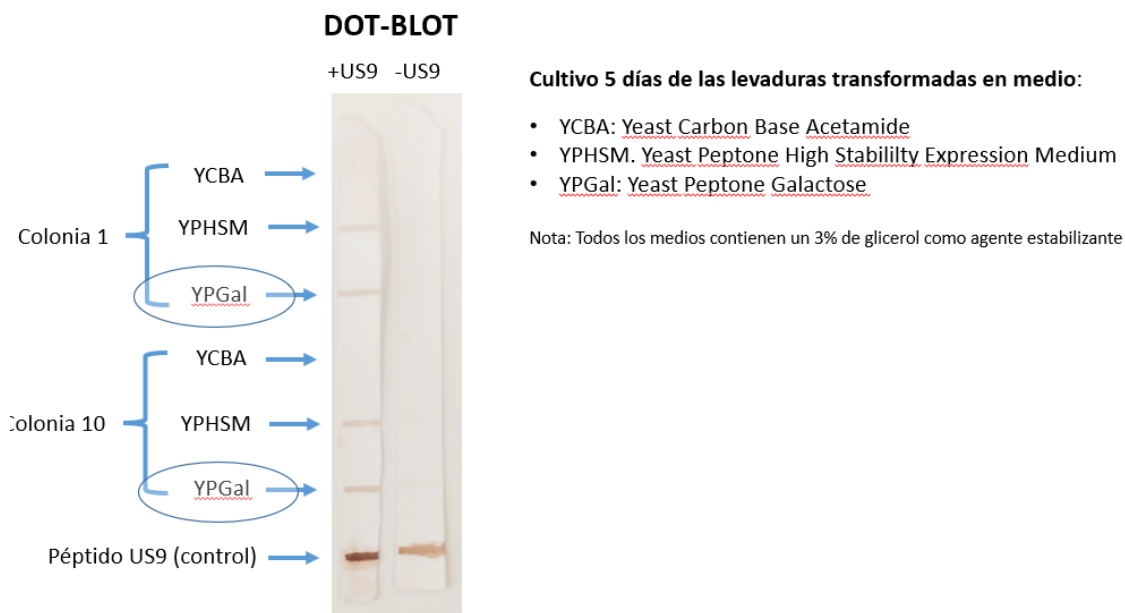


Figure 13. Expression of the recombinant protein PQLleish2 using different culture media on day 5 of incubation.

Colonies 1 and 10 that presented a cassette of integration in multiple copies in the yeast genome (Fig. 12) were used to optimize the production of recombinant protein using 3 culture media containing 3% glycerol as a protein stabilizer gene (Fig. 13). As can be seen, the maximum production occurred after 5 days of culture using YPGal medium (Fig. 13).

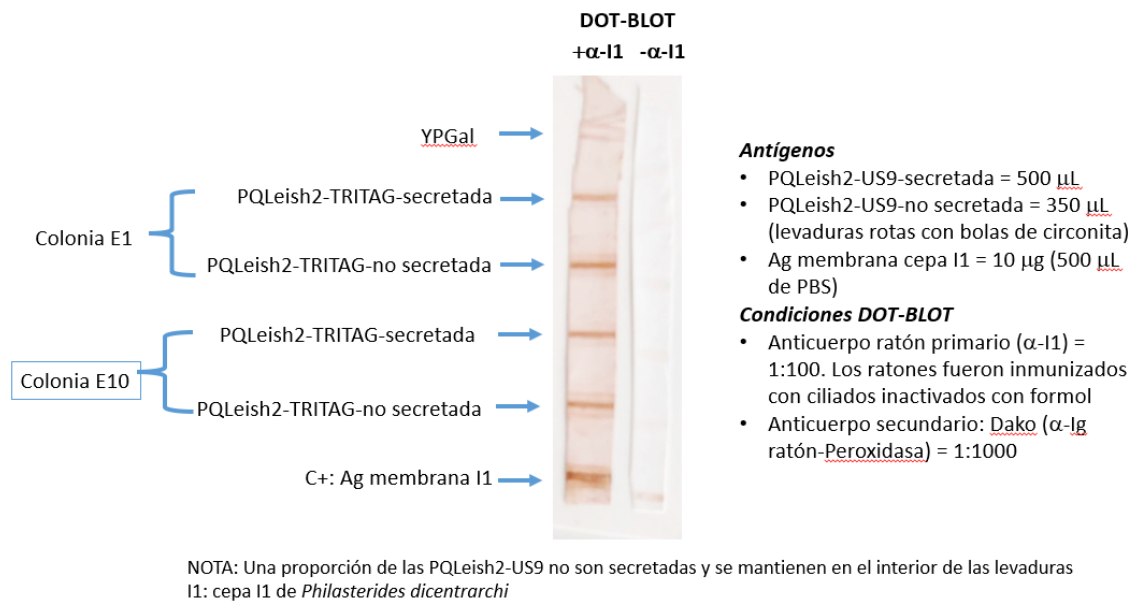


Figure 14. Confirmation of the recognition of the recombinant protein PQLeish2 by DOT-Blot by sera from mice immunized with inactivated complete ciliates emulsified in Freund's adjuvant.

Transforming yeast colonies secrete the PQLeish2 protein into the culture medium; however, a proportion of the protein produced by yeast is maintained intracellularly and is not secreted (Fig. 14).

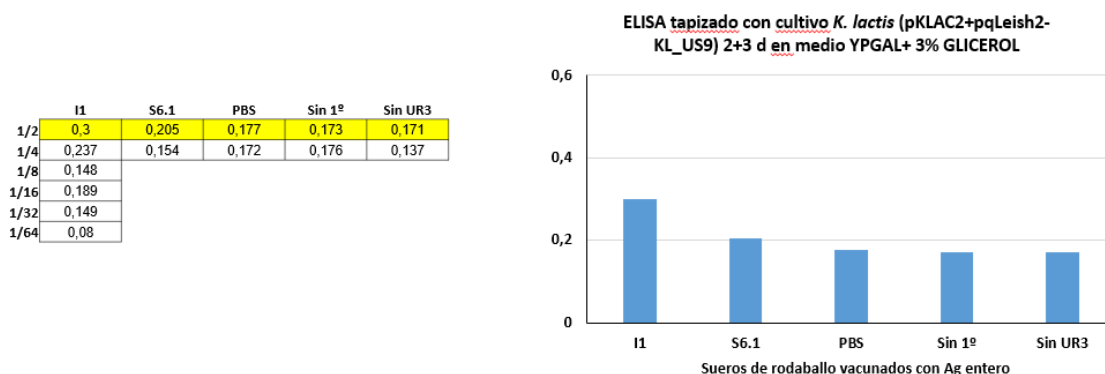


Figura 15. Inmunoensayo ELISA para la detección de anticuerpos anti-PQLeish2 por rodaballos vacunados con ciliados inactivados de las cepas I1 y S6.1 de *P. dicentrarchi*.

The recombinant protein PQLeish2 produced by *K. lactis* is recognized by the sera of mice immunized with the whole ciliates of *P. dicentrarchi*. Anti-strain I1 antibodies recognize the recombinant protein better than anti-strain S6.1 antibodies. This result is fully explainable because the construction of the recombinant protein was carried out based on the amino acid sequences of the I1 strain.

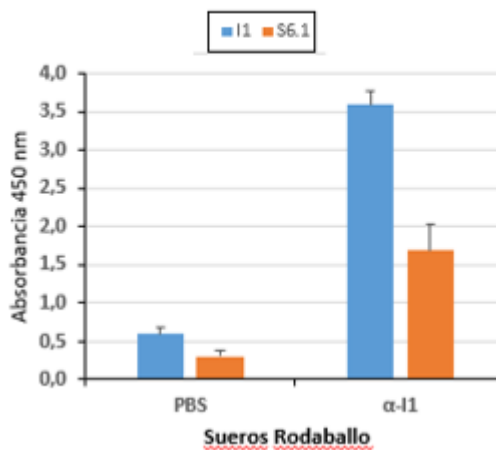


Figure 16. ELISA assay to determine the degree of recognition of inactivated anti-ciliated turbot antibodies of strain I1 on total antigens of I1 and S6.1.

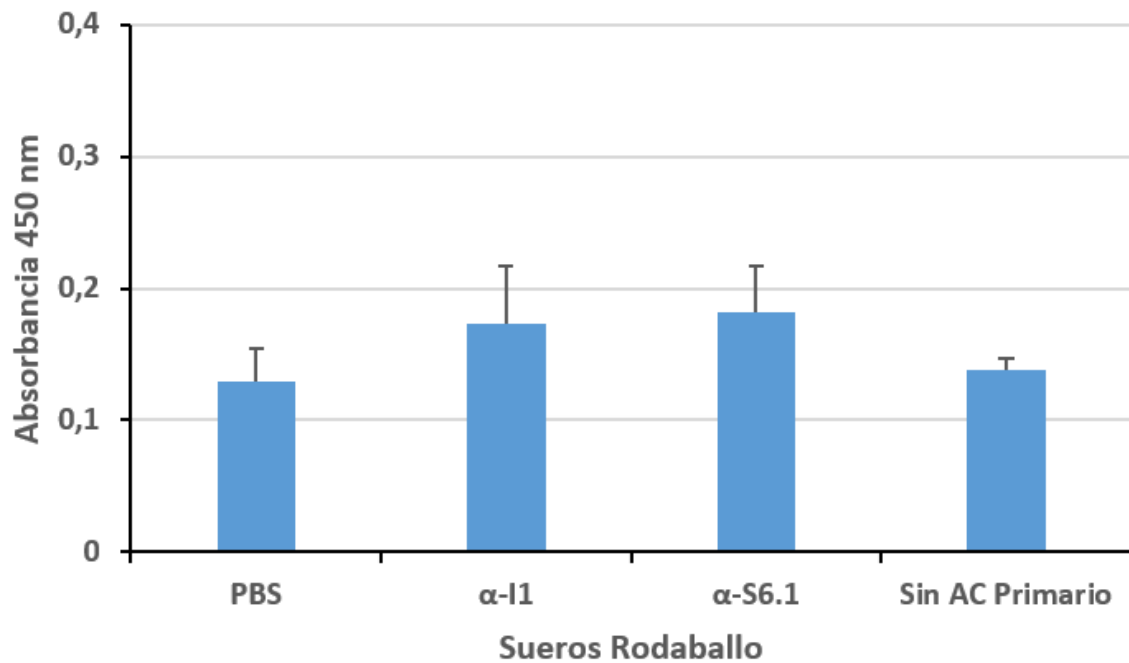


Figure 17. ELISA assay coating the wells with the recombinant protein PQLeish2 versus rhodabal sera immunized with whole ciliates of strains I1 and S6.1.

The antigenic difference between strains I1 and S6.1 is also demonstrated because turbot immunized with whole ciliates of strain I1 recognize antigens of the self-strain better than antigens of strain S6.1 (Fig. 16). However, VSPs 1 and 3 and LSFs 5 and 14 appear to be no different between the two strains (Fig. 17).

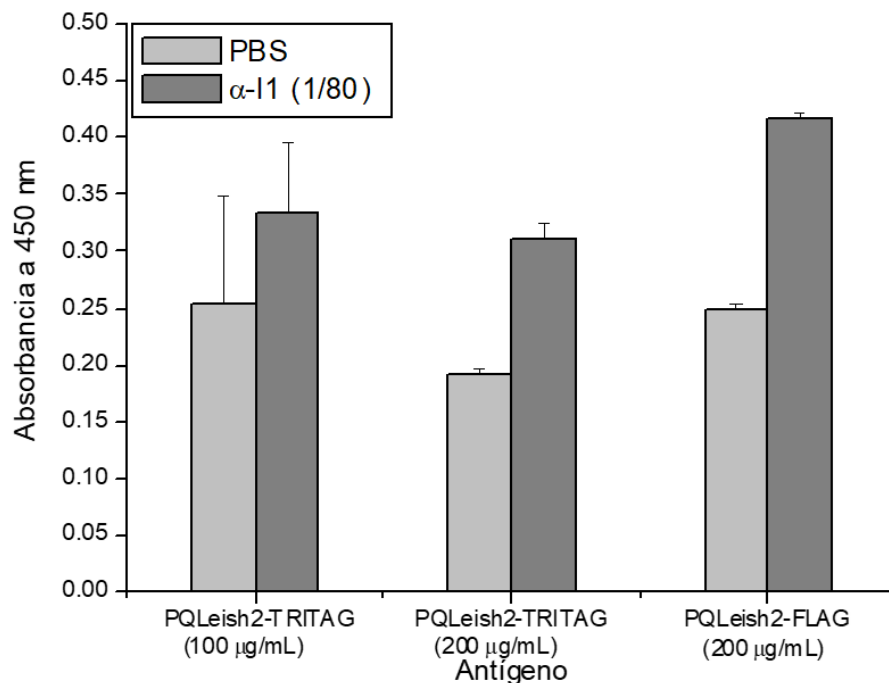


Figure 18. ELISA assay to determine antibody levels of turbot immunized with different concentrations of the recombinant protein PQLeish2 against total antigens of ciliates of strain I1.

To obtain a significant anti-*P. diacentrarchi* antibody response, turbot must be immunized with doses of the recombinant protein PQLeish2 greater than 200 µg/mL (Fig. 18).

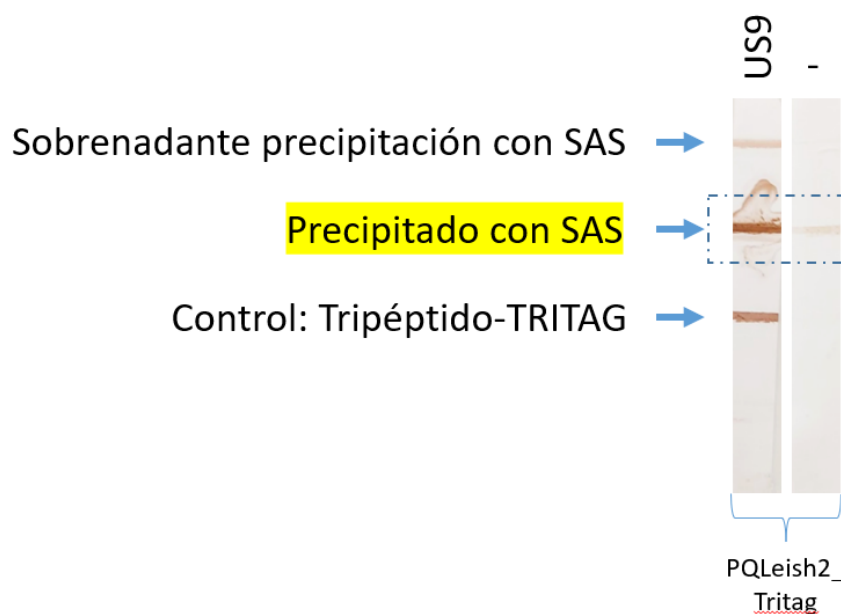


Figure 19. Concentration of the recombinant protein PQLeish2 containing the TRITAG tag from *K. lactis* culture medium ultrafiltered through a 10 kDa membrane and precipitated with saturated

ammonium sulfate (SAS). For the detection of the recombinant protein, the monoclonal antibody US9 that recognizes the TRITAG peptide was used.

In order to obtain the highest possible concentration of the recombinant protein PQLeish2 to be used for vaccination trials, the culture medium was ultra-concentrated by means of tangential ultrafiltration (lateral (Vivaflow® Tangential Flow Filtration (TFF) cassettes). Once the culture medium containing the recombinant secretion protein had been ultrafiltered, it was precipitated by 63% saturated ammonium sulfate (SAS). As can be seen in Figure 19, tangential ultrafiltration followed by precipitation with SAS significantly concentrates the recombinant protein that, following these methods, can be used for the production of the vaccines used in the protection experiments.

As can be seen in Fig. 14, the colonies of the yeast *K. lactis* secrete one part of the recombinant protein PQLeish2 and the other part is stored intracellularly. Taking advantage of this circumstance, we have designed a vaccination experiment in which they analysed its effects on the following parameters:

1. *Growth*
2. *Cytotoxic effect (complement)*
3. *Antibody production*
4. *Protection*

TURBOT VACCINATION EXPERIMENT WITH CHIMERIC RECOMBINANT proteinS PQLeish2

1. VACCINE COMPLETE CILIATES = 5×10^6 ciliates / mL = 5×10^5 ciliates / fish
2. PROCESSED YEASTS = 20 mg/mL = 2 mg/fish
3. CHIMERIC RECOMBINANT protein = 10 mg/mL (SAS) PRECIPITATE = 1 mg/fish (approx. 700 µg/fish).
4. CONCENTRATION OF ADJUVANT IN THE VACCINE = 50%
5. VACCINE COMPLETE CILIATES = 5×10^6 ciliates / mL = 5×10^5 ciliates / fish
6. PROCESSED YEASTS = 20 mg/mL = 2 mg/fish
7. CHIMERIC RECOMBINANT protein = 10 mg/mL (SAS) PRECIPITATE = 1 mg/fish (approx. 700 µg/fish).
8. CONCENTRATION OF ADJUVANT IN THE VACCINE = 50%

GRUPO EXPERIMENTAL	VACUNA	PECES
GRUPO 1	PBS	50
GRUPO 2	CILIADOS INACTIVADOS+MONT ISA 763A VG	50
GRUPO 3	LEVADURAS TRANSFORMADAS	50
GRUPO 4	LEVADURAS TRANSFORMADAS + MONT ISA 763A VG	50
GRUPO 5	PQLEISH_VSP_FLAG + MONT ISA 763A VG	50
GRUPO 6	LEVADURAS TRANSFORMADAS + PQLEISH_VSP_FLAG + MONT ISA 763A VG	50
TOTAL		300

Table 7. Vaccination trial with 6 groups of fish with different combinations, including groups 1) PBS, 2) inactivated whole ciliates and adjuvant, 3) yeasts presenting intracellular chimeric protein, 4) yeasts and adjuvant, 5) protein obtained from the supernatant (PQLeish2) and adjuvant, 6) yeasts plus protein from the supernatant and adjuvant.

Table 7 presents the experimental groups vaccinated with different vaccine formulations. The fish were vaccinated with two doses of vaccine on day 0 and 30, and the serums were obtained on day 60 post-immunization. On this day, the fish were also experimentally infected with 2×10^5 ciliates and the mortality obtained was recorded daily to determine the levels of survival and protection obtained after the administration of these vaccines.

1. Growth

Grupo experimental	Peso (g)	Nº Peces
1.PBS	110 c	50
2.Ciliados inactivados cepa S6.1 + M763	104 bc	50
3.Lev_Transformadas	100 abc	50
4.Lev_Transformadas + M763A VG	80 a	50
5.PQLeish_VSP_FLAG + M763A VG	102 abc	50
6.Lev_Transformadas + PQLeish_VSP_FLAG + M763A VG	87	50

Table 8. Weights of vaccinated turbot obtained at the end of vaccination in the trial outlined in Table 7. Different letters indicate significant variations in fish weights.

After the completion of the experiment, the effect of the different vaccines on fish growth was first evaluated. Altered fish weight could be related to adverse side effects of the vaccine that could affect animal welfare and also the commercial profitability of the fish. As shown in Table 8, vaccines that exclusively have the oily adjuvant M763A VG do not adversely affect fish growth; However, fish vaccinated with yeast and oily adjuvant have very low growth levels, indicating that the formulation of vaccines with these two components together negatively affects fish growth.

2. Cytotoxic effect (complement)

For the evaluation of the vaccine, the different vaccination experiments included for the first time a test to analyze the ability of the sera to cause the destruction/death of trophozoites through antibody-mediated cytotoxicity (AMC). Ciliates were incubated with various dilutions of serum at 25°C and the percentage of dead ciliates at 24 h of the S6.1 and I1 strains was determined. As can be seen in Tables 9 and 10, sera from fish vaccinated with inactivated whole ciliates and Montanide M763A VG oily adjuvant generated the highest CMA activity. However, sera from yeast-vaccinated fish containing the recombinant protein PQLeish2 did not significantly increase CMA compared to controls immunized with PBS.

	SUEROS frente a S6.1 (24 h)										MEDIA ± SD
VACUNA/GRUPO	1	2	3	4	5	6	7	8	9	10	
G1. PBS	1/4	1/8	1/4	1/4	1/4	1/8	1/8	1/4	1/8	1/4	5,6 ± 2,1 ^b
G2. S6.1 + M763	1/32	1/16	1/64	1/16	1/64	1/16	1/8	1/32	1/32	1/32	31,2 ± 19,4 ^a
G3. Lev T.	1/4	1/16	1/16	1/8	1/8	1/4	1/4	1/4	1/4	1/4	7,2 ± 4,9 ^b
G4. Lev T. + M763	1/4	1/4	1/4	1/4	1/4	1/4	1/2	1/2	1/8	-	4,3 ± 1,7 ^b
G5. pqLeish_VSP-Flag + M763	1/4	1/4	1/4	1/8	1/4	1/4	1/4	1/4	1/4	-	4,4 ± 1,3 ^b
G 6. Lev T. + pqLeish VSP-Flag + M763	1/4	1/2	1/4	1/4	1/4	1/2	1/4	1/4	1/8	1/8	4,4 ± 2,1 ^b

Table 9. Level of antibody-mediated cytotoxicity (CMA) (destruction of ciliates by complement activation) of sera from turbot vaccinated with different vaccines on ciliates of the SP6.1 strain. The cytotoxicity values presented in the table indicate the maximum dilution of the serum (titer) that produces a 100% mortality of *P. dicentrarchi trophozoites*. The right-hand column shows the average of the reverse dilution that kills 100% of the ciliates at 4 h. The greater the dilution of the serum, the greater the parasitocidal activity of the serum. In the right-hand column, groups with different letters (a, b) indicate that there are significant differences between groups.

	SUEROS frente a I1 (24 h)										MEDIA ± SD
VACUNA/GRUPO	1	2	3	4	5	6	7	8	9	10	
G1. PBS	1/8	1/8	1/8	1/8	1/4	1/16	1/8	1/16	1/8	1/4	8,8 ± 4,1 ^b
G2. S6.1 + M763	1/16	1/16	1/32	1/16	1/32	1/16	1/16	1/32	1/64	1/32	27,2 ± 15,2 ^a
G3. Lev T.	1/4	1/32	1/8	1/8	1/16	1/8	1/8	1/4	1/4	1/4	9,6 ± 8,7 ^b
G4. Lev T. + M763	1/8	1/8	1/4	1/4	1/4	1/4	1/4	1/4	-	-	4,8 ± 2,1 ^b
G5. pqLeish_VSP-Flag + M763	1/8	1/8	1/8	1/16	1/8	1/4	1/4	1/4	1/8	-	7,5 ± 3,7 ^b
G 6. Lev T. + pqLeish VSP-Flag + M763	1/4	1/4	1/4	1/8	1/8	1/4	1/4	1/4	1/8	1/8	5,6 ± 2,1 ^b

Table 10. . Level of antibody-mediated cytotoxicity (CMA) (destruction of ciliates by complement activation) of sera from turbot vaccinated with different vaccines on ciliates of the I1 strain. The cytotoxicity values presented in the table indicate the maximum dilution of the serum (titer) that produces a 100% mortality of *P. dicentrarchi trophozoites*. The right-hand column shows the average of the reverse dilution that kills 100% of the ciliates at 4 h. The greater the dilution of the serum, the greater the parasitocidal activity of the serum. In the right-hand column, groups with different letters (a, b) indicate that there are significant differences between groups.

3. Antibody production

When the levels of antibodies induced by vaccines composed of inactivated ciliates and transformed yeasts were analyzed, it was found that only vaccines with inactivated

ciliates generated significantly higher levels of antibodies than those injected only with PBS (Fig. 20).

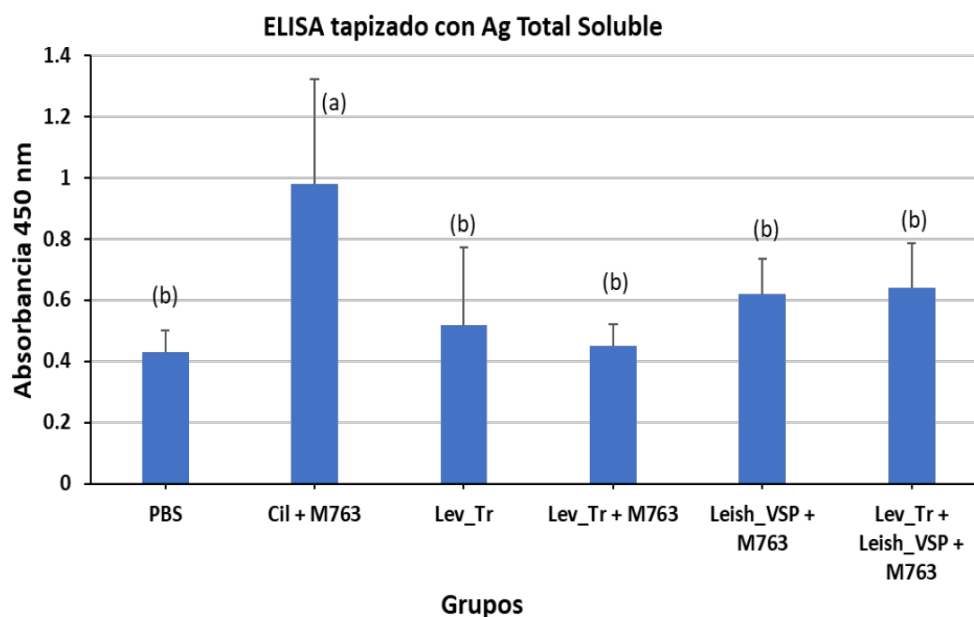


Figure 20. Antibody levels, determined by ELISA, in the serum of turbot vaccinated with inactivated ciliates emulsified in Montanide ISA 763 adjuvant (S6.1 + M763). Yeast processed with the recombinant PQLeish_VSP-flag (Lev_T) or emulsified with M763 (Lev_T + M763), the recombinant combined with montanide (PQLeish-VSP + M763) and a combination of yeast, recombinant and montanide (Lev_T + PQLeish-VSP + M763). The values represent the mean \pm standard deviation (SD). Bars with different letters mean statistically significant differences between groups.

4. Protection

When the level of protection of the vaccines formulated in the oily adjuvant Montanide 763 AV was analyzed, it was observed that only turbot vaccinated with inactivated ciliates were significantly protected from experimental infection, while all other groups of fish vaccinated with yeasts expressing the recombinant protein were not protected and, even, mortality levels were significantly higher than controls (Fig. 21). As a relevant fact, turbot that presented sera with the highest total antibody levels and the highest CMA response were those that presented the greatest protection against ciliate.

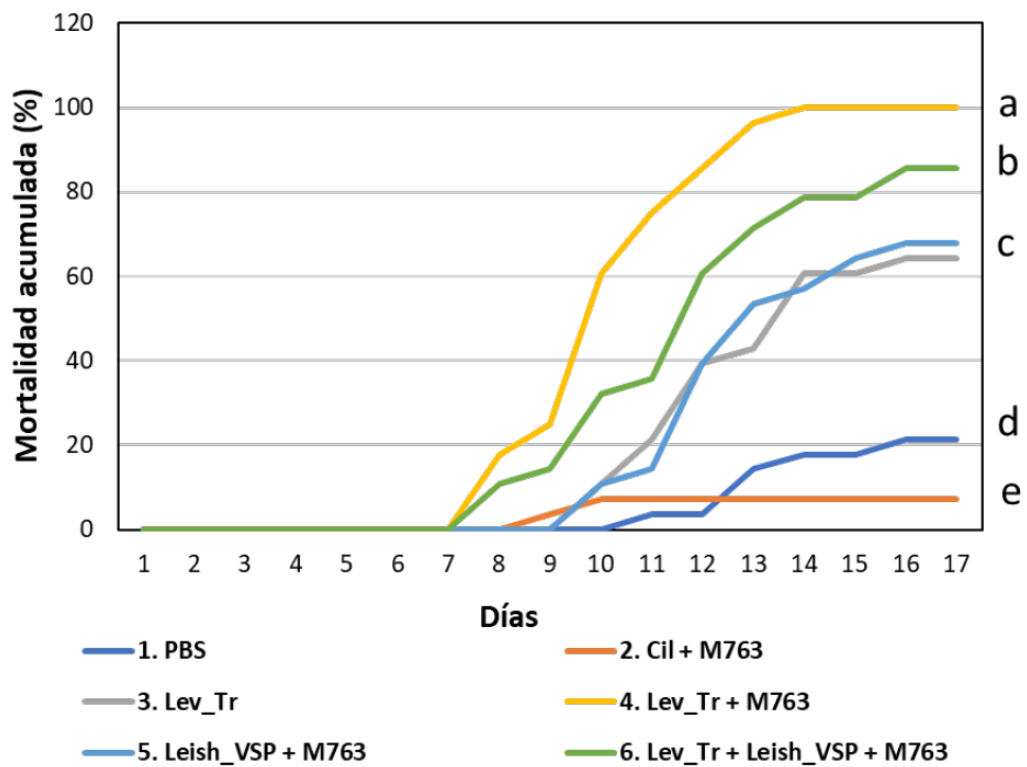
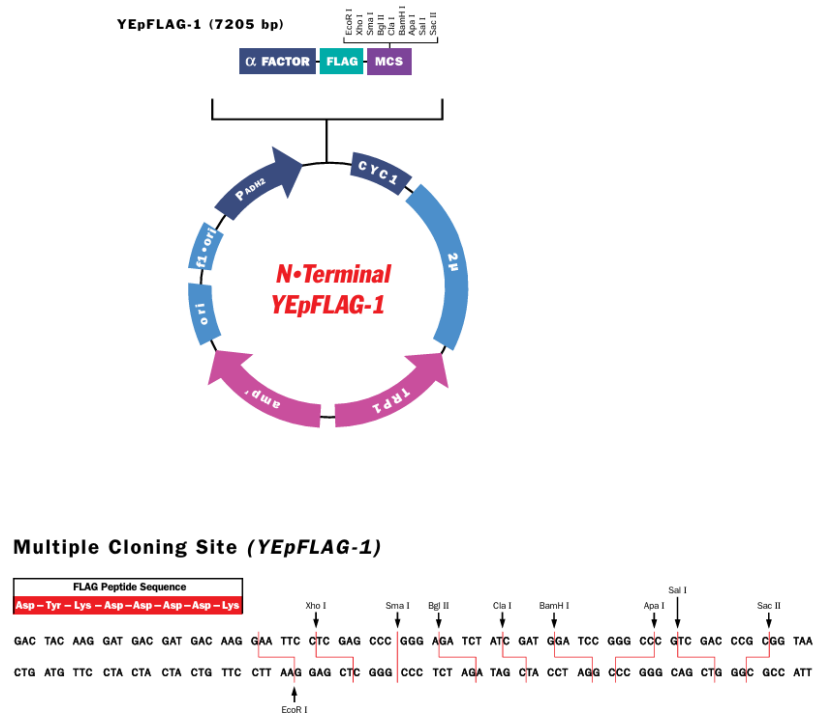


Figure 21. Cumulative mortality, as a function of time (days), in the 6 groups of fish after experimental infection. Turbot was injected with: PBS, inactivated ciliates + Montanide 763 (Cil + M763), yeasts transformed with the recombinant protein Leish-VSP (Lev_Tr), recombinant protein Leish_VSP + Montanide 763 (Leish_VSP+M763), transformed yeasts + recombinant protein + Montanide 763 (Lev_Tr +Leish_VSP+M763). Different letters (a-e) indicates statistically significant differences between groups.

Saccharomyces cerevisiae:



YEplFLAG-1 plasmid, previously linearized by digestion with **XhoI** and **SacII** (NZYTech)

AAC AGC ACA AAT AAC GGG TTA TTG TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA
GAA GAA GGG GTA CCT TTG GAT AAA AGA GAC TAC AAG GAT GAC GAT GAC AAG GAA TTC CTC
GAG CCC GGG AGA TCT ATC GAT GGA TCC GGG CCC GTC GAC CCG CGG TAA GCG GCC GCT GAT
CCG TCG AGC GTC CCA AAA CCT TCT CAA GCA AGG TTT TCA GTA TAA TGT TAC ATG CGT GAT
CCG TCG GAC TAC AAG GAT GAC GAT GAC AAG Péptido Flag

Primer_F_secuenciación: 5' C AGC ACA AAT AAC GGG TTA TTG 3'

Primer_R_secuenciación: 5' CGG ATC ACG CAT GTA ACA TTA TAC 3'

DISEÑO DE PRIMERS EN YEplFLAG-1

Forward:

Sin FLAG

5' AAA GAA GAA GGG GTA CCT TTG GAT AAA AGA ATG TTC GTT TTC TTG GTC CTG 3'

Con FLAG en N-terminal

5' AAA AGA GAC TAC AAG GAT GAC GAT GAC AAG ATG TTC GTT TTC TTG GTC CTG 3'

Sin FLAG + US9 (Triquina) en N-terminal

5' AAA GAA GAA GGG GTA CCT TTG GAT AAA AGA ATG ACT TTT AGT GTT CCA ATT AGC ATG

ACT TTT AGT GTT CCA ATT AGC 3'

Reverse:

Sin Tag en C terminal

Figure 22.- Design of recombinant proteins in the yeast *Saccharomyces cerevisiae*. The YepFlag-1 plasmid was used for the constructions.

Determination of the adjuvant effect of *Saccharomyces cerevisiae* yeasts, using ciliates or VSP proteins generated intracellularly in yeasts

After the analysis of the different VSPs, we have observed that VSP8 is a main component of one of the families of VSPs, which includes 7 components of it (from VSP2 to VSP8), and that it could be a good candidate for inclusion as an antigen in recombinant vaccines. In this experiment, WT yeasts (wild-type) or transformed yeasts have been included, which include the recombinant protein VSP8 located at the cytosolic level. The methodology used to express this protein is outlined in Fig. 22. In this construction, the original Flag tag of the YepFlag-1 plasmid was replaced by the TRITAG (MTFSVPIS peptide) tag at the N-terminal end. The VSP8 used in the experiment is included below:

```
>VSP1_sequence_DNA
ATGTTCAAGCAGATTGCTTTGTTGGCTTTGTTCTTGGCTGTTGTTTTCTCTGTTTGTGCTGATGGTCAAAACGGTTGTACTACTTGTG
CAGATGCTAACGATTGTCAAGTTTGTGATCAAGCCAACTTCTACTTCTTGAAGGTTAACAAGACTTGTGACTTGCAAACTGGTTGTAA
AACTTATGCTGGTGATGCTTGTACAGCTTGTGACGAACTAAGTTCTACTTCCCAAAGATCGCTAACAACTCTCTGAATGCAAGTTG
TTCTCTAACTGTAAGACCGTTGTGGATCAAAAGTGTACTGCTTGCCTGATGCTGTTACTGCTTCTCATTCTATTCAAGAGGGTGAAT
GTTTGAAAAACCTGCTTGAAGGATCAGAAACACAACGCCTTGAAGAACTGATTGTGAAGCTGATCCAGAAAAGGTTAATGGTATCGTTGG
TTGTACTGCTAAAGCTGATGCTCCAAATGCTACTACTCAATGTAAGACTTGAAGTCCGGTACTACTTTGGCTATTTCTGCTACTAAG
TGTGTTACCGATGTGCAAACTGTCAAACCTACAATGATACCGGTTGTAACGAATGCAAGACTGGTTTCAAGTTGAAGTCTCTCTACTA
AGGCTTGCAGAGCTGAAGGTGGTGTCTGAAAATGTTAACAATATTGCTGGTTGTACCGCACAAGCAGATGGTGTCAACGCTGCTAC
ACAATGTAAACATGCAAACTCTGGTACAACCTTGGCTATCTCAGCAACAAAATGTGTGACTGATGTCCAAAATTGCCAGACTTATAAC
GATACTGGTTGTGAACAATGTAACACCGGATTCAAATTGAACACTACCTCAAAGGCTTGTGAAGTTGATGCTGCTCAAAACGTTGAACA
ACATTGTGGGTTGTACAGCAAAGGCTGACGGTGCAAAATGCTGATACAAAGTGTAAAGCATGTATCGCAGGTACTACCTTAGCTACTAG
TGCTACAAAATGCGTTACCAACGTTAAGGATTGTGAGACATACAACGACACAGGATGTGAAAAGTGTAAAACGGGTTTCAAGCTTAAC
ACCACTTCTAAGGCATGTGAACAAGATAAGACCTCTCTCTTCACTGCTTCTCTGCTATCGTTTCTGCTTCTTTGGCAGTTATTT
TGGCTGTTTTTCGCTTTGGCTTTG
```

```
>VSP1_sequence_protein
MTFSVPISDGQNGCTTCADANDCQVCDQANFYFLKVNKTCDLQTGCKTYAGDACTACDETKFYFPKIANNFSECKLFSNCKTVVDQKC
TACADAVTASHSIQEGECLKTKLDQKHNAKLTDCADPEKVNIGVCTAKADAPNATTQCKTCKSGTTLAISATKCVTDVQNCQTYN
DTGCNECKTGFKLNSSTKACEAEGGASENVNNIAGCTAQADGANAAATQCKTCKSGTTLAISATKCVTDVQNCQTYNDTGCEQCNTGFK
LNTTSKACEVDAAQNVNNIVGCTAKADGANADTKCKTCIAGTTLATSATKCVTNVKDCQTYNDTGCEKCKTGFKLNTTSKACEQDKTS
SSSTASSAIVSASLAVILAVFALAL
```

Number of amino acids: 369

Molecular weight: 38391.88

Theoretical pI: 5.59

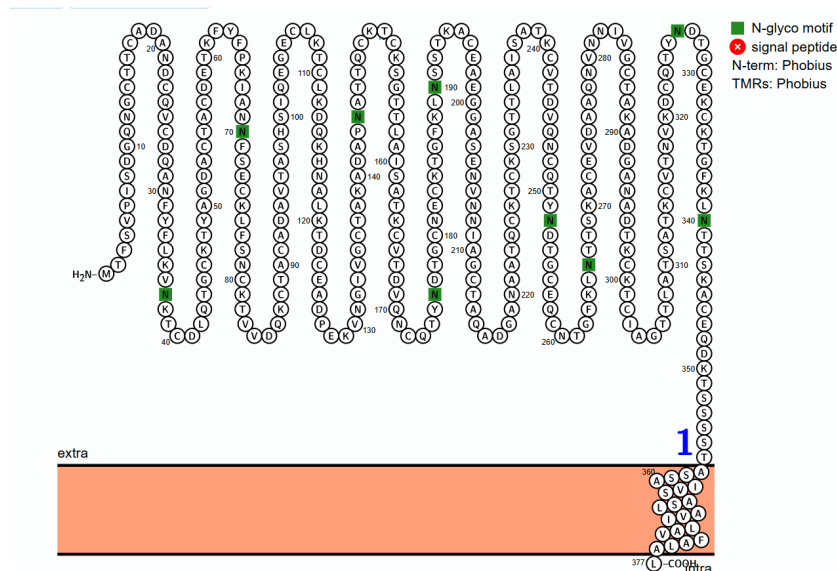


Figure 23. CDS proteoform corresponding to VSP8 designed for intracellular expression in *S. cerevisiae* yeast.

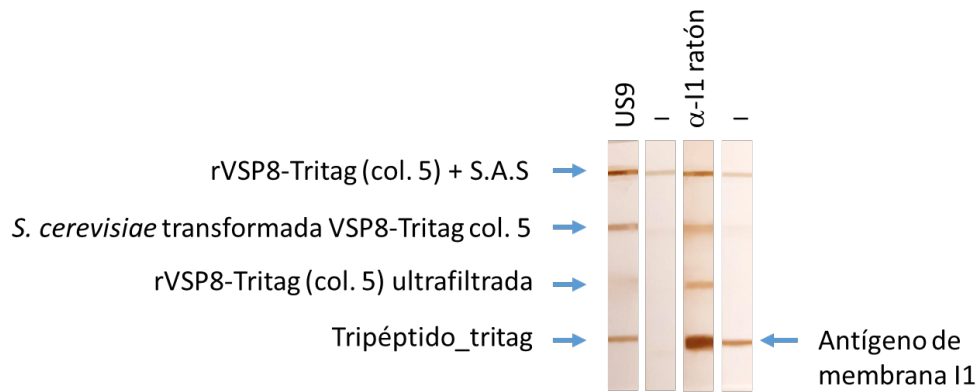


Figure 24. DOT-BLOT assay to determine the expression of the recombinant protein rVSP8 in the culture medium and/or intracellularly inside yeasts. For the concentration of the protein, tanGenecial ultrafiltration and precipitation with saturated ammonium sulfate (S.A.S.) were used.

As can be seen in Fig. 24, the recombinant protein rVSP8 is expressed intracellularly in *S. cerevisiae* and can be easily concentrated by precipitation in SAS. This protein is recognized by the mAB US9 that recognizes the TRITAG label and by serum from mice immunized with inactivated ciliates.

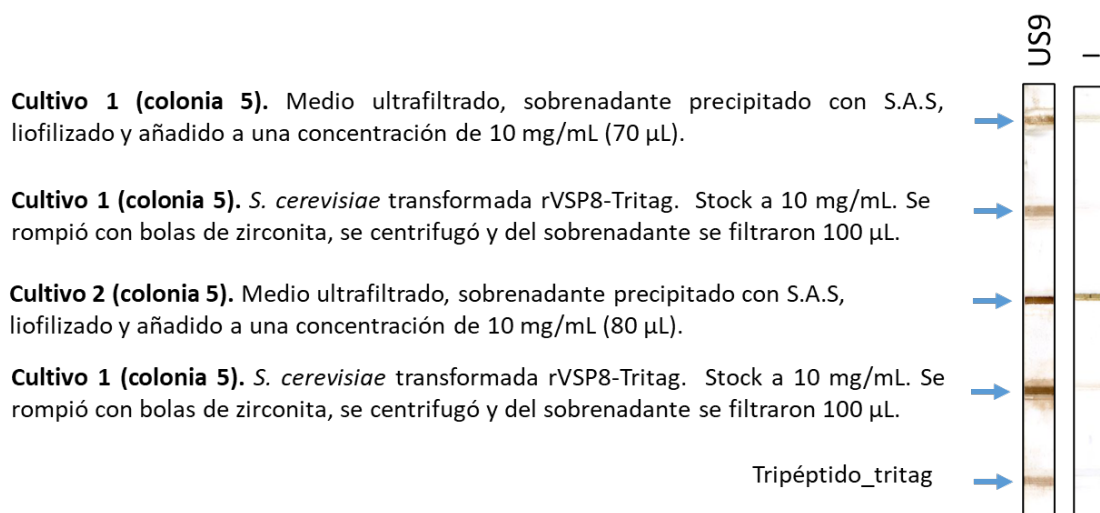


Figure 24. Dot-Blot: recombinant protein rVSP8-TRITAG expressed by several transforming colonies of *S. cerevisiae*. For the detection of the recombinant protein, the US9 mAB was used. The control was used with the chimeric tripeptide T3 containing the epitope TRITAG (MTFSVPIS KK VTKAYEKARDRA KK AVPESIDWDYYYVTEVKNQGQC)

To achieve optimal expression of the recombinant protein, two culture systems were used: **Culture 1**. A primary culture was performed directly in YPHSM production medium inoculated with a culture grown in YPD medium (Yeast Peptone Dextrose) and scaled up to 1 L with the production medium. The culture was incubated for 72 h. **Cultivation 2**. A primary culture was performed in the tryptophan non-auxotrophic medium (CM-TRP) and transferred to 1 L of the YPHSM (yeast peptone high stability expression medium) production medium. The culture was incubated for 96 h. As can be seen in Fig. 24, the maximum concentration of recombinant protein is obtained after the culture of the yeasts using system 2.

Vaccination trials

In order to simplify the production of recombinant vaccines, in this assay we used as an antigen the transformed yeasts themselves containing the recombinant protein rVSP8 of *P. dicentrarchi*. The experimental design of vaccination is presented in Table 11. The protocols for administering the vaccine, obtaining serums, cytotoxicity analysis and experimental infection were similar to the previous experiment.

GRUPO EXPERIMENTAL	VACUNA	PECES
GRUPO 1	PBS	40
GRUPO 2	CILIADOS INACTIVADOS + MONT ISA 763A VG	40
GRUPO 3	LEVADURAS WT	40
GRUPO 4	LEVADURAS TRANSFORMADAS VSP8	40
GRUPO 5	LEVADURAS WT +CILIADOS INACTIVADOS	40
GRUPO 6	LEVADURAS TRANSFORMADAS VSP8 +CILIADOS INACTIVADOS	40
TOTAL		240

Table 11. Groups used in the experiment. Groups: 1, PBS; 2, inactivated ciliates + Montanide adjuvant 763; 3, WT yeasts (wild-type, unprocessed); 4, yeasts processed with VSP8; 5, wild-type yeasts + inactivated ciliates; 6, Yeasts transformed with VSP8 + inactivated ciliates.

1. Growth

Grupo experimental	Peso azar (g)	Nº Peces
1.PBS	142	40
2.Cil + M763	134	40
3.Lev_WT	144	40
4.Lev_Tr	145	40
5.Lev_WT + Cil	133	40
6.Lev_Tr + Cil	138	40

Table 12. Average weight of the fish in each group. Fish groups: PBS, inactivated ciliates + Montanide 763 (Cil + M763), wild-type yeasts (Lew_WT), VSP8 processed yeasts (Lev_Tr), unprocessed yeasts + ciliates (Lev_WT + Cil), processed yeasts + ciliates (Lev_Tr + Cil).

At the end of the experiment, the fish from all the experimental groups were weighed and it was observed that there were no significant differences between them (Table 12).

2. Cytotoxic effect (complement)

	SUEROS (S6.1)										
VACUNA/GRUPO	1	2	3	4	5	6	7	8	9	10	MEDIA \pm SD
G1. PBS	1/2	1/4	1/4	1/4	1/4	1/4	1/8	1/2	1/4	1/4	4,0 \pm 1,6 ^b
G2. Cil + M763	1/16	1/4	1/16	1/4	1/32	1/16	1/16	1/8	1/16	1/32	16,0 \pm 9,8 ^a
G3. Lev_WT	1/2	1/2	1/2	1/4	1/4	1/2	1/4	1/4	1/4	1/4	3,2 \pm 1,0 ^b
G4. Lev_Tr	1/4	1/4	1/2	1/2	1/2	1/4	1/4	1/4	1/4	1/4	3,4 \pm 1,0 ^b
G5. Lev_WT + Cil	1/8	1/16	1/16	1/8	1/16	1/8	1/8	1/16	1/8	1/8	11,2 \pm 4,1 ^a
G 6. Lev_Tr + Cil	1/8	1/4	1/4	1/16	1/8	1/4	1/32	1/8	1/8	1/16	11,6 \pm 8,7 ^a

Table 13. Level of antibody-mediated cytotoxicity (CMA) (destruction of ciliates by complement activation) of sera from turbot vaccinated with different vaccines on ciliates of the S6.1 strain. The cytotoxicity values presented in the table indicate the maximum dilution of the serum (titer) that produces a 100% mortality of *P. dicentrarchi* trophozoites. The right-hand column shows the average of the reverse dilution that kills 100% of the ciliates at 4 h. The greater the dilution of the serum, the greater the parasitocidal activity of the serum. Fish groups: PBS, inactivated ciliates + Montanide 763 (Cil + M763), wild-type yeasts (Lev_WT), VSP8 processed yeasts (Lev_Tr), unprocessed yeasts + ciliates (Lev_WT + Cil), processed yeasts + ciliates (Lev_Tr + Cil). In the right-hand column, groups with different letters (a, b) indicate that there are significant differences between groups.

When analyzing the CMA capacity of turbot sera vaccinated against the S6.1 strain of *P. dicentrarchi*, we observed that the groups vaccinated with ciliates inactivated in M763 oily adjuvant, ciliates inactivated with wild yeasts and with inactivated ciliates and transforming yeasts have a significantly higher CMA activity than the other groups tested (Table 13). These results indicate that both the oily adjuvant and the yeasts (wild or processed) have adjuvant capacity increasing the CMA activity in turbot serums.

3. Antibody production

The level of antibodies produced by turbot vaccinated with inactivated ciliates and oily adjuvant M763 was the highest of all the groups tested. Likewise, antibody levels in rhodolal sera vaccinated with inactivated ciliates and wild or transformed yeasts also produced an above-control antibody level (PBS), but significantly lower than those vaccinated with inactivated ciliates and oily adjuvant, indicating that yeasts also have adjuvant activity, but quantitatively lower than that of the oily adjuvant (fig. 25).

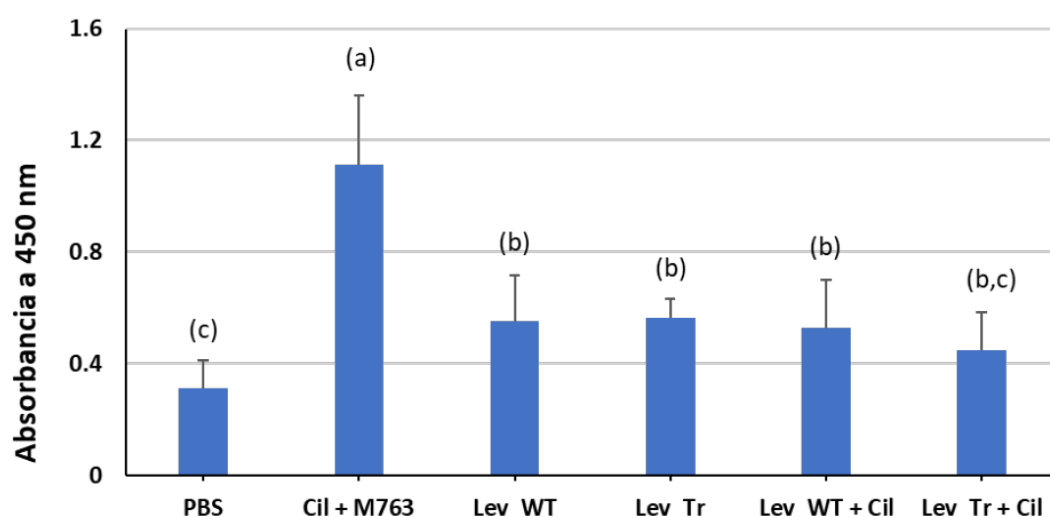


Figure 25. Antibody levels, estimated by ELISA, in the serum of turbot injected with PBS, inactivated ciliates + Montanide 763 (Cil+ M763), wild-type yeasts (Lev_WT), transformed yeasts (Lev_Tr), unprocessed yeasts + ciliates (Lev_WT + Cil), or with transformed yeasts + ciliates (Lev_Tr + Cil). The bars indicate the mean \pm standard deviation (SD) (n= 5). Only the group injected with whole ciliates and Montanide 763 showed significantly higher antibody levels than the control group (PBS). Groups with different letters (a-c) indicates that there are significant differences between groups.

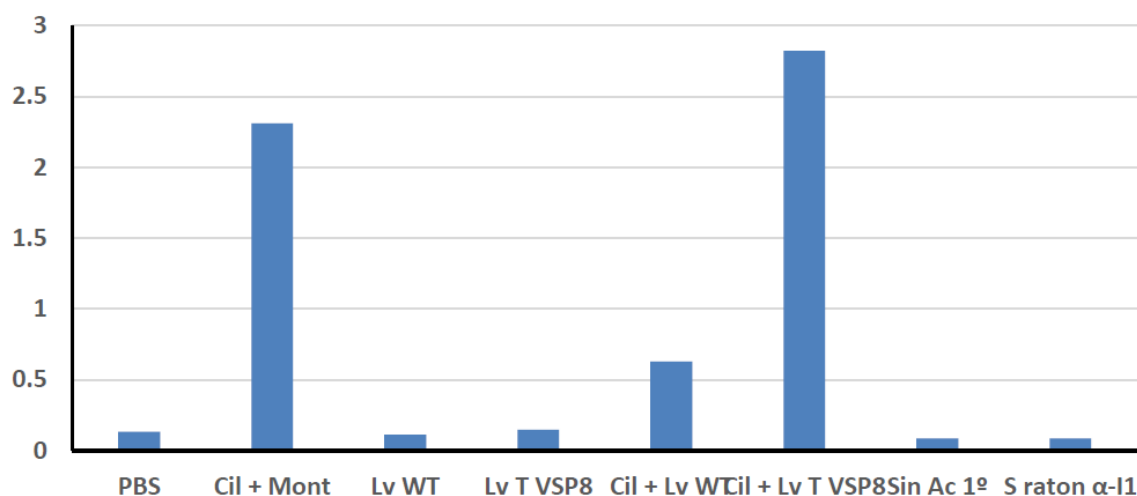


Figure 26. Levels of antibodies present in turbot serum against total polysaccharides extracted from a total ciliate extract. The serums tested were as follows: turbot serum injected with PBS (PBS), with ciliate + Montanide ISA 763 (Cil+M763), with unprocessed yeasts (Lev_WT), yeasts processed with VSP8 (Lev_T VSP8), unprocessed yeasts + ciliates (Lev_WT + Cil) or yeasts processed with VSP8 + ciliates (Lev_T VSP8 + Cil).

As in the previous case (Fig. 25), the ciliate and Montanide M763 groups, unprocessed yeast and ciliates and transformed yeasts and ciliates showed higher levels of antibodies against oligosaccharides than the rest.

4. Protection

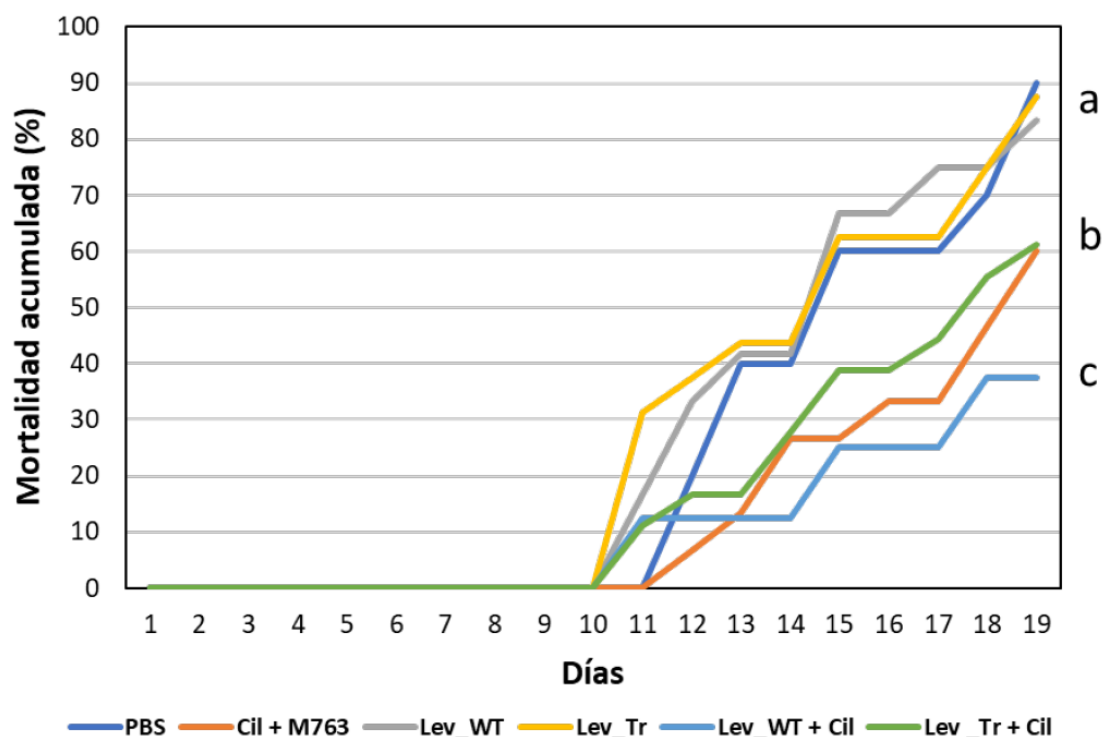


Figure 27. Cumulative mortality in turbot groups after experimental infection. The groups tested were: PBS, ciliates + Montanide ISA 763 (Cil+M763), unprocessed yeasts (Lev_WT), yeasts processed with VSP8 (Lev_T VSP8), unprocessed yeasts + ciliates (Lev_WT + Cil) or yeasts processed with VSP8 + ciliates (Lev_T VSP8 + C). Groups with different letters (a-c) are significantly different from each other.

With regard to the experiment of protection after infection of fish with a dose of 2×10^5 ciliates/fish of the S6.1 strain, the groups that presented the greatest protection were those vaccinated with yeasts (wild or processed) and ciliates or with inactivated ciliates and oily adjuvant M763 (Fig. 27). From these results it can also be deduced that there is a very good correlation between the protection results and the generation of CMA (Table 13). On the other hand, a good correlation is also observed between the levels of anti-polysaccharide antibodies (Fig. 26) and the protection obtained (Fig. 27), suggesting that an important part of the response generated in turbot against *P. dicentrarchi* is mediated by antibodies that recognize polysaccharides. This response, which is normally ignored in most studies of vaccination against scuticociliates, could be more relevant in protection than *one might think*.

Construction of recombinant VSPs proteins in *S. cerevisiae* and protection assays with synthetic peptides

Two plasmid constructions were designed in the YEPflag-1 vector containing CDS corresponding to VSP1, VSP2 and a chimeric protein containing antigenic fragments of all *P. dicentrarchi* VSPs for their intracellular expression in the yeast *S. cerevisiae*. In all cases, the TRITAG tag was added at the n-terminal end to detect the recombinant protein by the monoclonal antibody US9.

Chimeric recombinant protein containing all VSPs (expressed as *S. cerevisiae*):

VSP1 (68 aa):

LQGSFAAYDQACTNNLGCTANKCEAGNLKCDAAQCKAGYTRDAATEICNANN
CTTLGTTDPNKGQCTT

VSP2 (140-229; 90 aa):

TTTHSIQEGECLKTCLKDQKHNAADKTD CVADTEKVNGIVGCTAQADGADAAT
KCKTCKTGTSLAT SATKCVTDVQNCQTYNDTECQTCKT

VSP3 (102-134; 33 aa): DDTKSYIPKVADNFSECKLLTNCKTVVEQKCTA

VSP4 (46 aa):

KDQKHNAAKTACVADTEKVNGTAKADGADADTKCKTCKTGTSLAT

VSP5 (175-239, 65 aa):

IKCVTDVQDCKTYNDTGCELCNSGFKLNAATKACEAEGEATENINNISGCTAQ
ADGADAATKCKT

VSP6 (63 aa):

KSYEKHNADKTACEADPEMVNGIVGCITKAEGADAETKCKACVDGKTLATSAT

VSP7 (52 aa):

KTVVDQKCTACADAVNTTHSIQEGECLKTCEKDQKHNAEKTDCVADIENVNN

VSP8 (41 aa): LKDQKHNAALKTDCEADPEKVNGTAKADAPNATTQCKTCKSG

VSP9 (20 aa): NGYKTNNNEACTACDETKFY

484 aa recombinant protein including all VSPs and with -KK- separators and the TRITAG label:

MTFSVPISLQGSFAAYDQACTNNLGCTANKCEAGNLKCDAAQCKAGYTRDAA
TEICNANNCTTLGTTDPNKGQCTTKKTTTHSIQEGECLKTCLKDQKHNAADKTD
CVADTEKVNGIVGCTAQADGADAATKCKTCKTGTSLAT SATKCVTDVQNCQ
TYNDTECQTCKTKKDDTKSYIPKVADNFSECKLLTNCKTVVEQKCTAKKKDQKH
NAAKTACVADTEKVNGTAKADGADADTKCKTCKTGTSLATSKKIKCVTDVQD
CKTYNDTGCELCNSGFKLNAATKACEAEGEATENINNISGCTAQADGADAATK
CKTKKKSIEKHNAADKTACEADPEMVNGIVGCITKAEGADAETKCKACVDGKT
LATSATKKKT VVDQKCTACADAVNTTHSIQEGECLKTCEKDQKHNAEKTDCV
ADIENVNNKKLKDQKHNAALKTDCEADPEKVNGTAKADAPNATTQCKTCKSGK
KNGYKTNNNEACTACDETKFY

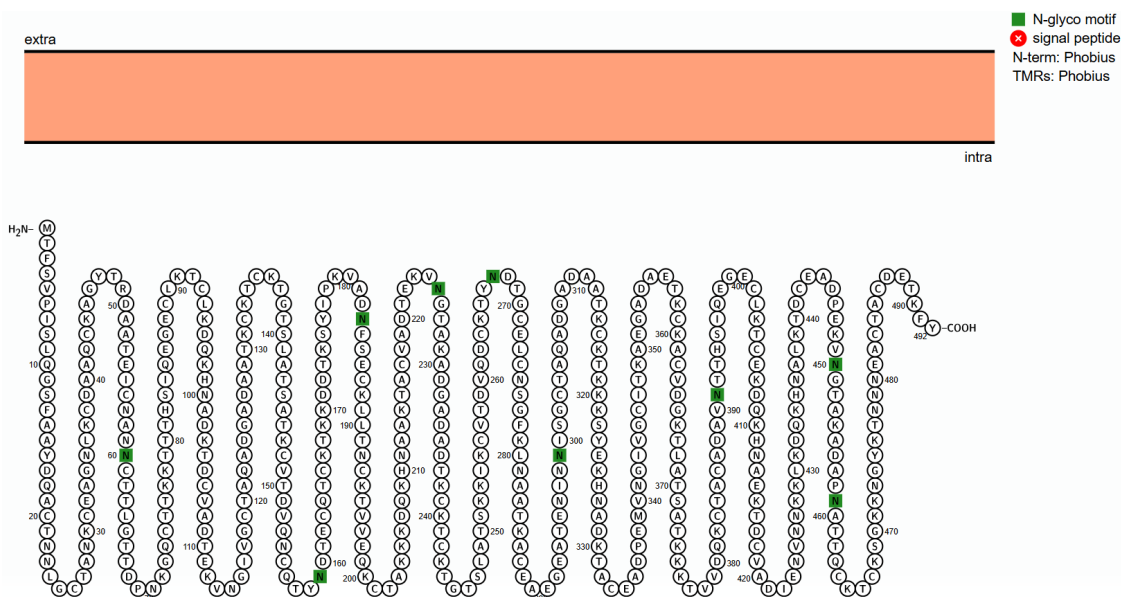


Figure 28. Proteoform of the chimeric recombinant protein containing the antigenic fragments of all VSPs.

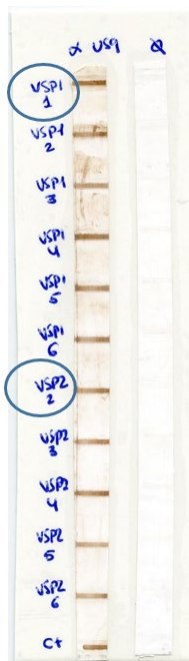


Figure 29. DOT-BLOT assay to determine the expression of the recombinant proteins rVSP1 and rVSP2 in the yeast *S. cerevisiae*. The transforming yeasts were cultured in YPHSM production medium for 4 days from a tryptophan-free CM plate. Several colonies were selected and grown in CM-Trp medium, yeasts were disrupted with zirconia balls, 100 μ L of the supernatant was added to a nitrocellulose strip and the strip was incubated with the monoclonal antibody US9.

The recombinant proteins containing the CDS of the VSP1 and VSP2 proteins expressed in the yeast *S. cerevisiae* were tested by a Dot-Blot immunoassay against the monoclonal antibody US9 that specifically recognizes the TRITAG tag carried by recombinant proteins. As can be seen, several colonies isolated from the selective medium generate a positive signal in the immunoassay indicating that yeasts are producing these proteins (Fig. 29).

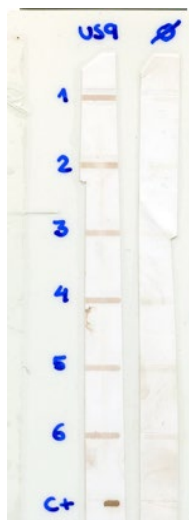


Figure 30. Dot-blot analysis of chimeric protein expression containing antigenic fragments of *P. dicentrarchi* VSPs in several *S. cerevisiae* yeast colonies using the US9 antibody that specifically recognises the TRITAG tag carried by the recombinant protein.

By means of a dot-blot assay, the expression of a chimeric protein containing fragments of all VSPs has been tested by transformed *S. cerevisiae* yeasts. As can be seen in Fig. 30, all the colonies tested give a positive signal in Dot-blot indicating that they are producing the chimeric protein.

Next, we designed an experiment in which we intend to evaluate the protective response induced by vaccines containing yeasts expressing the recombinant proteins VSP1 and a chimeric recombinant protein containing antigenic fragments of all the VSPs of *P. dicentrarchi* (Table 14), and compare it with combinations of yeasts and inactivated ciliates and also with synthetic peptides generated from the 8 VSPs of the ciliate (Table 15).

Grupo experimental	Peso azar (g)	Nº Peces
1.PBS	120 ± 19,5	35
2.Cil + M763	115 ± 17,4	40
3.Lev_VSP1	110 ± 17,8	40
4.Lev_VSPs	111 ± 19,3	40
5.Péptidos + M763	128 ± 20,3	40
6.Cil + Lev_VSPs	108 ± 12,2	40
7. Cil + Lev_WT	107 ± 23,4	40

Table 14. Experimental groups used in vaccinations that include the number and weight achieved by the fish at the end of the experiment. Cyl: inactivated ciliates; M763: Montanide ISA 763 adjuvant; Lev_VSP1: *S. cerevisiae* yeasts transformed with Gene encoding variable surface protein 1 (VSP1); Lev_VSPs: yeasts of *S. cerevisiae* transformed with a nucleotide sequence encoding a protein of 484 aa and including all VSPs; Lev_WT: unprocessed wild yeast. In the design of this experiment, 7 groups of fish have been used, whose number and final weights (in grams) are indicated in the table.

Nombre péptido	Secuencia aminoacídica
VSP1	NLKCDAAQCKAGYTRDAA
VSP2	LKDQKHNAKTDKCVADTEKVNG
VSP3	DDTKSYIPKVADNFSCKLLTN
VSP4	ACVADTEKVNGTAKADGADAD
VSP5	LKDQKHNAKDTACEADAE
VSP6	KSYEKHNAKDTACEADPEMVN
VSP7	ACTTCHEDRSYFPKTQNDASV
VSP8	ACADAVTASHSIQEGECLKT

Table 14. Amino acid sequences of linear peptides predicted by the Bepripped tool of variable surface proteins (VSPs) identified in *P. dicentrarchi*. The peptides were chemically synthesized and coupled to limpet hemocyanin (KLH) as a carrier protein.

1. Growth

As shown in Table 14, vaccinations do not significantly affect fish growth.

2. Cytotoxic effect (complement)

	SUEROS (S6.1)										
VACUNA/GRUPO	1	2	3	4	5	6	7	8	9	10	MEDIA \pm SD
G1. PBS	1/4	1/2	1/4	1/4	1/4	1/4	-	-	-	-	3,7 \pm 0,8 ^b
G2. Cil + M763	1/32	1/16	1/32	1/8	1/32	1/4	1/16	1/16	-	1/16	19,1 \pm 10,5 ^a
G3. Lev_VSP1	1/4	1/8	1/2	1/2	1/2	1/4	1/4	1/8	1/2	1/4	4,0 \pm 2,3 ^b
G4. Lev_VSPs	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	4,0 \pm 0,0 ^b
G5. Péptidos + M763	1/4	1/4	1/8	1/4	1/4	1/4	1/8	1/4	1/4	1/4	4,8 \pm 1,7 ^b
G 6. Cil + Lev_VSPs	1/4	1/4	1/4	1/4	1/4	1/8	-	-	-	-	4,7 \pm 1,6 ^b
G 7. Cil + Lev_WT	1/4	1/4	1/8	1/4	1/4	1/4	1/4	-	-	-	4,6 \pm 1,5 ^b

Tabla 15. Efecto citotóxico del suero de los rodaballos vacunados mediados por anticuerpos (CMA). Para realizar el ensayo se realizaron diluciones seriadas de los sueros a partir de la dilución $\frac{1}{4}$ y el título fue calculado como la dilución máxima del suero que provoca el 100% de la muerte de los ciliados. Los resultados se expresan como los valores enteros medios \pm la desviación estándar de la media (SD) de los títulos. Las letras iguales indican que no existen diferencias estadísticamente significativas y las letras desiguales indican diferencias estadísticamente significativas ($P < 0,05$).

The results of the CMA activity of the sera of the vaccinated turbot indicate that only vaccination with inactivated ciliates in oily adjuvant generates significant CMA activity (Table 15). Neither turbot vaccinated with yeast containing VSP1 nor turbot vaccinated with yeast containing the chimeric recombinant protein with all VSPs generate a significant CMA with respect to control (PBS).

3. Antibody production

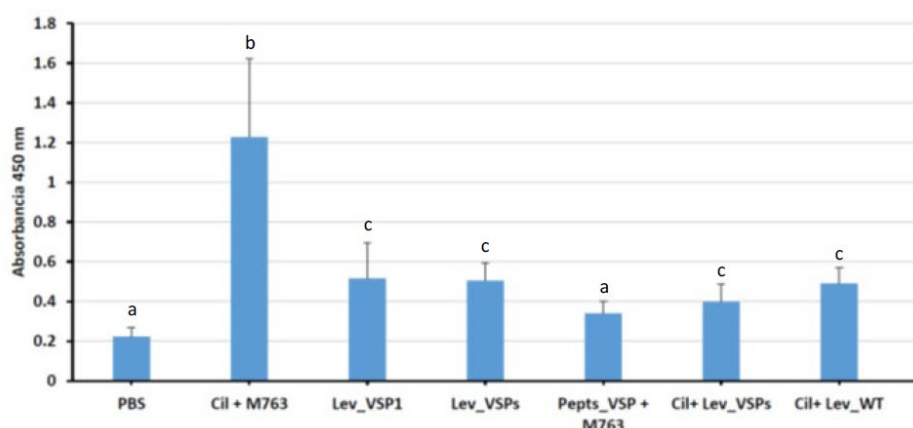


Figure 31. Membrane surface antigen antibody levels Generated by vaccinated fish determined by ELISA after spectrophotometer reading at 450 nm. The bars indicate the mean absorbance values (n=10) \pm the standard deviation from the mean. Uneven letters indicate statistically significant differences ($P < 0.05$) between groups.

In this experiment, the maximum antibody levels against surface antigens are obtained with the vaccination of inactivated ciliates in oily adjuvant M763; however, antibody levels in the groups vaccinated with yeasts containing the VSPs are significantly increased compared to the control (PBS) but turbot vaccinated with the peptide mixture containing sequences of all the VSPs in oily adjuvant do not generate a significant level of antibodies (Fig. 31).

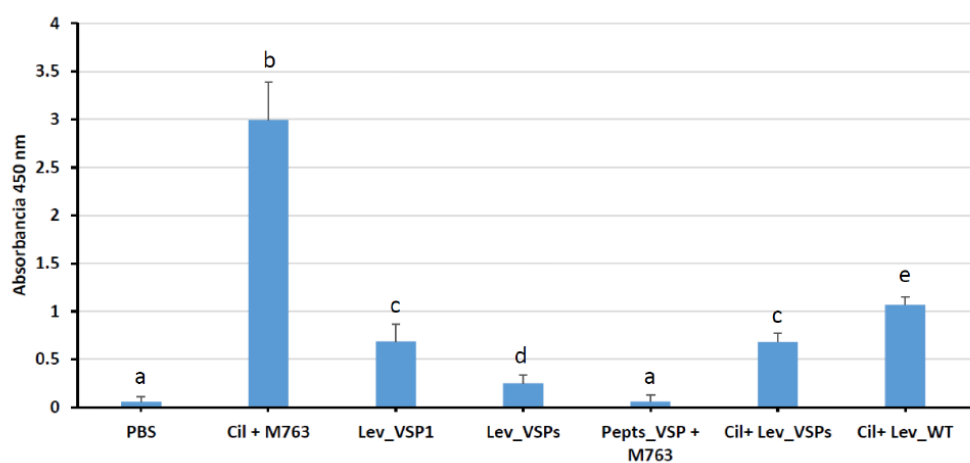


Figure 32. Production of anti-epitope glycan antibodies by vaccinated fish determined by a capture ELISA using solid-phase-coupled rabbit IgG and an anti-FITC-Peroxidase antibody to resolve the enzyme immunoassay. The bars show the mean absorbance values at 450 nm \pm the standard deviation (n=10).

When the antibody response generated by turbot vaccinated with inactivated ciliates and M763 oily adjuvant was analyzed, with yeasts transformed with the VSP1 and with yeasts containing the chimeric protein containing fragments of all the VSPs, the levels of antibodies generated against carbohydrate epitopes increased significantly; however, vaccination with chimeric peptides of the VSPs did not generate antibodies against glycan epitopes (Fig. 32).

4. Protection

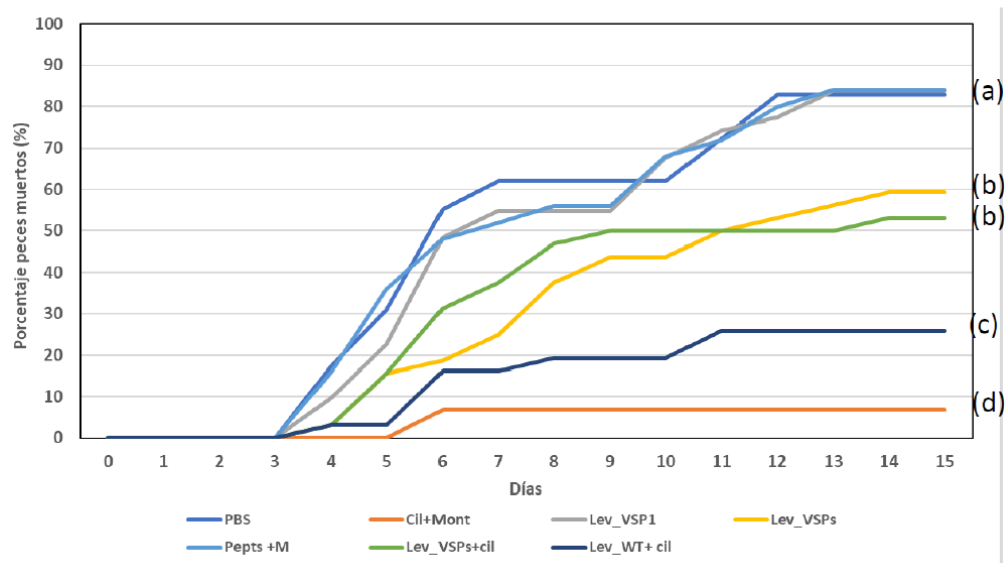


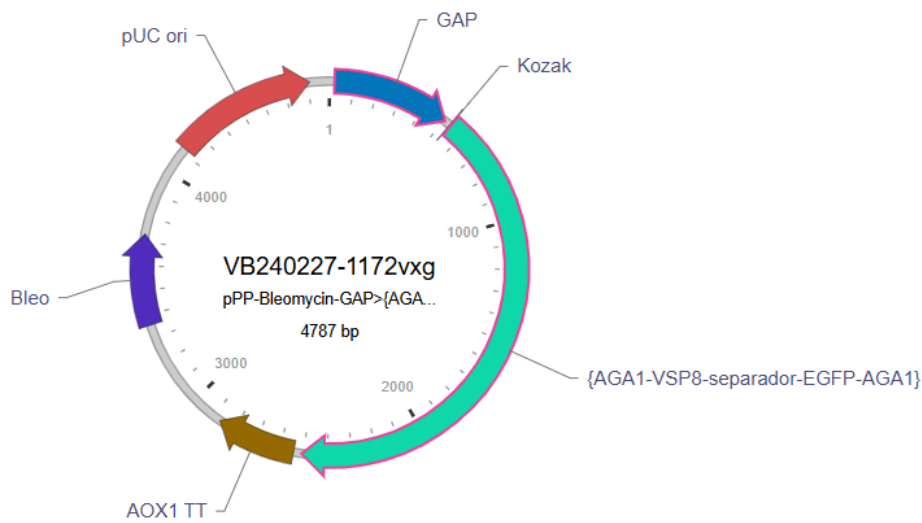
Figure 33. Cumulative survival rate of vaccinated turbot determined within 15 days of experimental infection. Uneven letters indicate statistically significant differences ($P < 0.05$) between experimental groups.

The results of the vaccine protection experiment (Figure 33) indicate that fish vaccinated with inactivated ciliates and Montanide ISA 763 have the highest survival rate, followed by the group of fish vaccinated with inactivated ciliates and the wild yeast of *S. cerevisiae*. Fish vaccinated with yeasts transformed with VSPs and with inactivated ciliates and yeasts transformed with VSPs also have a certain degree of protection but less than experimental groups 2 and 7. On the contrary, groups vaccinated with synthetic peptides + Montanide ISA 763 and with yeasts transformed with VSP1 do not produce any protection in the fish against infection with *P. dicentrarchi*.

***Komagataella pastoris* (sin. *Pichia pastoris*):**

Vector Summary

Vector ID	VB240227-1172vxg
Vector Name	pPP-Bleomycin-GAP>{AGA1-VSP8-separador-EGFP-AGA1}
Vector Size	4787 bp
Vector Type	<i>P. pastoris</i> Yeast Protein Expression Vector
Inserted Promoter	GAP
Inserted ORF	{AGA1-VSP8-separador-EGFP-AGA1}
Plasmid Copy Number	High
Antibiotic Resistance	Bleomycin, Phleomycin or Zeocin(TM)
Cloning Host	VB UltraStable (or alternative strain)



Vector Components					
Name	Position	Size (bp)	Type	Description	Application notes
GAP	■ 22-507	486	Promoter	Glyceraldehydes-3-phosphate dehydrogenase promoter from Pichia pastoris	Strong constitutive promoter.
Kozak	■ 532-537	6	Miscellaneous	Kozak translation initiation sequence	Facilitates translation initiation of ATG start codon downstream of the Kozak sequence.
{AGA1-VSP8-separador-EGFP-AGA1}	■ 538-2508	1971	CDS	None	None
AOX1 TT	■ 2541-2869	329	Transcription_terminator	Alcohol oxidase 1 transcription terminator.	Allows transcription termination and polyadenylation of mRNA transcribed by Pol II RNA polymerase in yeast.
Bleo	■ 3357-3731	375	CDS	Bleomycin resistance gene	Allows cells to be resistant to bleomycin, phleomycin or Zeocin(TM).
pUC ori	■ 4119-4707	589	Rep_origin	pUC origin of replication	Facilitates plasmid replication in E. coli; regulates high-copy plasmid number (500-700).

CDS: VSP8-EGFP

MTLSFAHFITYLFTILLGLTNIALADGQNGCTTCADANDCQVCDQANFYFLKVNKTCDLQTGCKT
YAGDACTACDETKFYFPKIANNFSECKLFSNCKTVVDQKCTACADAVTASHSIQEGECLKTCCLK
DQKHNAKLTDCOADPEKVNGIVGCTAKADAPNATTQCKTCKSGTTLAISATKCVTDVQNCQTYN
DTGCNECKTGFKLNSSTKACEAEGGASENVNNIAGCTAQADGANAATQCKTCKSGTTLAISATK
CVTDVQNCQTYNDTGCEQCNTGFKLNTTSKACEVDAAQNVNNIVGCTAKADGANADTKCKTCIA
GTTLATSATKCVTNVKDCQTYNDTGCEKCKTGFKLNTTSKACEQDKTSSSSSTSGGGSGGGGSG
GGGSMVSKGEELFTGVVPIVLVDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVPWPT
LVTTLTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKNGIKVNFKIRHNIEDGSGVLADHYQQNTP
IGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYKYMGSQSQTRLPLG
KLVFAMAVACNVIFS-

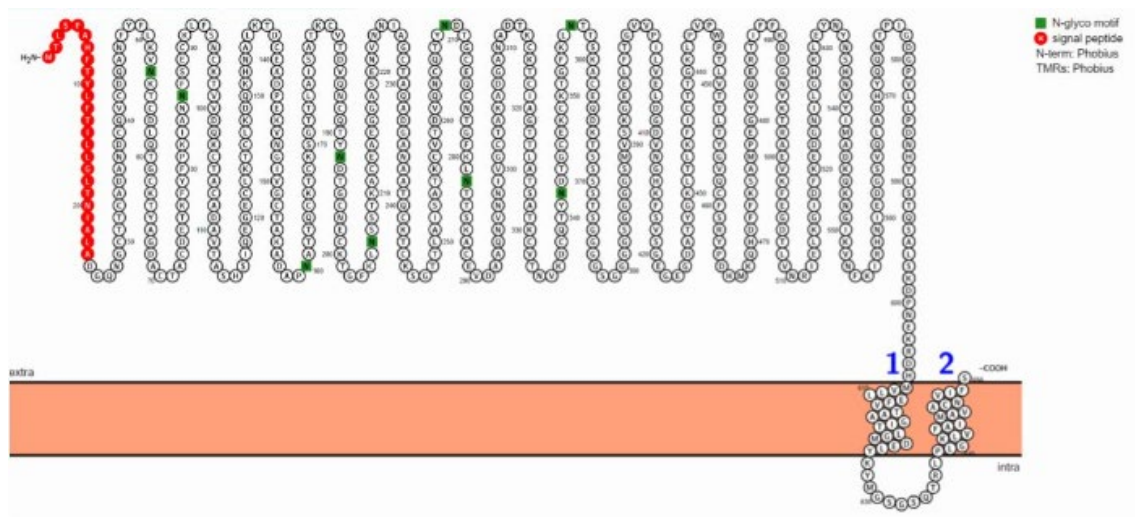


Figure 34. Plasmid construction for expression of the VSP8 protein of *P. dicentrarchi* in the yeast *P. pastoris*, components of the vector, sequence of the recombinant protein and its proteoform, containing the signal peptide, the glycosylation sites and 2 transmembrane domains.

We have expressed several VSPs proteins of *P. dicentrarchi* that present a high degree of glycosylation in the yeast *P. pastoris* because it has been shown that this yeast is capable of producing a higher degree of fidelity in glycosylation than in the case of the yeast *S. cerevisiae* which usually generates hyperglycosylations in recombinant glycoproteins. In this case, we have expressed a polycistronic CDS containing the glycoprotein VSP8 of *P. dicentrarchi* and the green fluorescent protein EGFP. The design of the polycistronic protein has been carried out with the aim of expressing it in the yeast membrane. Once the yeasts have been transformed, we have corroborated that the protein is expressed in the cell membrane (Fig. 35).

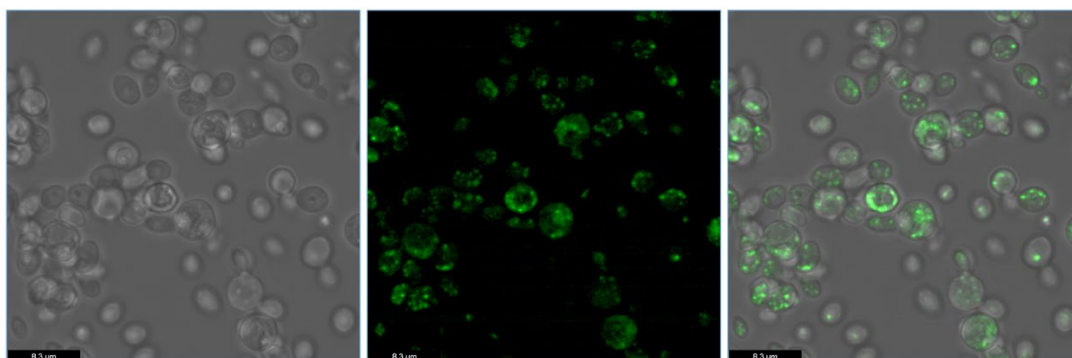
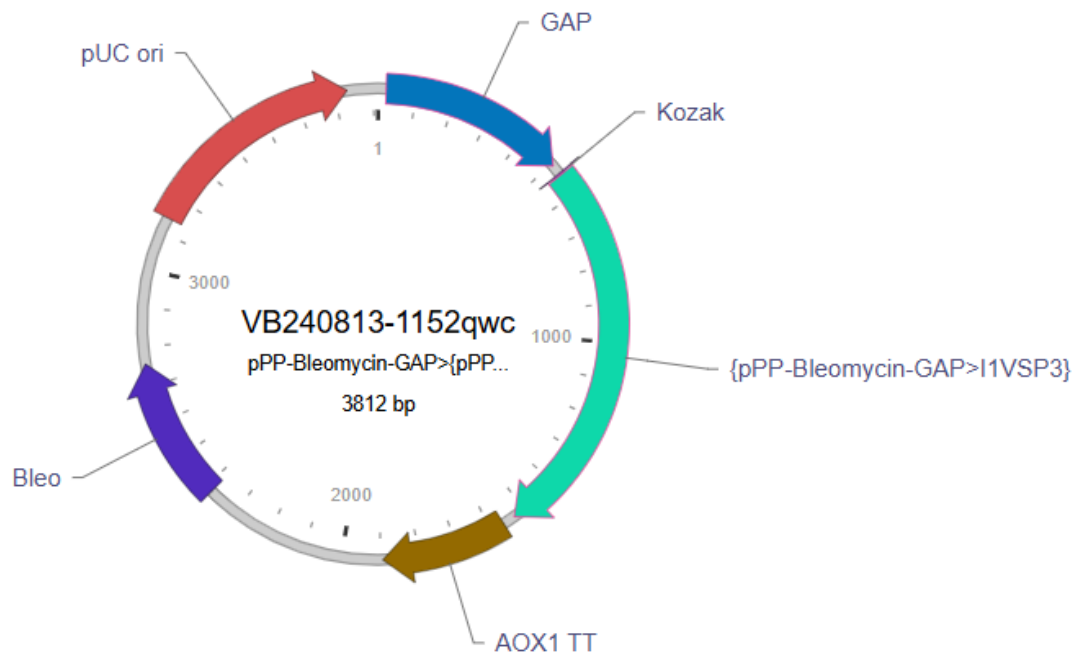


Figure 35. Microphotographs showing the location of the green fluorescent protein that is co-expressed with the VSP8 of *P. dicentrarchi*.

Once it was verified that the design of the plasmid constructs for expression in the yeast membrane were correct (Fig. 35), we went on to design a new construct containing the CDS of the VSP3 protein (Fig. 36).



Vector Components					
Name	Position	Size (bp)	Type	Description	Application notes
GAP	■ 22-507	486	Promoter	Glyceraldehydes-3-phosphate dehydrogenase promoter from Pichia pastoris	Strong constitutive promoter.
Kozak	■ 532-537	6	Miscellaneous	Kozak translation initiation sequence	Facilitates translation initiation of ATG start codon downstream of the Kozak sequence.
{pPP-Bleomycin-GAP>I1VSP3}	■ 538-1533	996	CDS	Proteína variable superficie 3-VSP3-	None
AOX1 TT	■ 1566-1894	329	Transcription_terminator	Alcohol oxidase 1 transcription terminator.	Allows transcription termination and polyadenylation of mRNA transcribed by Pol II RNA polymerase in yeast.
Bleo	■ 2382-2756	375	CDS	Bleomycin resistance gene	Allows cells to be resistant to bleomycin, phleomycin or Zeocin(TM).
pUC ori	■ 3144-3732	589	Rep_origin	pUC origin of replication	Facilitates plasmid replication in E. coli; regulates high-copy plasmid number (500-700).

CDS: TRITAG-POLIHIS-VSP3

MTL~~SFAHFTYLFTILLGLTNIALAMTFSVPIS~~HHHHHHKIIQQKCSKKSPYQLSSQLLFSL
 PARKNTNLQIFYNYNFLNFCFYERVGEGGCTACNSEADPACTACDAAENYFIKEGAC
 VLITGCTAATAGVCDTCDKAKFYFKANNTCDLQTGCKTYTGDACTACDDTKSYIPKV
 ADNFSECKLLTNCKTVVEQKCTACADAVTTTHSIQEGECLITCKSNEKHNADKTACEA
 DAEKVNGIVGCITKPDGADADTKCKACKTGTTLAT~~SATKCVTDVQNCQTYNDTECQT~~
 CKTGFKLNTTTKACDADKSSSSAIVSASLAVILAVFALAL

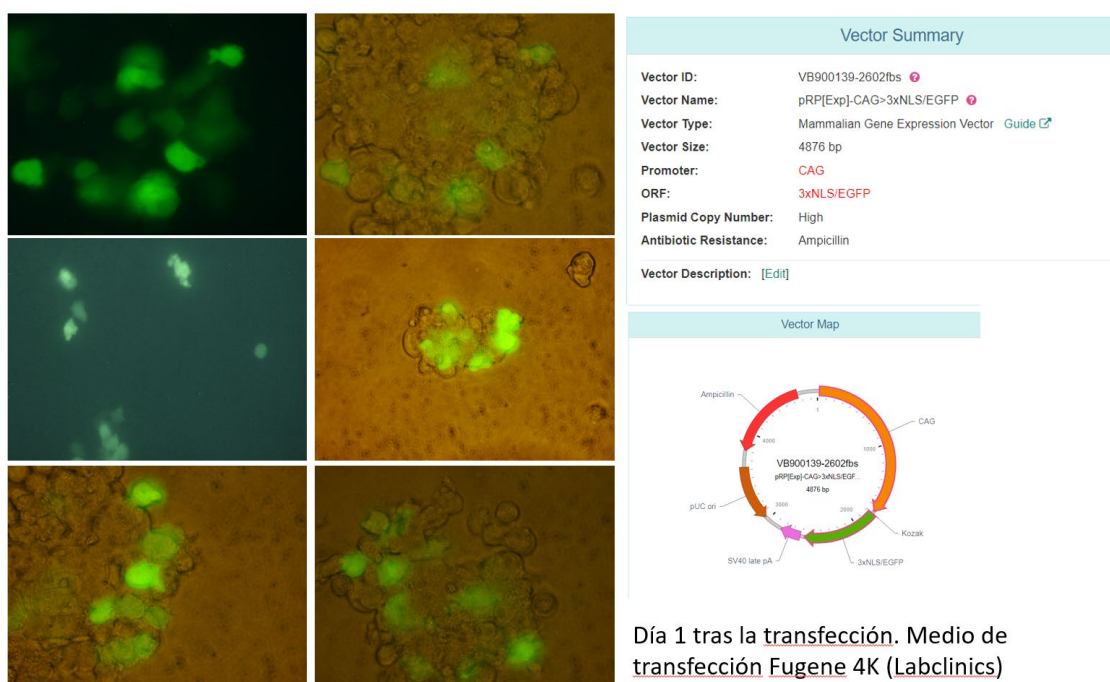


Figure 37. Expansion of green fluorescent protein (EGFP) in HEK293 cells.

48 horas	Control	EGFP	
	5314	27776	485/20,530/25
	5323	26798	485/20,530/25
	5324	25912	485/20,530/25
Media	5320,33	26828,67	

4 días	Control	EGFP	
	4628	5066	485/20,530/25
	4626	5063	485/20,530/25
	4771	5100	485/20,530/25
Media	4675,00	5076,33	

Table 16. Expression of green fluorescent protein (EGFP) in HEK293 cells. Fluorometric quantification of fluorescence emitted by transfected and non-transfected HEK293-F cells with the pRP[Exp]-CAG>3xNLS/EGFP plasmid at 46 and 96 h post-defection. Each well contains 105 cells in Free Style medium and fluorescence quantification was performed in triplicate.

As can be seen from the results obtained and presented in Table 16, maximum fluorescence (maximum expression of the EGFP protein) is obtained after 48 h of culture and, after 96 h, the cells practically stop expressing this protein.

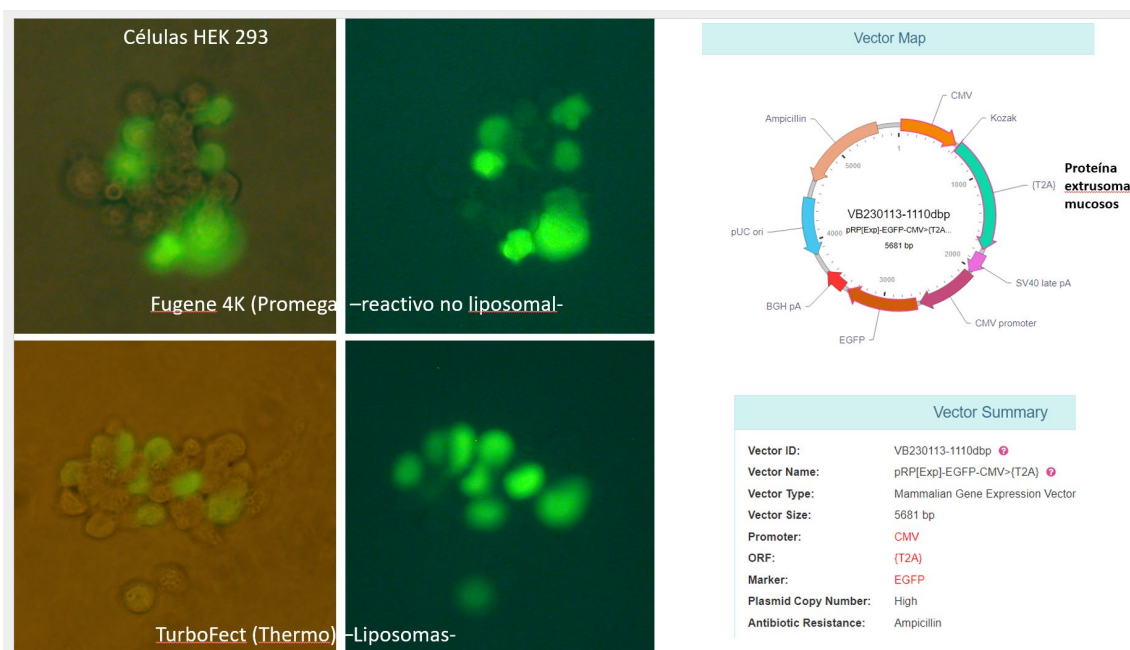


Figure 38. Effect of the transfection reagent on the expression of the polycistronic protein T2A (extrusome protein) / green fluorescent protein EGFP.

24 horas	WT	FuGENE 9uL	FuGENE 7uL	TurboFect 2uL	TurboFect 3uL	
	5197	19565	13499	5634	7692	485/20,530/25
	5116	20101	13787	5784	7737	485/20,530/25
	5174	17863	13610	5723	6575	485/20,530/25
MEDIA	5162,33	19176,33	13632,00	5713,67	7334,67	485/20,530/25

48 horas	WT	FuGENE 9uL	FuGENE 7uL	TurboFect 2uL	TurboFect 3uL	
	5194	73839	36077	10369	26672	485/20,530/25
	5282	53691	35270	9461	26834	485/20,530/25
	5049	46189	32721	8862	24848	485/20,530/25
MEDIA	5175,00	57906,33	34689,33	9564,00	26118,00	485/20,530/25

Table 17. Quantification of fluorescence emitted by HEK293 cells transfected with the pRP[Exp]-EGFP-CMV>{T2A} plasmid at 24 and 48 h using TurboFect™ (ThermoFisher Scientific) and FuGENE 4k (FUGENEE®) transfection reagents.

In a subsequent experiment, it was intended to determine the transfection efficiency for the expression of a polycistronic protein composed of the extrusome protein of *P. dicentrarchi* (T2A) and the green fluorescent protein (EGFP) using two transfection reagents: TurboFect and FuGENE 4k (Fig. 38). When the fluorescence results emitted by the cells transfected with both reagents were analyzed, it was observed that the FuGENE 4k reagent is the one that generates the best transfection of HEK293 cells (Table 17).

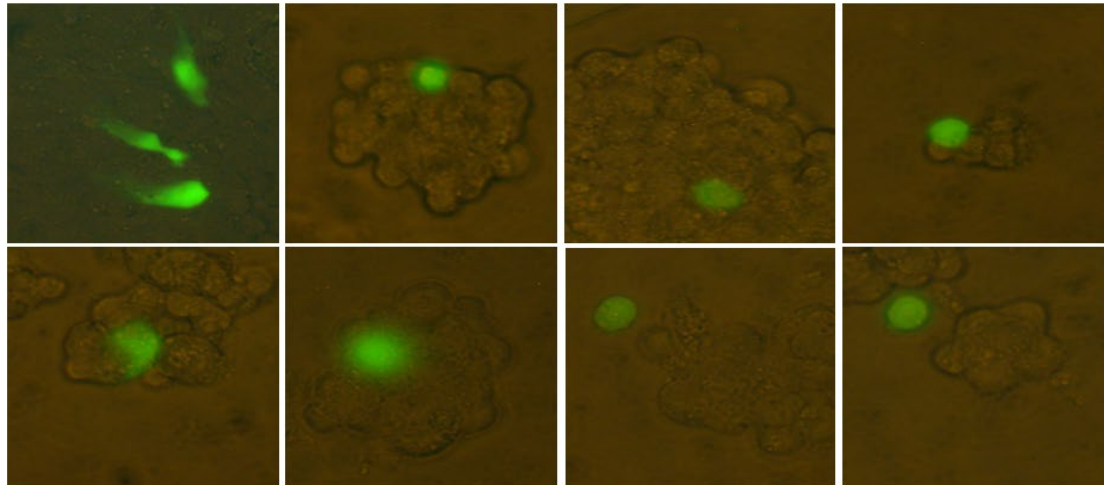


Figure 39. Expression of green fluorescent protein (EGFP) in EPC (Papulous Cyprini Epithelioma) cells of bighead carp (*Pimephales promelas*) using the expression plasmid in eukaryotes pRP[Exp]-CAG>3xNLS/EGFP.

We have also performed an additional transfection experiment using the FuGENEE 4k transfection reagent in carp papular epithelioma (ECP) cells for the expression of the green fluorescent protein. In this experiment, 7 and 10 μL of reagent and 2 μg of plasmid construction were used from cells kept in MEM medium (with Hank's salts) and without fetal bovine serum. The mixture of the transfection reagent and plasmid DNA was carried out in FreeStyle medium (Gibco). From 24-48 h onwards, the expression of the EGFP protein was observed; however, the transfection levels obtained in these cells are lower than in HEK293 cells, indicating that additional optimization experiments are still needed to improve the efficiency of the expression of recombinant proteins (Fig. 39).

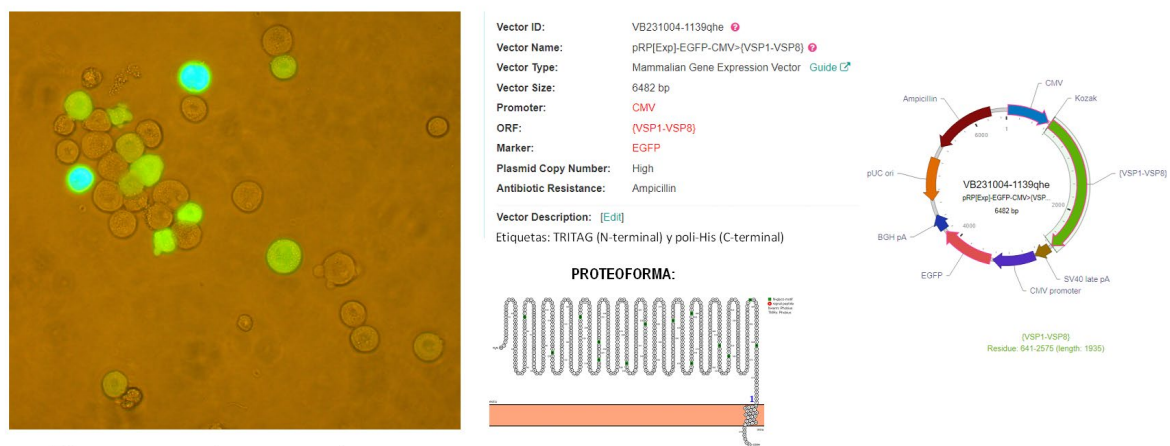
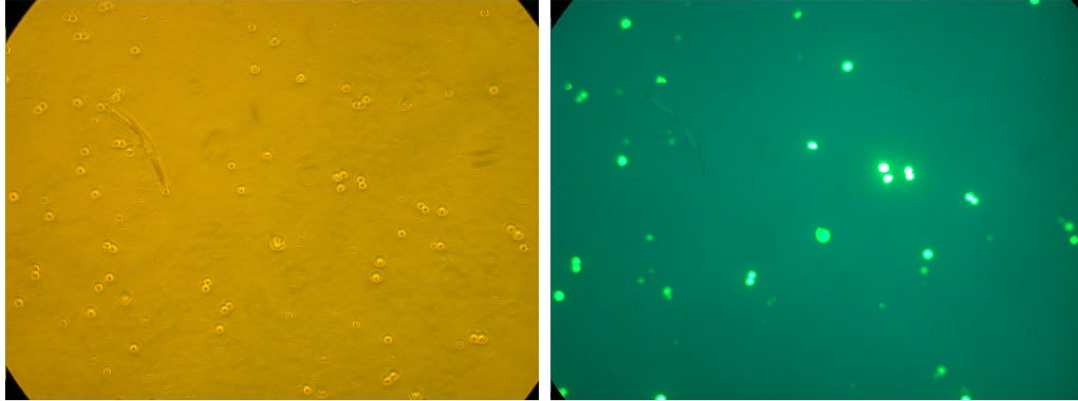


Figure 40. HEK293 cells transfected with the pRP[Exp]-EGFP-CMV>{VSP1-VSP8} plasmid.



HEK 293 transfectadas con 2 μ g plásmido + 8 μ L de agente transfectante (Fugene 4K –Promega-). 48 h

Figure 41. HEK293 cells transfected 2 μ g of pRP[Exp]-EGFP-CMV>{VSP1-VSP8} plasmid and 8 μ L of the transfectant agent FuGENEE 4k at 48 h of culture.

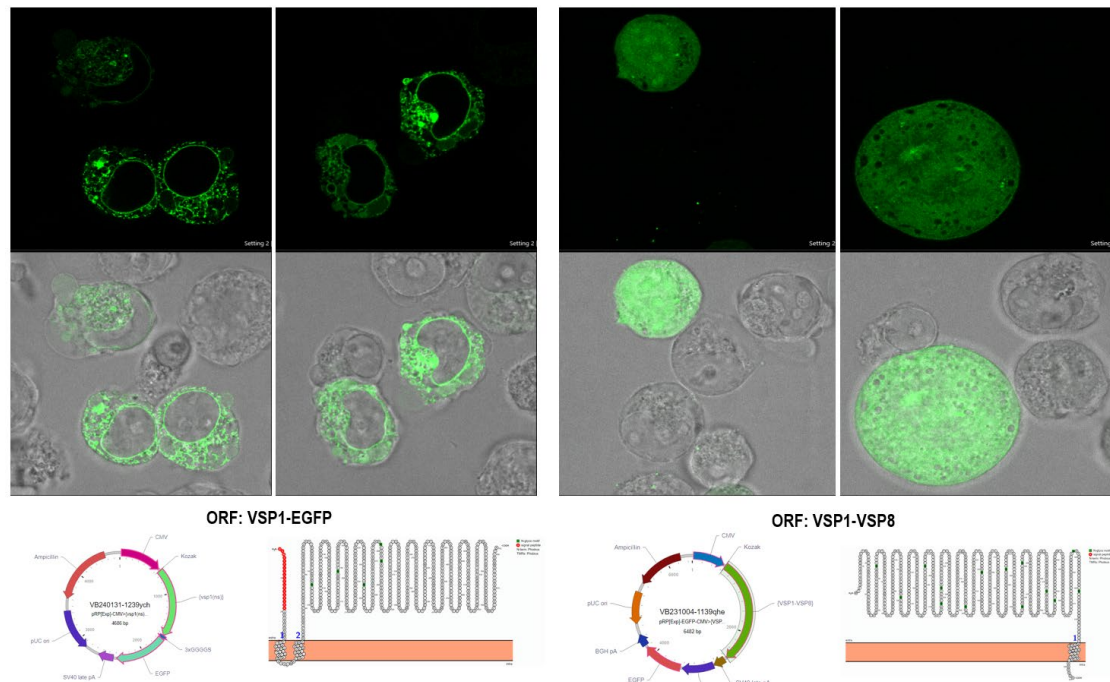


Figure 42. Expression of chimeric proteins VSP1-EGFP and VSP1-VSP8-EGFP in HEK293 cells.

In a subsequent experiment we designed two polycistronic plasmid constructs containing protein VSP1 and proteins VSP1/VSP8 and, as a marker protein, EGFP. In the case of the VSP1-EGFP construct, a CDS containing a signal peptide and a transmembrane domain was designed for the expression of chimeric protein in the cell membrane (Fig. 42) and, in the case of chimeric protein VSP1-VSP8-EGFP, a plasmid construction without signal peptide was designed for the transfected cells to produce the recombinant proteins intracellularly (Fig. 42). In addition, protein VSP1-EGFP should be expressed glycosylated, while VSP1-VSP8-EGFP should be expressed without the presence of glycans. In both cases, the transfected HEK293 cells efficiently produced proteins at 48 h of culture (Figs. 41-42).

We vaccinated fish with HEK293 cells expressing VSP1 proteinS and chimeric VSP1-VSP8 protein (Fig. 42) and determined CMA and antibody production against the S6.1 strain.

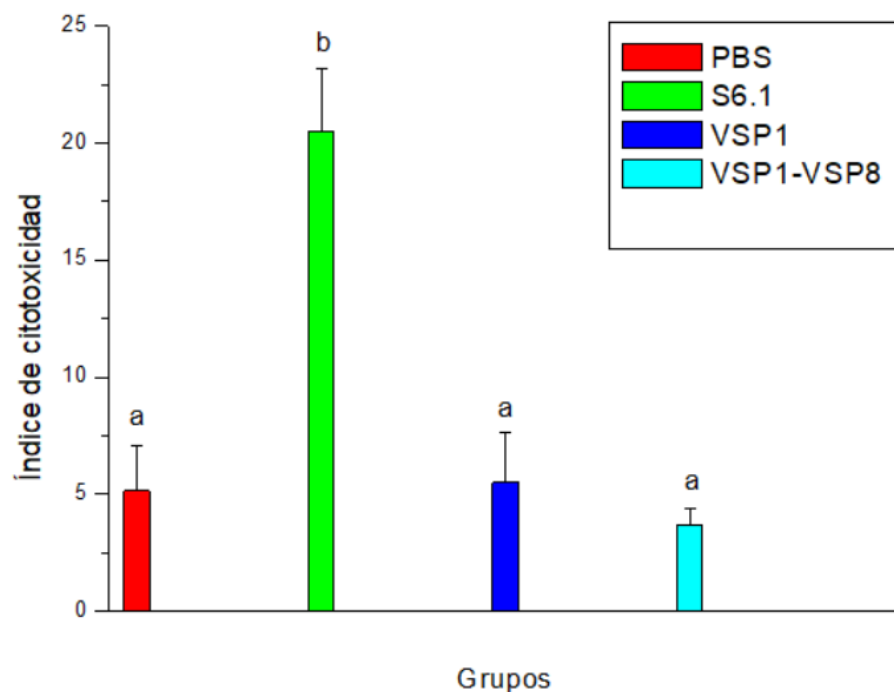


Figure 43. Antibody-mediated cytotoxicity Generated by rhodolal sera immunized with inactivated Ciliates of *P. dicentrarchi* -strain S6.1-, HEK293 cells expressing the recombinant proteinS VSP1 and VSP1-VSP8 and PBS (Control). Uneven letters indicate statistically significant differences ($P < 0.05$) between groups.

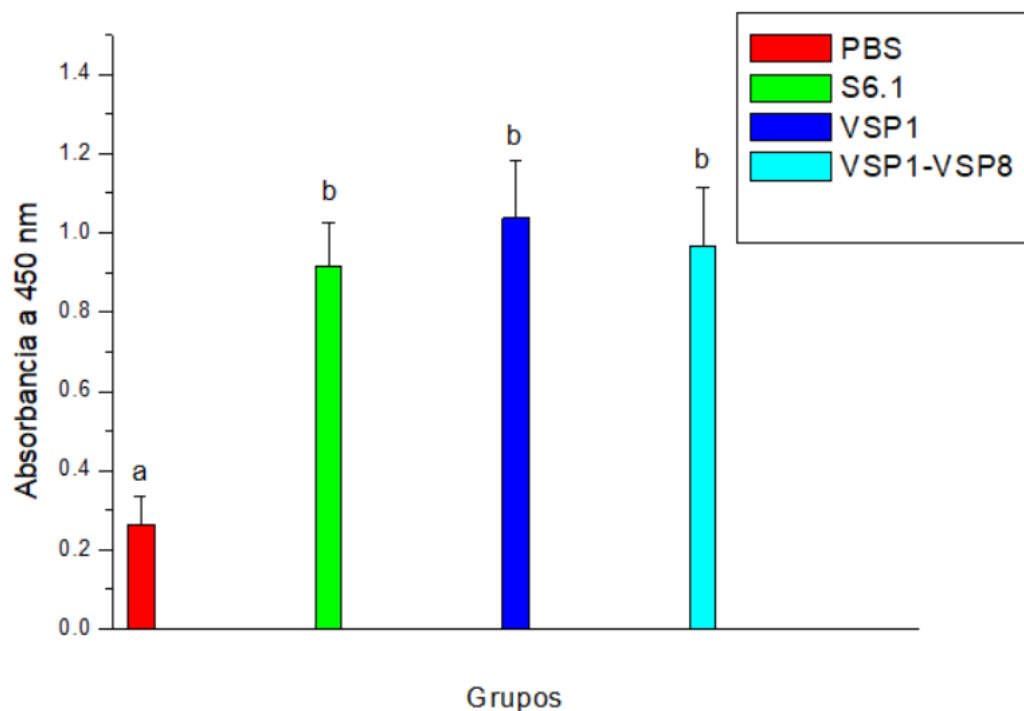


Figure 44. ELISA assay to determine the level of anti-S6.1 antibodies by PBS-vaccinated turbot sera (control), inactivated ciliates of the S6.1 strain, HEK293 cells expressing protein VSP1 and VSP1-VSP8. The bars express the mean values \pm standard deviation ($n=5$) and the different letters indicate statistically significant differences ($P<0.05$) with respect to the control (PBS).

Los resultados de la vacunación con los ciliados inactivados y con las células HEK293 que expresan las proteínas VSP1 y VSP1-VSP8 indican que únicamente los sueros de los rodaballos vacunados con ciliados inactivados generan anticuerpos que generan citotoxicidad frente a los trofozoitos de *P. dicentrarchi* (Fig. 43). Por el contrario, cuando se testaron los sueros de los rodaballos vacunados para determinar el nivel de anticuerpos frente a los antígenos totales de la cepa S6.1, se observó que todos los grupos generan niveles de anticuerpos significativamente más elevados. The results of vaccination with inactivated ciliates and HEK293 cells expressing VSP1 and VSP1-VSP8 proteins indicate that only sera from turbot vaccinated with inactivated ciliates generate antibodies that generate cytotoxicity against *P. dicentrarchi* trophozoites (Fig. 43). On the contrary, when the sera of vaccinated turbot were tested to determine the level of antibodies against the total antigens of the S6.1 strain, it was observed that all groups generated significantly higher levels of antibodies than the control (PBS) but not significantly different between them (Fig. 44).

Objective 3.- To develop a vaccine formulation containing biodegradable adjuvants that generate protection against homologous and heterologous serotypes.

Vaccination trials with biodegradable adjuvants

In this project we have also tested the effect of two adjuvants admitted for veterinary use: a) Montanide ISA 763A VG oily adjuvant and b) Montanide gel 02 PR -Seppic-. Montanide ISA 763A VG adjuvant is a formulation of non-mineral oil in water and Montanide gel 02 PR adjuvant is a polymeric adjuvant consisting of sodium polyacrylate gel particles in water. Both adjuvants have been developed for use in parenteral vaccines.

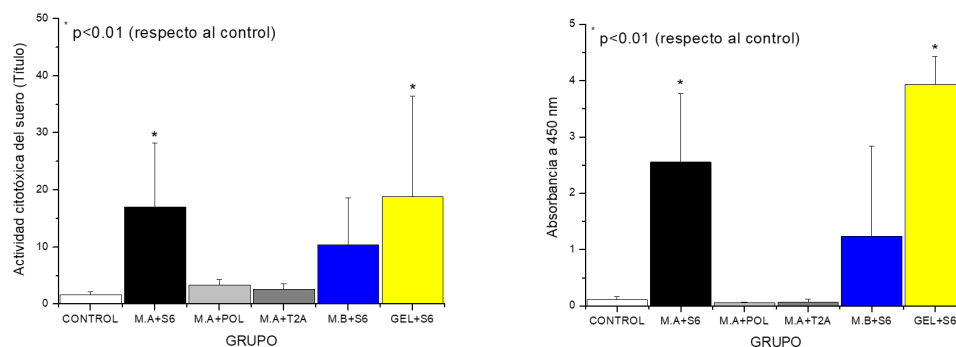


Figure 45. Cytotoxic effect and level of antibodies present in rhodore sera immunized with a fraction of total polysaccharides (POL) obtained from trophozoites of *P. dicentrarchi*, a recombinant protein of the extrusome of *P. dicentrarchi* protein (T2A) expressed in HEK 293 cells and inactivated ciliates (S6) formulated in Montanide ISA 763 A VG (M.A.) oil adjuvant and in Montanide gel 02 PR (GEL). The bars show the means \pm standard deviation ($n=5$). Asterisks indicate statistically significant differences from control.

The results shown in Fig. 45 indicate that the vaccine formulated with inactivated ciliates and adjuvant Montanide gel 02 PR stimulates the highest levels of antibodies and AMC.

Isolation of membrane glycoproteins anchored to glycosyl-phosphatidylinositol (GPI)

Many eukaryotic cell proteins are anchored to the cell membrane by covalent bonding to glycosyl-phosphatidylinositol (GPI). These proteins lack transmembrane domains, have no cytoplasmic tail, and are therefore found exclusively in the extracellular portion of the plasma membrane. Molecules anchored to glycosylated phosphoinositol are a structurally diverse family of proteins that includes: protozoan coat components, activation antigens, complement regulatory proteins, adhesion molecules, membrane-associated enzymes, and many other glycoproteins. In the final phase of this project, we have evaluated the immunogenic capacity of GPI-anchored glycoproteins of *P. dicentrarchi* and evaluated their potential usefulness for the development of a vaccine against scuticociliasis. To obtain GPI-bound glycoproteins, we have used the enzyme phospholipase C and the protocol used to obtain this antigenic fraction has been optimized to obtain it in the ciliate *P. dicentrarchi*.

To obtain the glycoproteins linked to GPI, we have used the enzyme phospholipase C and, in a first experiment, the enzyme concentration and incubation time were optimized to determine the maximum extraction of glycoproteins anchored to GPI.

CONCENTRACIÓN GPI (ug/uL)	0,5 uL	1 uL
30 minutos	3,6	3,5
1 hora	3,4	3,8
2 horas	4	4

Table 18. Evaluation of the extraction level of GPI-anchored glycoproteins (gli-GPI) by different concentrations of phospholipase C (PLC) by incubating the ciliates (5×10^7 ciliates/mL) with the enzyme at 30°C and at different times. 0.5 μ L of PCL corresponds to 0.05 U.

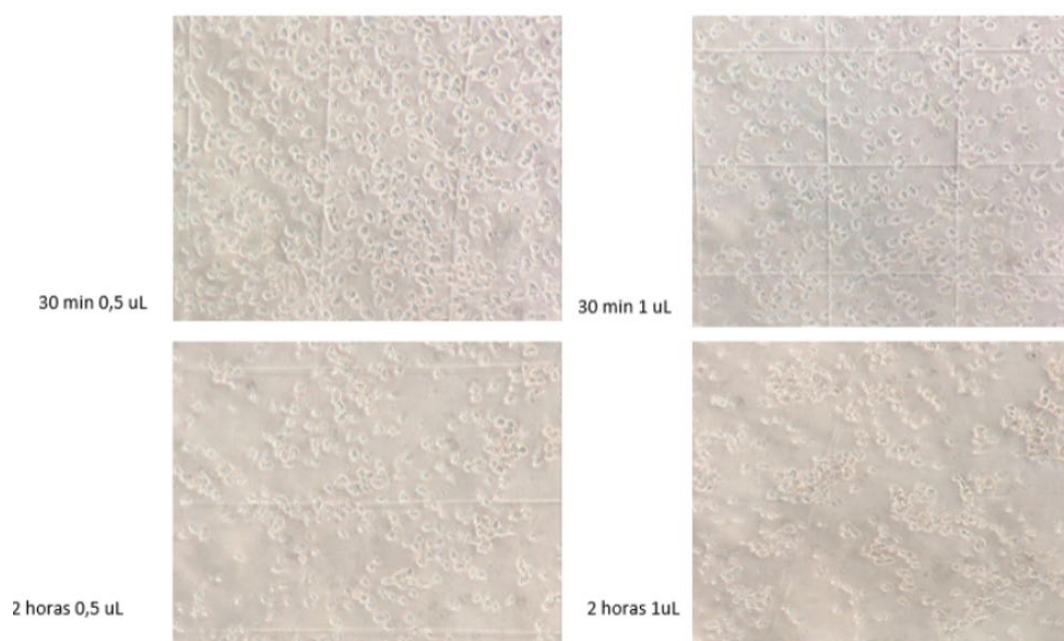


Figure 46. Morphology of *P. dicentrarchi* trophozoites incubated with different concentrations of PLC at various times.

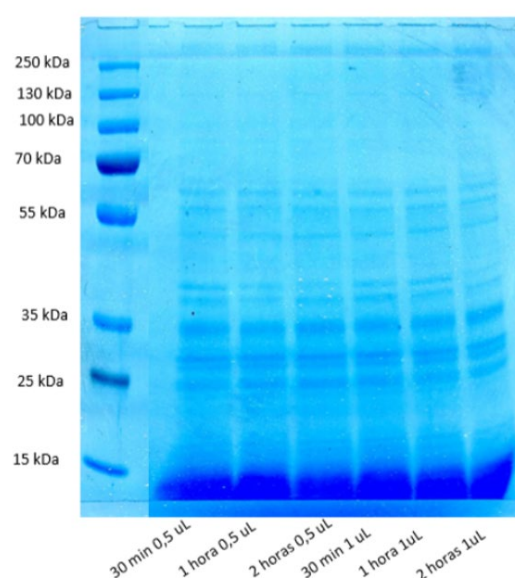


Figure 47. SDS-PAGE (12.5%) under gli-GPI reducing conditions extracted with different concentrations of PLC and at various incubation times.

As can be seen in Table 18, the optimal conditions for obtaining the maximum PLC concentration and incubation time ratio without affecting the viability of the ciliates (Fig. 46) was 0.05 U/mL of PLC and an incubation time of 30 min. In all cases, the polypeptide profile is very similar (Fig. 47).

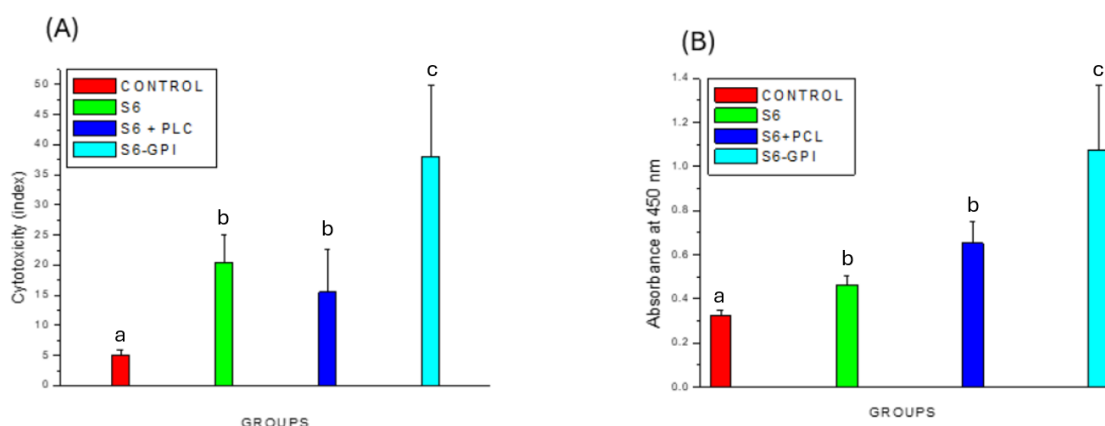


Figure 48. CMA and total antibody levels in rhodabal sera vaccinated with inactivated ciliates (S6), with inactivated ciliates and treated with PLC (S6+PLC) and gli-GPI (S6-GPI). The bars indicate the means \pm SD (n=5). Different letters indicate statistically significant differences (P<0.05).

Taking into account the possibility that the VSPs and leishmanolisins of *P. dicentrarchi* are part of the gli-GPI and that the obtaining of this fraction is highly conservative while maintaining the native structure of these components, we have carried out an experiment of turbot vaccination to assess its potential protective capacity. Montanide gel 02 PR was incorporated as an adjuvant in the vaccines. As can be seen in Fig. 48, the CMA activity of the sera of turbot vaccinated with gli-GPI was the highest and significantly higher than that generated by the sera vaccinated only with inactivated ciliates. This result is also repeated in the case of the determination of total antibodies by ELISA (Fig. 48).

	WGA	ConA	PNA	
Con GPI con SR	0,946	0,209	0,7	450
Con GPI con SR	0,661	0,245	0,579	450
Con GPI sin SR	0,085	0,069	0,308	450
Con GPI sin SR	0,121	0,09	0,299	450

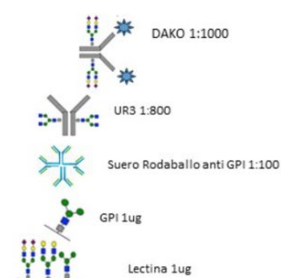


Table 19. ELISA-capture of the gli-GPI of *P. dicentrarchi* with the lectins WGA (*Triticum vulgaris* lectin), ConA (*Canavalia ensiformis* lectin) and PNA (*Arachis hypogaea* lectin). UR3: turbot anti-Ig monoclonal antibody; DAKO: anti-mouse Ig rabbit antibody conjugated with peroxidase. SR: immunized turbot serum.

GPI-coupled proteins are captured by the WGA lectin which has an affinity for N-acetyl- β -D-glucosaminil residues and N-acetyl- β -D-glucosamine oligomers, by the ConA lectin, which has an affinity for α -D-mannosyl and α -D-glucosyl terminal residues, and by PNA, which has specificity for β residues of -D-Gal(1-3)-D-galNAc (Table 19). The binding of GPI-coupled proteins by lectins indicates that they are ciliate membrane glycoproteins.

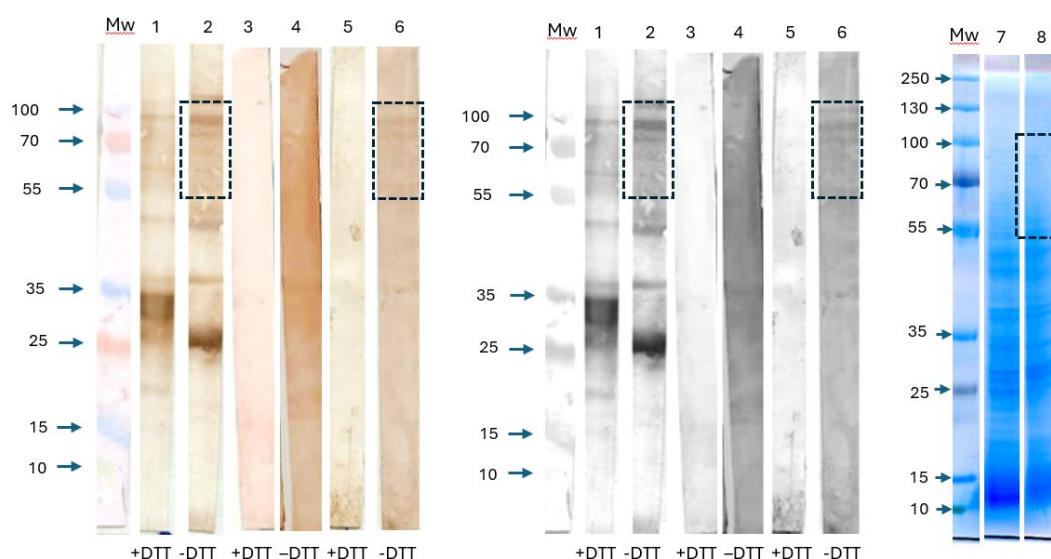


Figure 49. Effect of the reduction of disulfide bonds on antigenicity in turbot and mice of gli-GPI determined by Western-blot analysis. lanes1-2: inactivated a-ciliates (mouse); lanes 3-4: inactivated a-ciliates (turbot); lanes 5-6: a-GPI (turbot); lane 7: SDS-PAGE GPI reduced; lane 8: SDS-PAGE GPI not reduced. Mw: Molecular weight markers (kDa). antigen: GPI-S6 treated (reducing buffer) and untreated (non-reducing buffer) with DTT (+/-DTT). Inactivated a-ciliated mouse serum: 1:500. Inactivated a-ciliated turbot serum and a-GPI: 1:50.

Finally, we have conducted a study on the immunogenicity of gli-GPIs. As can be seen in Fig. 49, while the antibodies produced in mice vaccinated with inactivated ciliates recognize several polypeptides present in the gli-GPIs under both reducing and non-reducing conditions of these proteins, turbot; however, the antibodies produced by turbot vaccinated with gli-GPI only recognize unreduced proteins and this recognition occurs mainly in proteins with a size range of 55-100 kD (Fig. 49). Currently, we are carrying out an identification of these proteins through mass spectrometry analysis and we are also developing an experiment to determine the level of protection of these gli-GPI in turbot by vaccination in fish farms and subsequent experimental infection.

Objective 4.- To evaluate the immune response generated by the vaccine.

Evaluation of the immune response generated by the vaccine against *P. dicentrarchi*

The analysis of the immune response was carried out in turbot weighing 60 g, which were immunized with different antigens from *P. dicentrarchi*, or with other antigens, as a comparison. To determine antibody (IgM) levels, serum (at 30 and 60 days after the first dose) and peritoneal fluid (at 7, 14 and 21 days after the first dose) were obtained. To study the immune response in the spleen and in the clusters of cells and vaccine that are generated in the peritoneal cavity, samples were obtained at 4, 7, 14, 21 and 30 days after the first dose, and at 7, 14 and 21 days after the second dose. Cell populations (B IgM+ cells, B IgT+ cells, and CD4-1 and CD4-2 cells) were studied by immunofluorescence and *in situ* hybridization.

Immunization of turbot with *Philasterides dicentrarchi* antigens and other antigens, used comparatively, generates the production of specific IgM against these antigens in the peritoneal cavity and in the blood

We have analysed the levels of antibodies in the blood and peritoneal fluid of turbot vaccinated with one or two doses of vaccine. For comparison purposes, we use three antigens; *Philasterides dicentrarchi* (whole ciliates, as described in the project), phycocorithrin and NP-KLH (4-hydroxy-3-nitrophenylacetyl (as hapten) and limpet haemocyanin); the latter two antigens have been used as a model in numerous immunisation studies in fish and mammals. We observed a significant increase in serum specific IgM levels 14 days after the administration of the first dose (Fig. 1), obtaining results similar to those described in other immunization studies in fish (Esteve-Gassent et al., 2004; doi: 10.1016/S1050-4648(03)00036-6. The presence of IgM in serum in the groups immunized with the different antigens indicates that the immunization has worked correctly, and that we can analyze the changes in the spleen cell populations belonging to these groups of fish. In the case of peritoneal fluid, we observed an increase in specific IgM levels at 7 days (Fig. 2). Although there are very few studies on the dynamics of IgM production in the peritoneal cavity after immunization by this route, it has been observed that B cells migrate rapidly to the cavity after immunization, with a significant increase from day 6 of the first dose (Castro et al., 2017; doi: 10.1016/j.dci.2017.01.012; Granja and Tafalla, 2019; doi: 10.1016/j.fsi.2017.10.003); indicating a rapid adaptive response at the immunization site. Antibody levels in the cavity increase over time, reflecting a response like that obtained in serum. The studies carried out in this project, by immunofluorescence and *in situ* hybridization, on the cell and vaccine clusters generated in the peritoneal cavity of vaccinated turbot show abundant IgM+ and IgT+ B cells in these clusters a few days after immunization. We do not know if the Igs that appear in the cavity come from these cells, but given their abundance in the cavity it is very likely that they come, at least in part, from the B cells present in the clusters. We do not know the origin of these B lymphocytes, although judging by the intense proliferation of these cells in the spleen, as we will see later in memory, it is possible that this is their origin. On the other hand, Granja and Tafalla, 2019 (doi: 10.1016/j.fsi.2017.10.003) describe two populations of IgM cells in the peritoneum of rainbow trout; these two populations could be equivalent to B1 and B2 lymphocytes, described in the mammalian peritoneal cavity; B1 cells are predominant, which produce polyspecific antibodies. Whether a part of the IgM that we detect in the cavity is produced by this type of lymphocytes is something that needs to be investigated to better understand how this response occurs.

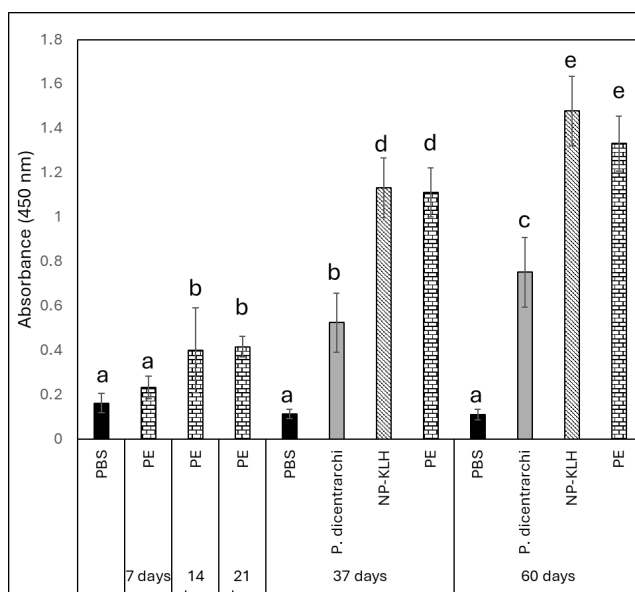


Fig. 1. IgM levels (measured as absorbance) in immunized turbot serum at days 0 and 30. Specific IgM against the antigens used can be detected from 14 days onwards, and their concentration is increased up to 60 days. Bars with different letters mean statistically different groups. The groups used were PBS, PE (phycoerythrin), *P. dicentrarchi* and NP-KLH. The fish were immunized at days 0 and 30.

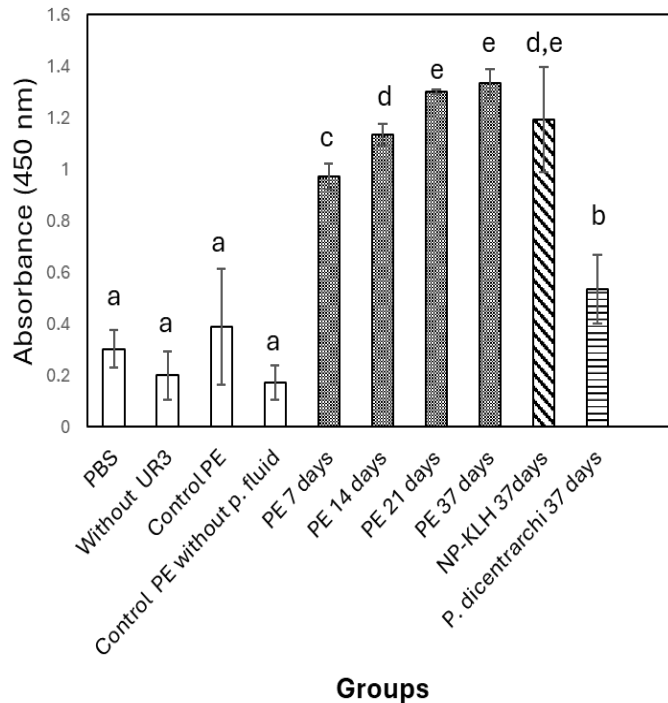


Fig. 2. IgM levels (measured as absorbance) in the peritoneal fluid of immunized turbot at days 0 and 30. Specific IgM against the antigens used can be detected from 7 days, and their concentration is increased up to 37 days. Bars with different letters mean statistically different groups. The groups used were PBS, PE (phycoerythrin), *P. dicentrarchi* and NP-KLH. The fish were immunized at days 0 and 30.

Intraperitoneal immunization with *P. dicentrarchi* antigens induced an increase in IgM+ and IgT+ B lymphocytes in the cell clusters and vaccine generated in the peritoneal cavity

As previously mentioned, intraperitoneal immunization generates the formation of vaccine cell clusters that adhere to the viscera or peritoneal wall. We have studied the populations of IgM+ and IgT+ B lymphocytes. In addition, using anti-PCNA antibodies, we evaluated whether B lymphocytes proliferated in the cavity. From day 4, we observed the presence of IgM+ B cells, whose number increases over time. Within the B IgM+ cells, we have observed cells with intense labelling and cells with weaker labelling, similar to that described in trout (Granja and Tafalla, 2019; doi: 10.1016/j.fsi.2017.10.003) (Fig. 3). These B cells appear scattered in the cluster of cells and vaccine, at a much lower density than that observed in the spleen. However, although the density is low, the clusters of cells and vaccine are spread throughout the cavity so the final number of B cells in the cavity can be very high, although it is difficult to determine by the distribution of these clusters. IgT+ B cells are very scarce in the first two weeks, but their number also increases over time. Some of the IgM+ and IgT+ B cells are PCNA+, indicating that they are proliferating (Fig. 4A, B).

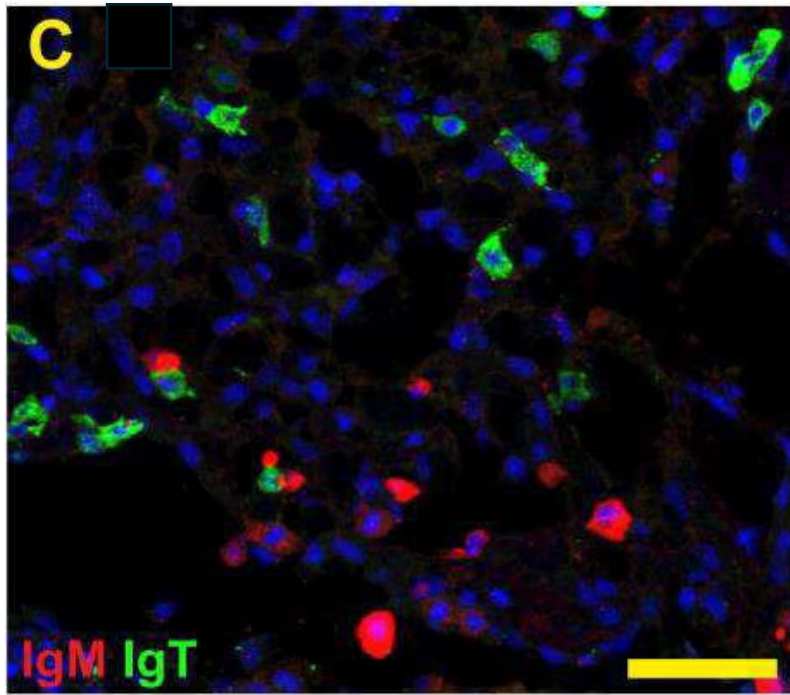


Fig. 3. Immunofluorescence image of a cluster of cells and vaccine in turbot vaccinated with *P. dicentrarchi* (day 60), showing IgM+ (red) and IgT+ B lymphocytes (green). Scale: 100 μ m.

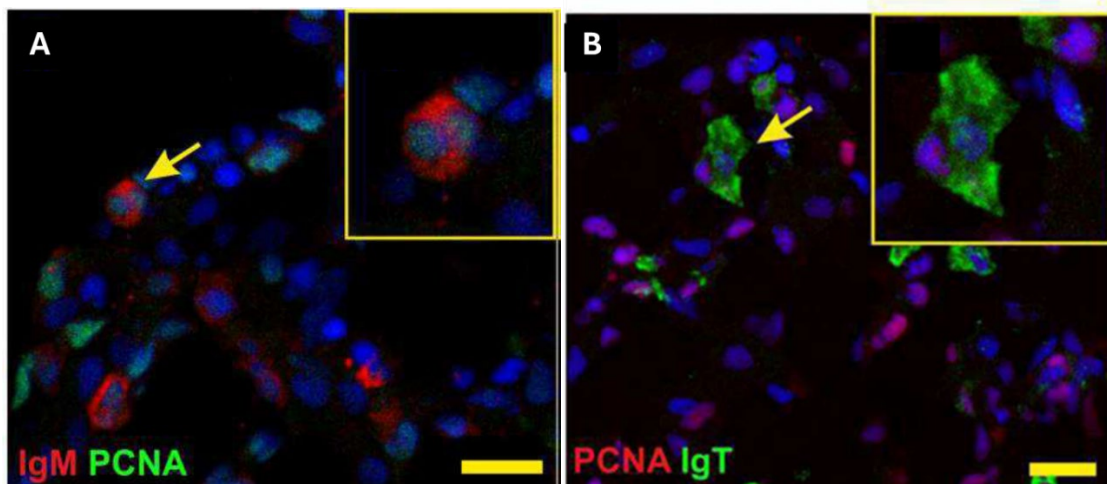


Fig. 4. A) Immunofluorescence image of a cluster of cells and vaccine in turbot vaccinated with *P. dicentrarchi* (day 60), showing B IgM+ lymphocytes (red cytoplasm) in division (PCNA+, in green). B) IgT+ lymphocytes (green cytoplasm), PCNA+ (purple). Scale: 10 μ m.

Immunization of turbot intraperitoneally generates important changes in the spleen, resulting in the transport of antigens to this organ and a marked increase in the populations of B lymphocytes (IgM+ and IgT+) and cells that express *cd4-1* and *cd4-2*.

Turbot has two *cd4* genes: *cd4-1* and *cd4-2*

Prior to the analysis of cell populations expressing *cd4* in vaccinated and unvaccinated fish, we have identified and characterized *cd4* genes in turbot. *Cd4-1* has a coding region (cfs) of 1410 nucleotides, 469 aa, and *cd4-2* has a cfs of 1080 nucleotides, 359 aa. When comparing the amino acid sequence with those of humans, chicken, frog, zebrafish and the spotted catan (*Lepisosteus oculatus*), we observe that all CD4 and CD4-1 proteins possess four immunoglobulin domains. CD4-2 of the spotted catan and zebrafish have three domains, while that of turbot contains only two (Fig. 5). Phylogenetic analysis shows that CD4-1 and CD4-2 form clades with homologous proteins in other fish species, while other vertebrates, which possess only CD4, form a separate clade. However, *cd4* and *cd4-2* have a more recent common ancestor than *cd4-1* (Fig. 6). We have also analyzed synteny, finding that *cd4-1* and *cd4-2* appear together on the same chromosome and share adjacent genes with other fish species and with *cd4* from other vertebrates. These data suggest that turbot possesses two *cd4* genes and that these, which we have used for in situ hybridization studies, are homologues of *cd4-1* and *cd4-2* from other fish species.

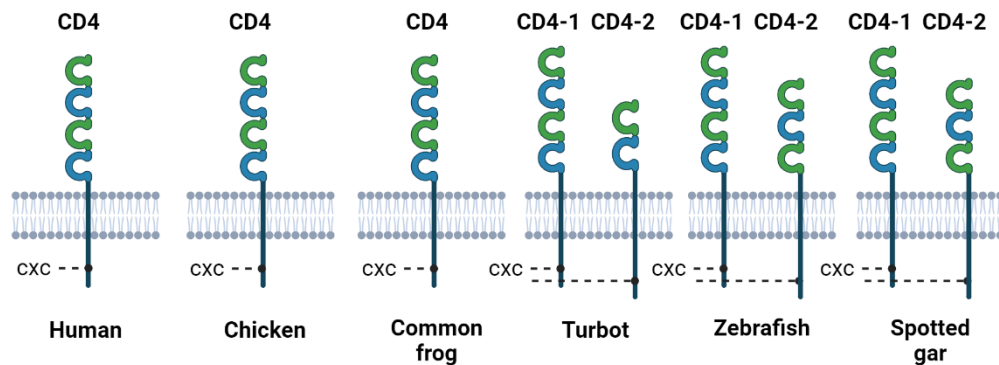


Fig. 5. Structure of CD4-1 and CD2 proteins from fish and CD4 from humans, chickens and frogs. All CD4 and CD4-1 proteins have four extracellular immunoglobulin-like domains, a transmembrane and an intracellular region. CD4-2 of the spotted gar and zebrafish contain three domains, while that of turbot has only two.

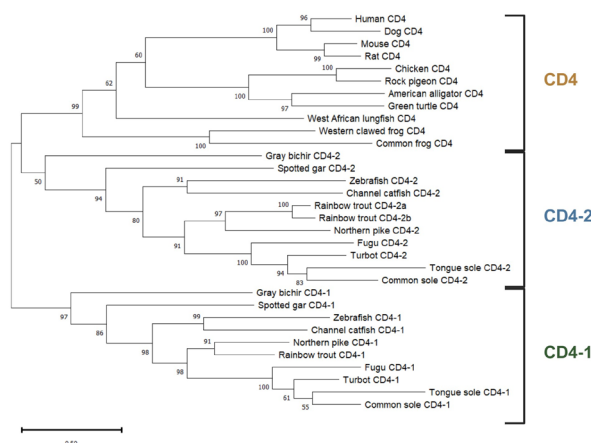


Fig. 6. Phylogenetic analysis of CD4, CD4-1 and CD4-2 of vertebrates, obtained using MEGA X with the maximum likelihood method. The tree is drawn to scale, with branch lengths measured in number of substitutions per site. The GeneBank access numbers for each stream are not included in the tree.

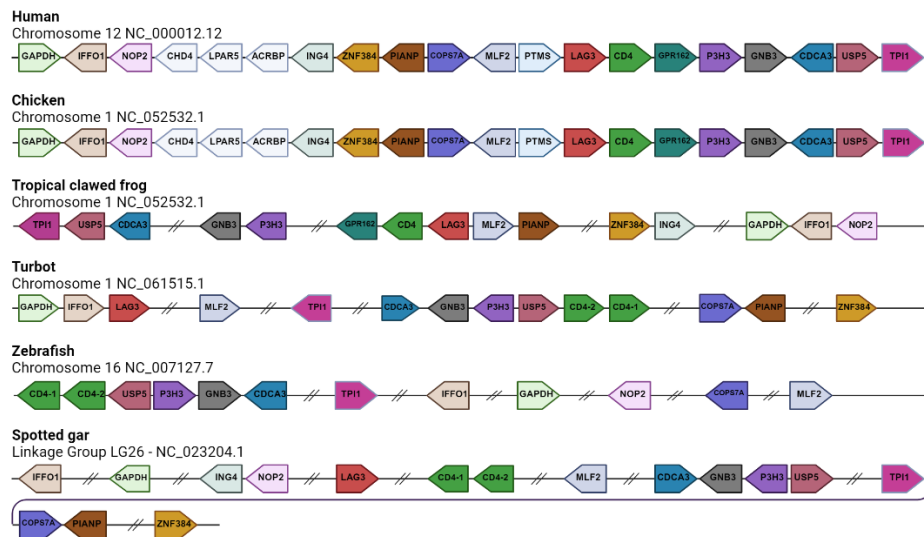


Fig. 7. Analysis of the synteny of the *cd4*, *cd-1* and *cd4-2* genes in several vertebrates. The data were obtained from Ensembl and Geneomius. The *CD4-1* and *CD4-2* genes of fish are located together on chromosome 1. The *cd4-1* and *cd4-2* turbot genes are flanked by several genes common to the three species analyzed. In the case of other vertebrates, these genes are also located in a position close to *cd4*. Double bars indicate the presence of other non-homologous genes found at the human locus GAPDH to TP11. The arrows represent the direction of gene transcription.

Unimmunized fish have underdeveloped centers of melanomacrophages (MMC) and associated lymphoid tissue (SLA)

IgM⁺ and IgT⁺ B cell populations in the spleen of control fish were analyzed by immunofluorescence and *in situ* hybridization. Cells expressing *cd4-1* and *cd4-2* were identified by *in situ* hybridization. Cell proliferation was analyzed using an anti-PCNA antibody. The white pulp was poorly developed in the spleen of juvenile non-immunized turbot (60 g), although lymphoid cells were identified in the wall of the blood vessels and associated with melanomacrophages centres (MMC).

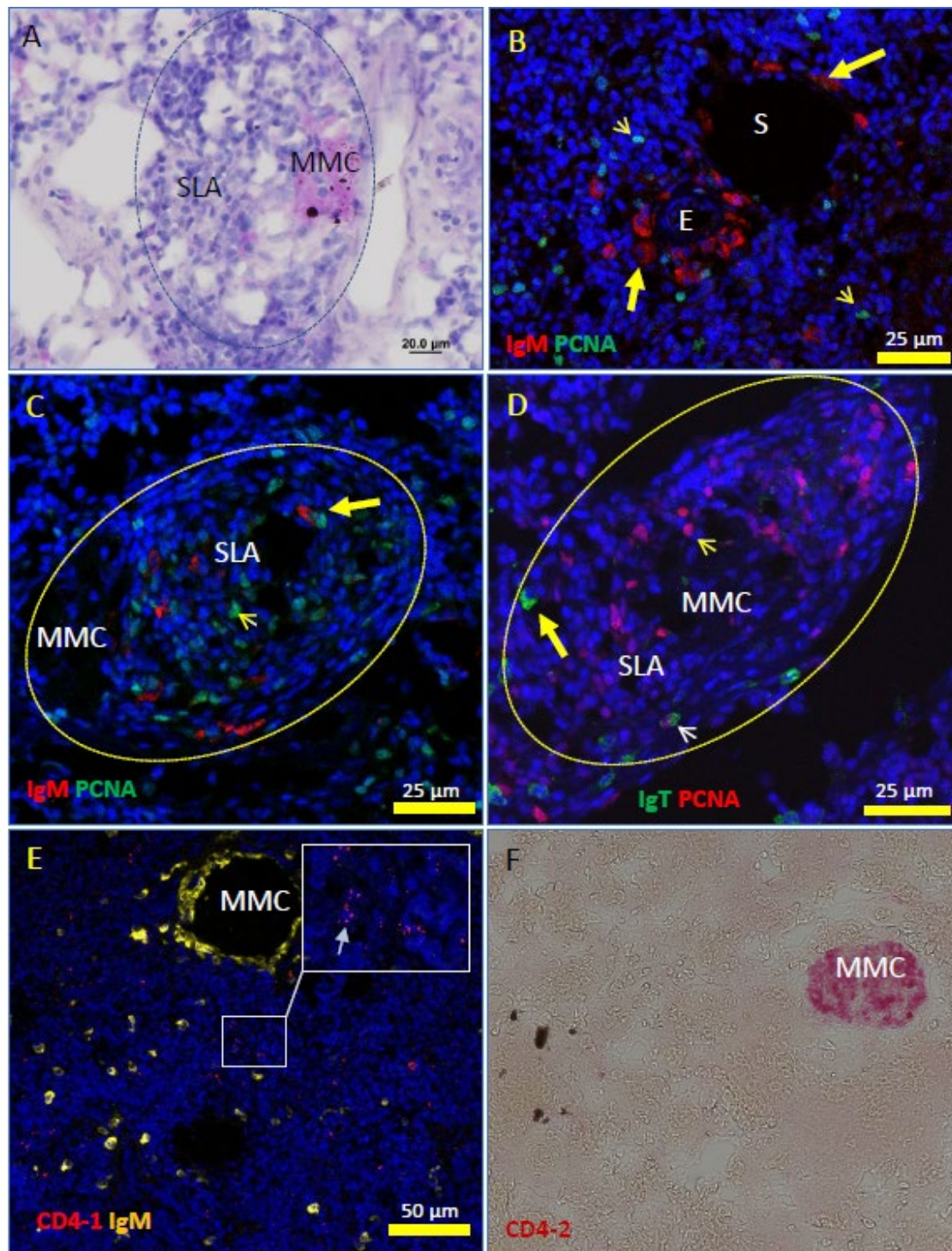


Fig. 8. Unimmunized fish spleen. A) Micrograph of a histological section stained with hematoxylin/periodic Schiff's acid (PAS) showing a center of melanomacrophages (MMC) and a lymphoid aggregate associated with MMC (SLA). The blue ellipse delimits the area occupied by both structures. Melanomacrophages contain PAS-positive material in the cytoplasm. B-D) Double-labeled immunofluorescence and confocal microscopy of spleen sections. The cores (blue) were stained with DAPI. B) IgM⁺ cells (large yellow arrow) can be observed on the walls of an ellipsoid and on a sinusoid. Very few PCNA⁺ (small arrow) cells were observed and the IgM⁺ B cells were mainly PCNA⁻. C) MMCs and adjacent lymphoid tissue (SLA), which contains several IgM⁺ and PCNA⁺ cells (large yellow arrow). The yellow ellipse delimits the area occupied by the MMCs and the SLA. The small arrow shows the nucleus of an IgM-PCNA⁺ cell. D) MMC and

SLAs containing very few IgT+ cells (large yellow arrow) and one IgT-PCNA+ cell (small yellow arrow). IgT+PCNA+ cells (small white arrow) are also shown. E) *In situ* hybridization in turbot spleen. Igm-expressing B cells appear surrounding an MMC and scattered in the red pulp. Cells expressing *cd4-1* are scattered throughout the spleen (arrow, insert), although they are rare. The cores (blue) were stained with DAPI. F) *In situ* hybridization of the turbot spleen. Cells that express *cd4-2* appear exclusively in MMCs.

MMCs are rare, small, and contain PAS-positive melanomacrophages, but the number of melanin granules is generally very low (Fig. 8A). Some of the MMCs had a small group of lymphoid cells adjacent to them (Figs. 8A-D). Clusters of IgM+ cells were found along the periarteriolar areas and in the ellipsoidal walls (Fig. 8B). They were also found near the thin walls of the venous system, especially those located in the centre of the organ. The highest concentration of IgM+ cells was found in the lymphoid aggregates adjacent to the MMCs (SLAs), and the cells are scattered or clustered, but in very low numbers (Fig. 8C). IgM+ cells were also found scattered in other areas of the spleen. IgM+PCNA+ cells were present in some SLAs, indicating some cell proliferation activity (Fig. 8C).

IgT+ cells are very scarce. Occasionally, IgT+ cells were observed in very low numbers in SLAs (Fig. 8D) and scattered in very low numbers in other parts of the spleen, usually near the walls of blood vessels. Like IgM+ cells, a small proportion of IgT+ cells are also PCNA+ and were most found in SLAs (Fig. 8D).

In the spleen of unimmunized turbot, cells containing *cd4-1* transcripts were scarce and with very low transcripts per cell. In this group of fish, cells containing *cd4-1* transcripts were distributed in the red pulp of the spleen. Similarly, cells with *cd4-1* transcripts were occasionally found in SLAs, which were poorly developed (Fig. 8E). In this group of fish, *cd4-2* transcripts were only found in the melanomacrophages of the MMCs (Fig. 8F).

Melanoma Centers (MMCs) and Associated Lymphoid Aggregates (SLAs) of the Spleen Undergo Major Changes in Immunized Fish

The most evident morphological changes in the spleen in response to vaccination were found in MMCs and SLAs, with a notable increase in cell size and number in both (Fig. 9A). At days 4 and 7, a marked increase in the size of MMCs and SLAs was observed in vaccinated fish. Although the response was heterogeneous, some of the SLAs were several times larger than in control fish on days 4 and 7 (Figs. 9B, C, D). On days 0 and 60 (30 days after the booster dose), the mean MMC size was 29.02 ± 7.5 and 63.58 ± 12.3 μm , respectively, in fish immunized with *P. dicentrarchi*. The MMCs of the vaccinated fish were less compact and more disorganized than in the controls on days 4, 7 and 37. The MMCs contained PAS-positive material and, at those sampling times, cells with similar morphology and dyeing characteristics were also found in the SLAs. MMCs and SLAs appear to share the same compartment, as a discontinuous layer of flattened cells often appears surrounding these structures. These cells appear to separate the MMCs and SLAs from the red pulp (Figs. 9C, D). Over time, the CMMs became more compact forming an oval to rounded structure (Figs. 9E, F).

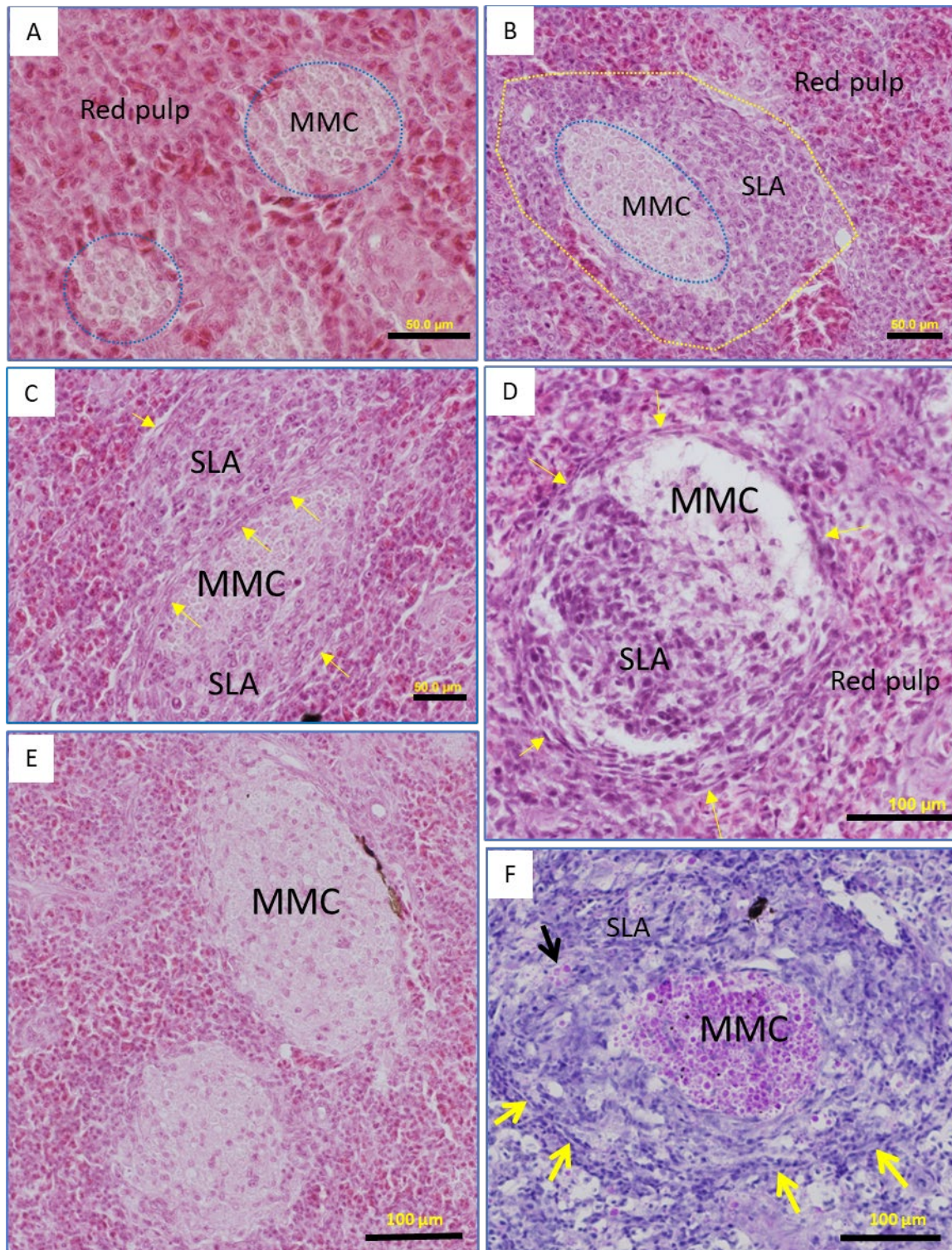


Fig. 9. Micrographs of spleen sections stained with hematoxylin/eosin (A-E) or hematoxylin/periodic Schiff's acid (PAS) (F). A) Spleen of an unimmunized fish showing small centers of melanomacrophages (MMCs), delimited by a blue circle, and inconspicuous SLAs. B) Spleen of a fish sampled on day 4 after vaccination with *P. dicentrarchi* and adjuvant showing a large MMC (blue line) and a very well-developed SLA (delimited by a yellow line). C and D) Day 7 after vaccine administration. The MMCs and SLA are bounded by elongated cells that form a compartment (yellow arrows) separate from the red pulp. E) Day 21 after vaccination. Vaccinated fish showed large MMCs, but SLAs decreased in size, compared to spleens obtained on day 7. F) Fish immunized and then injected with *P. dicentrarchi* (15 days after injection) showing a

compact MMC with melanomacrophages containing PAS-positive material. The SLA, well developed in this group, contained cells with PAS-positive material, probably melanomacrophages (black arrow). Elongated cells were observed separating the MMC and SLA compartment from the red pulp (yellow arrows).

The antigen is found in cells isolated in SLAs and MMCs

The antigen used to immunize fish was found mainly in the MMCs at 4 and 7 dpi, and in numerous cells located in the SLAs, between lymphoid cells, although we could not identify the cell types that carried this antigen (Figs. 10A, B). Antigen-containing cells were also seen scattered in other parts of the spleen. The changes generated in MMCs and SLAs by vaccines containing *P. dicentrarchi*, NP-KLH or PE were apparently similar.

Vaccination induced a drastic increase in the number of IgM+ cells in SLAs

To assess the effects of vaccination of fish on the spleen, turbot was immunized intraperitoneally with a vaccine containing the ciliated parasite *Philasterides dicentrarchi*, with NP-KLH or with PE and adjuvant. Samples were obtained at 4, 7, 14 or 21 days after the injection or at 7, 14, 21 and 30 days after the second immunization. In addition, some fish belonging to the group that received two doses of the *P. dicentrarchi* vaccine were experimentally infected with ciliate and sampled on day 14. The major vaccine-induced changes in the turbot spleen affected MMCs and SLAs. The changes in SLAs came very quickly. At day 4, SLAs showed a noticeable increase in the number of IgM+ cells, and most of them were IgM+PCNA+, indicating that they were actively proliferating (Fig. 10C). A similar image was observed on day 7, with SLAs containing many IgM+PCNA+ B cells (Fig. 10D). MMCs tend to be oval or rounded in shape, but lack B cells, although some IgM+ was often found surrounding the centre, especially the area in contact with SLAs. In SLAs, we do not find a clear distribution in light or dark areas and the proliferation of B cells seems to occur throughout the structure. IgM+ B cells were also found in other parts of the spleen, normally surrounding blood vessels like ellipsoids, but in small numbers and some of them were also in mitosis at day 7. The SLAs also contained IgM-PCNA+ cells, indicating that other cell types are proliferating as well, although the cell types undergoing this proliferation were not identified. IgM-PCNA+ cells were also found scattered in other areas of the spleen but appeared in clusters on the walls of large blood vessels. On day 7, a few cells containing *IgT* transcripts or IgT+ cells were also found among the cells containing IgM+-positive transcripts or surrounding the SLA zones, but in very low numbers (Fig. 10E). The levels of transcripts in these IgT cells were much lower than in IgM cells, and neither by immunofluorescence nor *in situ* hybridization was it observed that the *IgT* and *IgM* transcripts colocalized in the same cell. Cells containing *cd4-1* transcripts accumulated in the SLA zones at 4 and 7 dpi, usually mixed with B cells (Fig. 10F). It is unknown whether they migrate from surrounding areas or proliferate in SLAs, although a high level of cell proliferation was observed in SLAs.

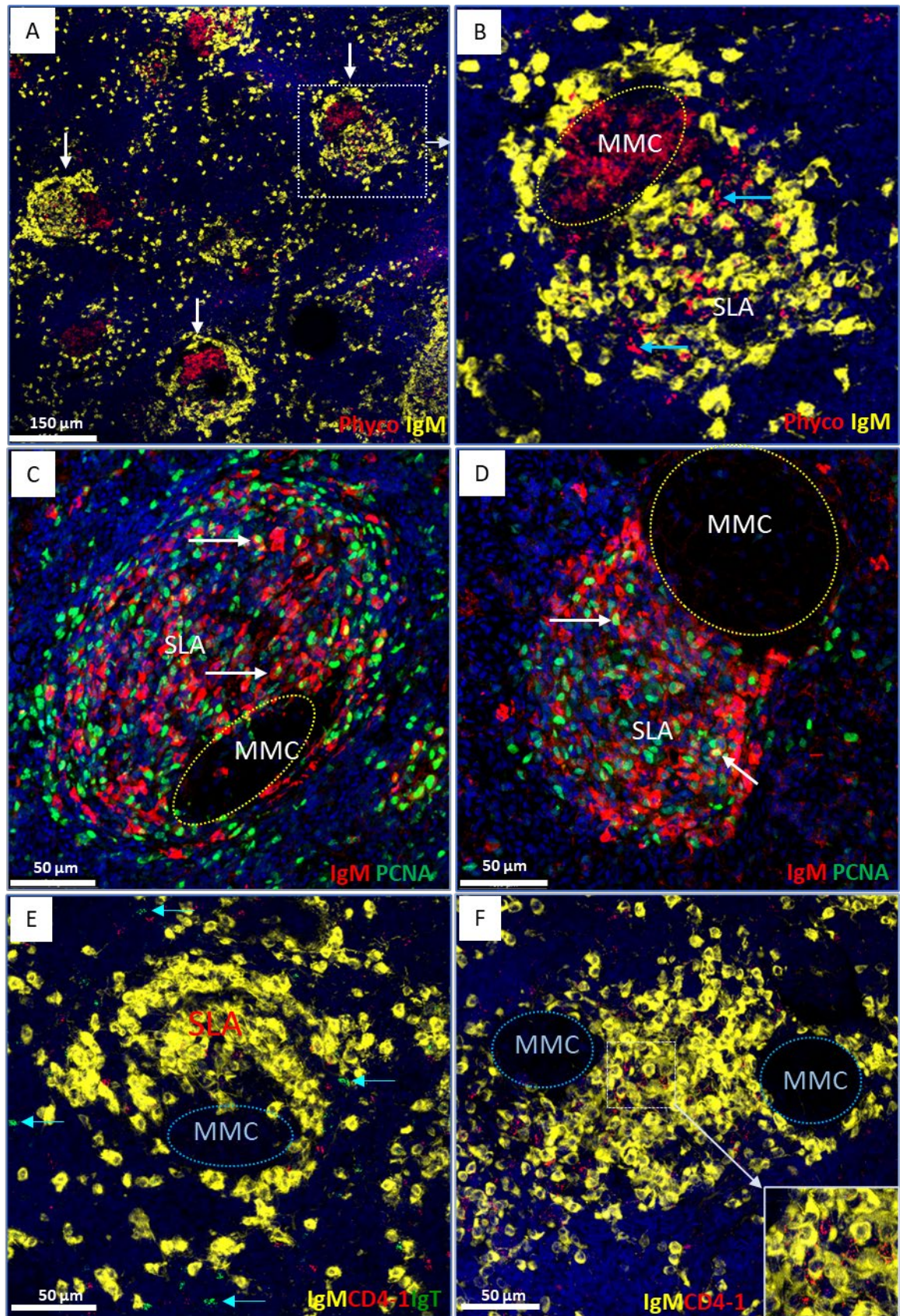


Fig. 10. Confocal microscopy. A, B) In situ hybridization images in the spleen of turbot injected with PE and adjuvant and sampled on day 7 p.i. Phycoerythrin occurs in MMC (white arrow) and SLA (blue arrows) melanomacrophages. IgM-expressing cells (yellow) appear mainly in SLAs but were also observed scattered in other parts of the

spleen. C, D) Immunofluorescence images with double labeling of IgM (red) and PCNA (blue) of fish vaccinated with NP-KLH and adjuvant. On days 4 (C) and 7 (D) p.i., the spleen showed highly developed SLAs, containing numerous IgM⁺ B lymphocytes and many of them were also PCNA⁺, indicating that they were proliferating (white arrows). The MMCs are delimited by a dashed yellow line. E, F) *In situ* hybridization in the spleen of fish immunized with NP-KLH. The *igm*, *cd4-1*, and *igt* transcripts appear in yellow, red, or green, respectively. On day 7 p.i., numerous cells containing *igm* transcripts were mainly located in SLAs. Cells with *CD4-1* transcripts are abundant and mixed with IgM-containing cells (see Figure F, insert). Some cells expressing *igt* were also found in peripheral areas, but in small numbers (blue arrows). In all the figures, the cores were stained with DAPI.

In addition to their location in the MMCs, cells containing *cd4-2* transcripts were found in very low numbers in the SLAs, and are probably MM. The size of the SLAs decreased considerably over time, with 76.6 ± 21.3 and 16.6 ± 4.1 IgM⁺ cells per SLA being found at 7 and 21 days after booster injection (Fig. 12A). The number of IgM⁺ cells also decreased over time in SLAs, and IgM⁺PCNA⁺ cells were also scarcer (Fig. 11B). IgT⁺ cells were also scarce and could be found mixed with IgM⁺ cells in the SLAs or in peripheral regions (Fig. 11B). Cells containing *CD4-1* transcripts were not very abundant and tended to appear mixed with B cells (Fig. 11B).

Relevant morphological changes were also observed in the spleen of fish sampled at days 7, 14 and 21 after the booster dose. As on day 7 after the first dose, a clear increase in the complexity and size of MMCs and SLAs was observed, compared to those of fish injected with PBS (Figs. 12C, D). Interestingly, using both immunofluorescence and *in situ* hybridization, we have also found IgT⁺ cells in some of the SLAs (Fig. 11E, F). Cells containing *cd4-1* transcripts were abundant and mixed with IgM⁺ cells in the SLAs or dispersed in other areas of the spleen (Fig. 11E). SLAs decreased considerably in size over time, decreasing the layers of cells surrounding the MMCs, being especially evident at 21 days after immunization.

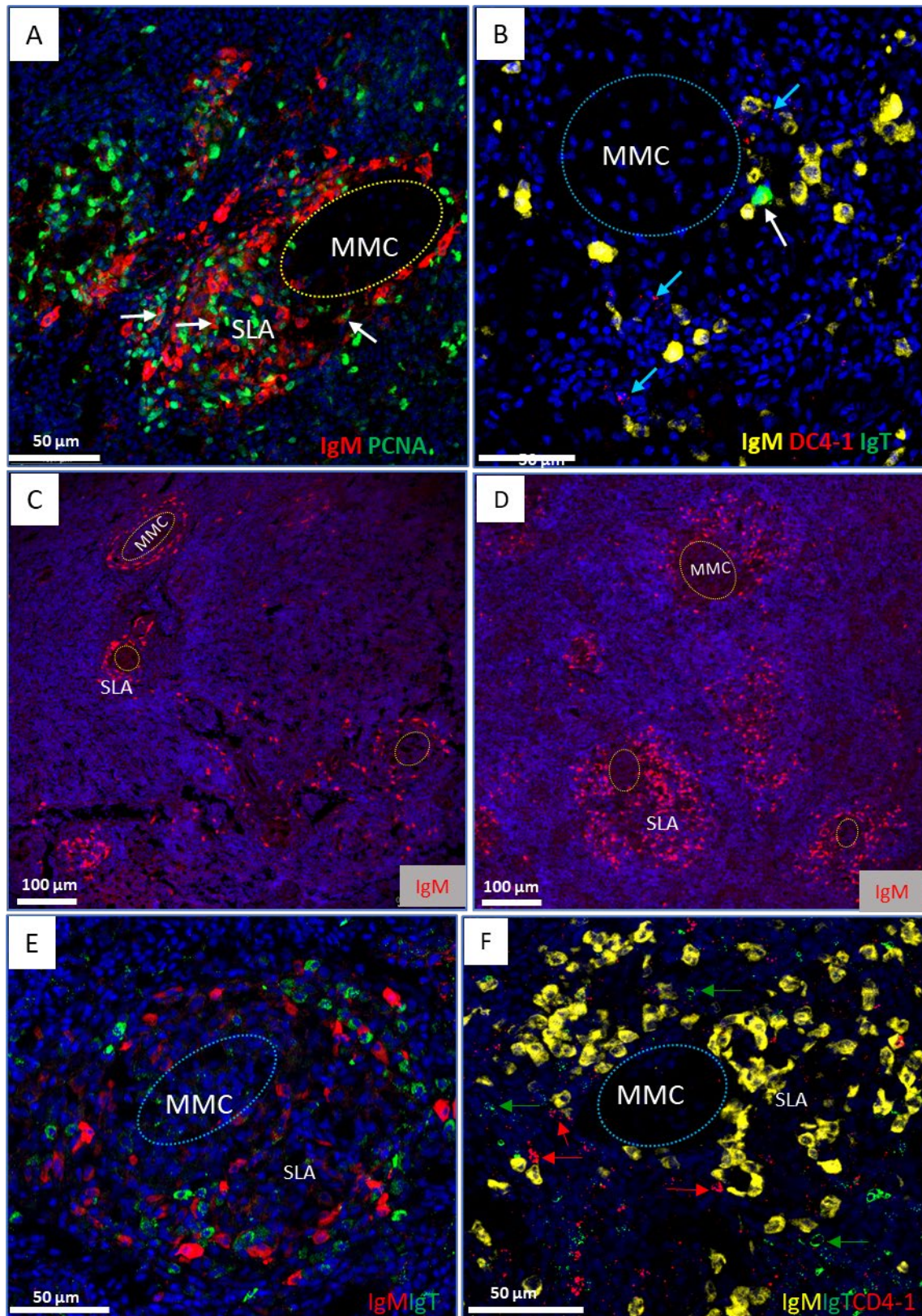


Fig. 11. Confocal microscopy. Immunofluorescence (A, C, D, E) and *in situ* hybridization (B, F) imaging of spleen sections. A) Fish immunized with NP-KLH and sampled on day 14 showed an SLA containing numerous IgM+ (red) cells, some of them PCNA+ (white arrows). On day 14, SLAs are typically smaller than on day 7. B) Similarly, SLAs from the same group of fish showed a lower number of B cells expressing IgM (yellow) and CD4-1 (blue arrows) than on day 7. IgT-expressing cells (white arrows) contained fewer

IgM-expressing B cells (yellow). IgT-expressing cells (white arrows) mixed with IgM cells were also observed. C, D) On day 37 after immunization with *P. dicentrarchi* (7 days after the second dose of vaccine), an increase in the size of SLAs and the number of IgM+ B cells in this structure and dispersed by the spleen was again observed, with notable differences with the PBS group. E) In addition to IgM+, we found a notable increase in the number of IgT+ cells in SLAs, which are usually mixed with IgM+ cells. F) On day 37, cells with *igm* transcripts were abundant in the SLA, usually mixed with cells containing *cd4-1* transcripts (red arrows). As in immunofluorescence, cells with *igt* transcripts (green arrows) were also found in the SLA of this group of fish. In all figures, the cores were stained with DAPI, and the dotted circles delimited the CMM. In all the figures, the cores were stained with DAPI.

At 14 days, B cells containing *IgM* transcripts were abundant surrounding the ellipsoids and were scattered in other areas of the spleen (Fig. 12A, B). Some of the SLAs contained IgT+ B cells and some of them were PCNA+, indicating that they are proliferating (Fig. 12C). *CD4-1* cells were scattered, often mixed with B cells containing *igm* transcripts (Fig. 12D). By day 60, most CMMs contained a compact group of MMs. The SLAs of immunized fish were higher than those of fish injected with PBS. The SLA areas were rich in IgM+ B cells, although IgT+ cells were also observed and, occasionally, IgT+ B cells were more abundant than IgM cells in some of the SLAs (Fig. 12E). IgM+ B and IgT+ cells were also observed scattered throughout the spleen, although the former were much more abundant than the latter. Finally, we studied the spleen of vaccinated fish that were infected with *P. dicentrarchi*. The fish that survived the infection showed an intense proliferation of cells distributed throughout the spleen, but which mainly affected SLA cells (Fig. 12F). Unsurprisingly, many of the IgM+ B cells were also PCNA+ (Fig. 13A). However, we have also observed IgT+PCNA+ B cells in SLAs, suggesting that IgT cell proliferation may also occur in these areas. Using double labeling with anti-IgM and IgT or by *in situ* hybridization, we found that most SLAs contained almost exclusively IgM+ B cells, but some contained a mixture of IgM+ and IgT+ B cells (Figs. 13B-D).

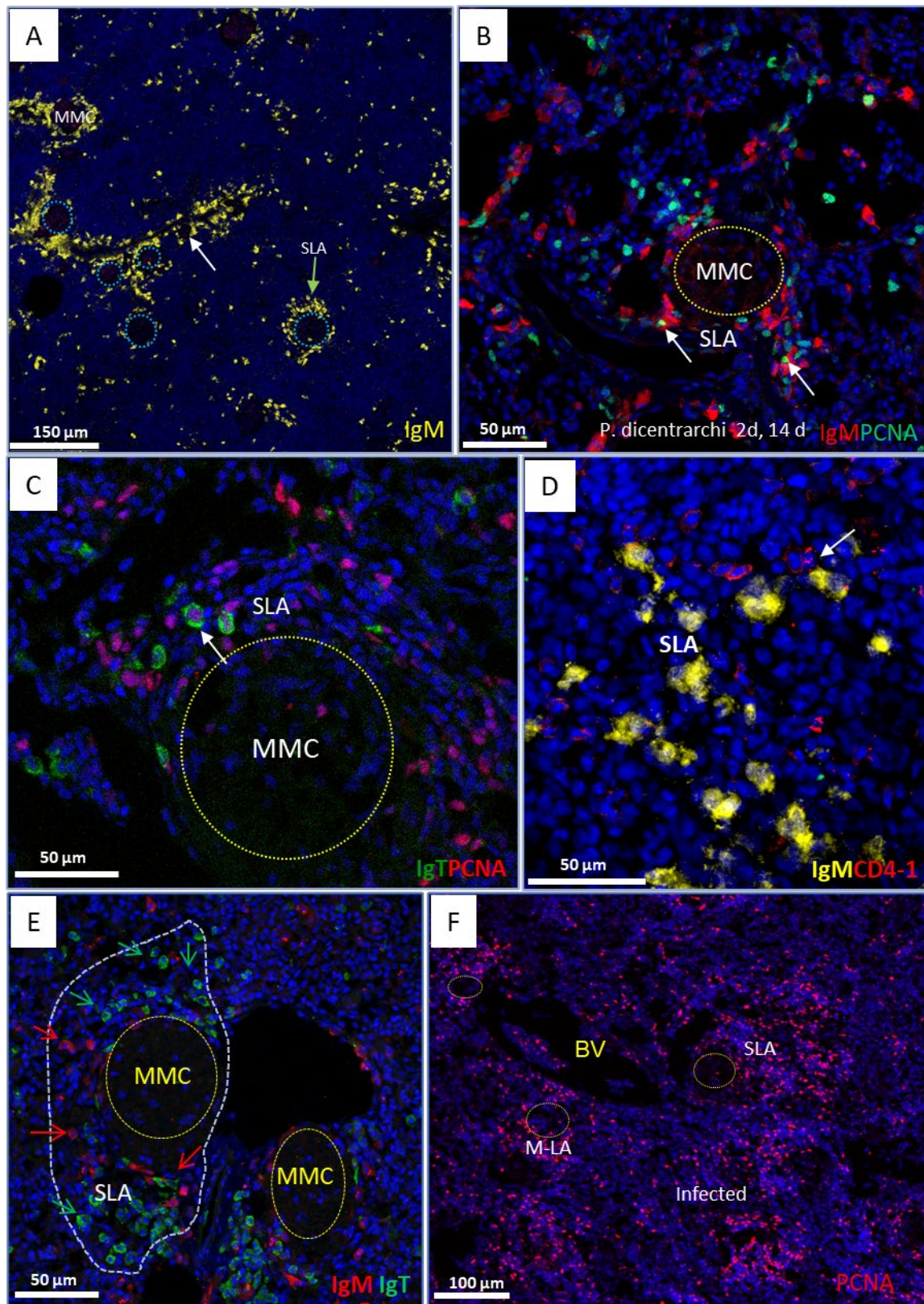


Fig. 12. Confocal microscopy. In situ hybridization (A, D) and immunofluorescence (B, C, E, F) images of spleen sections of fish sampled on day 14 after administration of the second dose of vaccine (A-D), on day 30 (E), or of fish recovered from infection (F). A) Turbot immunized with phycoerythrin. Cells containing IgM transcripts (yellow) appear in the SLA surrounding the melanomacrophages centers (blue circles), on the walls of the ellipsoids (white arrow), and scattered by the red pulp. B-E) Fish immunized with *P. dicentrarchi*. B) At this time of sampling, SLAs are less developed than at 4 or 7 days

after immunization. The figure shows some IgM+ B cells (red) surrounding the center of melanomacrophages (MMC, yellow circle) and some of them are PCNA+ (white arrow). C) The SLAs of the immunized contain some IgT+ B cells (green) and some of them are PCNA+, indicating that they are proliferating. The yellow circle shows the MMCs. D) Cells containing IgM (yellow) and *cd4-1* transcripts in an SLA, which appeared mixed with cells containing CD4-1 transcripts in an SLA. E) On day 30, some of the SLAs contained IgM+ cells (red arrows) mixed with IgT+ cells (green arrows). The fish recovered from the experimental infection, which had been previously vaccinated, showed a high degree of proliferating cells distributed throughout the spleen, and especially in the SLAs. The CMMs are outlined in yellow and the CMM+SLAs in white. In all the figures, the cores were stained with DAPI.

Using dual labeling with anti-IgM and IgT or using *in situ* hybridization, we found that most SLAs contained almost exclusively IgM+ B cells but a few of them contained a mixture of IgM and IgT B cells (Figs. 13B-D).

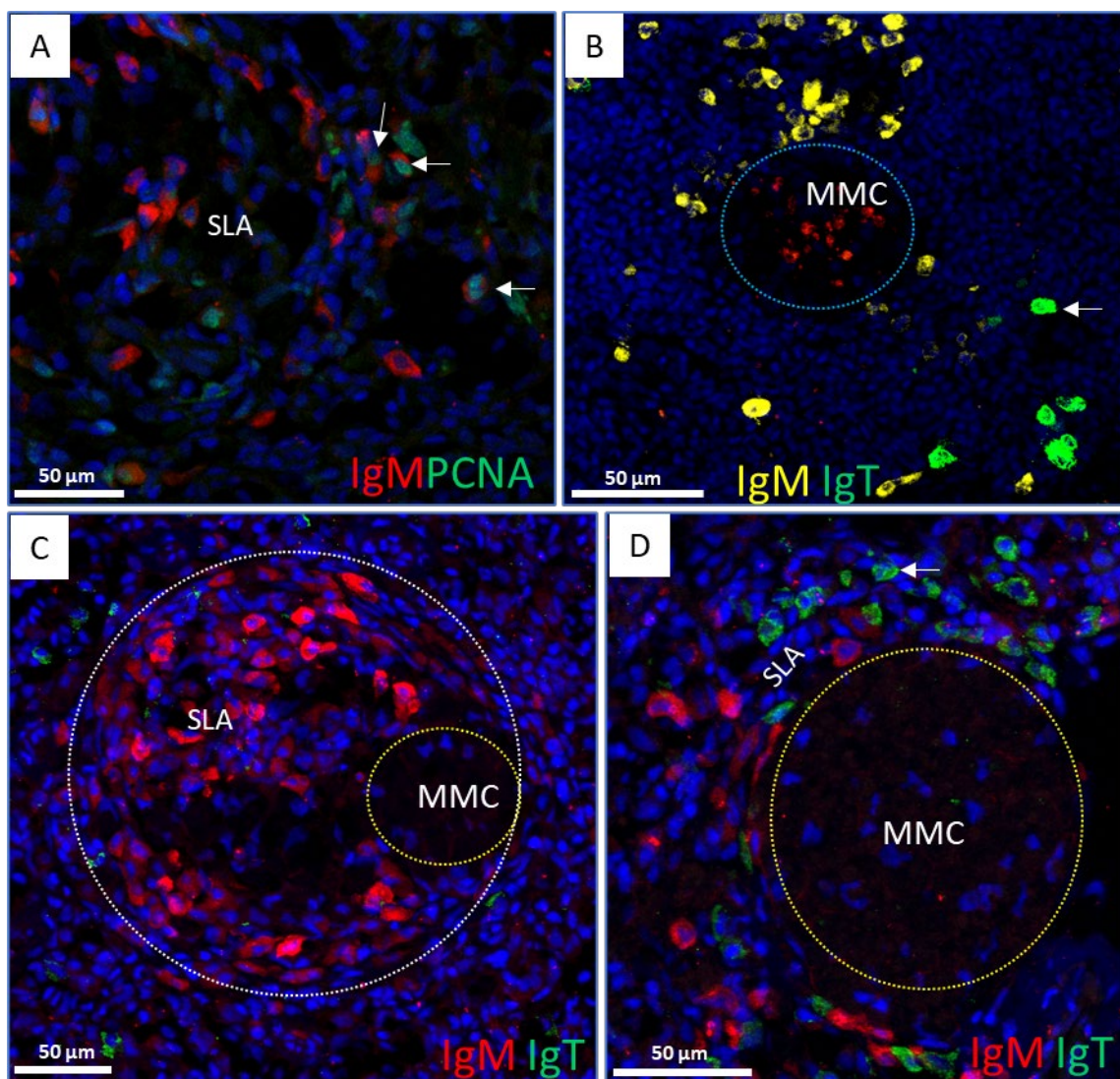


Fig. 13. Confocal microscopy. Immunofluorescence (A, C, D) and *in situ* hybridization (B) imaging of spleen sections of fish surviving experimental infection with *P. dicentrarchi* (day 14). These fish were previously immunized with two doses of a vaccine containing the parasite and infected on day 30 after the second dose. A) Lymphoid tissue associated with MMCs (SLAs) showing IgM+ B cells, some of them proliferating (white arrows). B) Section of the spleen showing cells containing *igm* (yellow) and *igt* (green),

white arrow) transcripts. In this case, both groups of cells were separated, but in other SLAs they appeared mixed. C) SLAs that contain numerous IgM+ B cells but lack IgT+ cells. The MMC is surrounded by a blue line and the MMC+SLAs are surrounded by a white line. D) SLA containing mixed IgM+ (red) and IgT+ B cells (green, white arrow). In all the figures, the cores were stained with DAPI.

CONCLUSIONS OF THE EXPERIMENTS CARRIED OUT IN THIS PROJECT

1.- In this Project we have identified and molecularly characterized 16 leishmanolisins (LSFs) that are expressed in *P. dicentrarchi*, all of them being glycoproteins.

P. dicentrarchi expresses 8 proteins that contain domains common to variable surface proteins (VSPs) characterized in other protozoa. Like LSFs, all identified VSPs are glycosylated.

3.- Both VSPs and LSFs are located on the surface of the ciliate and can be considered as relevant antigens when generating vaccines against this parasite; However, we have observed that there is a differential expression of these glycoproteins during infection, which makes it necessary to test them one by one, and this generates a very high number of combinations that makes it difficult to carry out all the required protection experiments, since it is necessary to know the previous result to design the new vaccination trial and this is obtained. at least every 3 months.

4.- The system of expression of recombinant proteins based on VSPs and LSFs through the secretion strategy in yeasts has been shown to be very inefficient. The intracellular and/or yeast membrane expression of recombinant proteins is a more practical strategy to achieve a vaccine because yeast expressing recombinant protein can also enhance its immunogenicity because it has an intrinsic adjuvant effect.

5.- To advance more quickly in the search for protective antigens, chimeric recombinant proteins have been generated consisting of peptides of VSPs and LSFs that have been used in the vaccination of fish. From a theoretical point of view, the use of chimeric proteins would have the advantage of testing, in a single vaccination, several VSPs and LSFs and this would allow us to have more complete information on which VSPs/LSFs are potentially involved in protection. Unfortunately, the protection results indicate that no significant protection is observed in fish vaccinated with these chimerical constructs versus controls. These results also highlight the complexity of designing proteins made up of peptides that may not contain the relevant epitopes in protection, even using bioinformatics tools for predicting antigenic peptides. In addition, in addition to the difficulties described, there is a lack of knowledge of the amount of protein needed for immunization, which should therefore also be optimized.

6.- In the protection experiments using the different vaccine formulations, in which the levels of cumulative mortality achieved after the experimental infection were determined, the results of the assays to determine the level of specific total antibodies generated by the infected fish and the results of the levels of antibodies involved in antibody-mediated complement cytotoxicity (AMC) were also included. In this regard, although there is usually a good correlation between the level of total antibodies and the protection obtained; however, the CMA trial generates results that predict with great accuracy those groups whose vaccination will generate the highest degree of protection in the fish.

7.- Vaccines formulated with peptides from all VSPs and conjugates to the limpet hemocyanin transporter protein (KLH) did not generate significant levels of antibodies against the total proteins of *P. dicentrarchi*, nor did they generate antibodies that increased the CMA against trophozoites, so we think that the strategy for the production of vaccines formed by synthetic peptides does not seem adequate to generate an effective vaccine against scuticociliatosis.

8.- Although yeasts could produce translational modifications at the level of glycosylation of proteins, this glycosylation may not coincide with the glycosylation presented by ciliate glycoproteins. In this regard, we have also generated recombinant proteins for

expression in expression systems in mammalian cells (specifically in human HEK293 cells). In this case, recombinant proteins that express VSP1 in the cell membrane of cells and a chimeric protein composed of two VSPs (1 and 8) that is produced intracellularly were tested. In both cases, although these recombinant proteins generated a high total antibody response against the parasite; however, the CMA response generated was not significantly different from the control, which indicates that these vaccines probably do not generate protection against *P. dicentrarchi* infection.

9.- The formulation of the vaccines with adjuvants allowed for veterinary use by parenteral route, as is the case of the Montanide ISA 763A VG oily adjuvant and the adjuvant in the form of sodium polyacrylate gel (Montanide gel 02 PR) generated a good total antibody and CMA response; however, the 02 PR gel adjuvant seems more suitable due to its ease of administration and apparent better assimilation by the fish, producing fewer side effects than the oily adjuvant.

10.- Since the VSPs and LSFs proteins are located on the cell surface and are most likely part of the glycoproteins coupled to the lipids of the cell membrane and, specifically, to the glycosyl-phosphatidylinositol (GPI) -gli-GPI-, we have developed a protocol for the extraction of these membrane glycoproteins by treatment with the enzyme phospholipase C. The presence of these glycoproteins in this antigen fraction is also confirmed by their recognition with lectins (e.g. WGA, ConA and PNA). After vaccination of turbot with vaccines containing GPI-coupled glycoproteins and Montanide gel adjuvant 02 PR, we have found that vaccinated fish generate very high levels of both total antibodies and antibodies involved in CMA, even in values higher than those induced by inactivated ciliates. On the other hand, the antibodies induced by gli-GPI in turbot are mostly of the conformational type that exclusively recognize epitopes on native glycoproteins of a size between 55-120 kDa. This last aspect could explain the difficulty in obtaining protective vaccines using recombinant proteins due to the more than probable inability to generate native proteins in the expression systems in yeasts and/or mammalian cells that have a conformation identical to ciliate proteins.

11.- Finally, from our point of view, it would be necessary to carry out an in-depth glycoproteomic study on these glycoproteins to determine their true nature and to verify the role, not only of the protein portion, but also that of their associated sugars on the protective response that would be vital to develop an effective vaccine against scuticociliatosis in the future. In this regard, we believe that we are close to achieving the goal of achieving such a vaccine to prevent and control scuticociliatosis.

12. At the immune level, the results of this work allow us to conclude that turbot presents lymphoid tissue microstructures associated with melanomacrophages (MMC) centres like those described in trout capable of processing the antigens from vaccinations.

13.- Turbot has associated lymphoid aggregates (SLAs) organized in the spleen. These SLAs contain B cells (IgM and IgT), CD4-1 and CD4-2 cells, cells with antigens, and cells with high proliferating activity, especially B cells, constituting a niche where activities such as those found in germinal centres can occur. Like germinal centres, turbot SLAs are transient structures, which can develop very quickly after immunization and, after reaching a certain size, gradually decrease.

On the other hand, we have observed a discontinuous layer of flattened cells surrounding the SLAs and MMCs, suggesting that these structures form a separate compartment from the red pulp.

15.- MMCs also increased in size after immunization and contain MM, which expresses *cd4-2*, with these antigens. However, it is necessary to establish the relevance of MMCs in the immune events that occurred in SLAs, as the former are surrounded by capsules in turbot and other fish species.

16.- Finally, IgT+ cells were also found in the turbot spleen and their presence in SLAs increased over time, suggesting that the spleen could also be a site of IgT production after immunization.