3. Detection of *Wolbachia* and *in silico* based structural and evolutionary analysis of *Wolbachia* Surface Protein (*wsp*) across *Trichogramma* sp.

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**Abstract**

*Wolbachia* surface protein (*wsp*) is a beta-barrel transmembrane structure which involves in host immune response, cell proliferation, pathogenicity and controlled programmed cell death. The protein having four extracellular loops containing hyper variable regions are separated by conserved regions. The *wsp* structure is homologous to *Neisseria* surface protein (Nsp A) and the recombination has a large impact on diversity of this protein including positive selection which is major role on protein evolution. The molecular mechanism through which *Wolbachia* induces various reproductive anomalies is unclear; a key feature observed for such anomalies might be because of *Wolbachia* undergoing extensive recombination. The specific amplification of *wsp* region enables the detection of *Wolbachia* presence in an organism. In this study, the *wsp* region was used to detect the presence of *Wolbachia* in *Trichogramma* and predicted the structural and functional variations in *wsp* sequences of *Wolbachia* present in *Trichogramma* sp.

**Key words**: *wsp*, OMP, Nsp A, Chelex

**3.1. Introduction**

*Wolbachia* are a group of endosymbiotic bacterium and is one of the most common parasitic microbes of many arthropods, nematodes and insect species. A survey conducted by a team of scientists detected *Wolbachia* in 16.9 percent of insect species which estimated to a total number of 1.69 – 5.07 million insect species present globally (Werren *et al.*, 1995). It is also estimated that about 25-70% of species of insects are its potential hosts, and is now known to be a remarkable genetic manipulator of the infected species (Kozek and Rao, 2007).

*Wolbachia* was first reported in 1924 by Marshall Hertig an entomologist and Samuel Wolbach, a pathologist, through a collaborative study on the presence and identification of microorganisms in arthropods (Hertig and Wolbach, 1924). It was
first considered as Rickettsiae like organism for its characteristic resemblance with other Rickettsiales. Until 1971, when Yen and Barr (1971) discovered Culex mosquito eggs were killed by cytoplasmic incompatibility, when the sperm of Wolbachia-infected males fertilized infection-free eggs, the role of Wolbachia was little known. The phylogenetic relationship of Wolbachia and its divergent hosts also remained unknown until early 1990s. Stouthamer et al. (1993) revealed that a bacterium that induces parthenogenesis in a Hymenopteran parasitoid shares a 95 per cent similarity with an incompatibility microorganism of α-proteobacteria group and is later identified to be Wolbachia. Parthenogenesis has higher impact on parasitoid wasps in classical and augmentative biocontrol process, and hence has a wide interest in parthenogenesis inducing Wolbachia strains (Stouthamer, 1993).

Wolbachia, for its obligatory intracellular nature, morphological and traditional identification procedures has become impractical and needs to be identified molecularly. Advancement of PCR based molecular methods greatly facilitated the studies of this bacterium. Bacterial identification in general was done by amplifying the 16S rRNA gene; however, the slow evolutionary rate of 16S rRNA gene has not made it possible to adequately resolve a well defined phylogeny of Wolbachia strains with these sequences (Zhou et al., 1998). Later Wolbachia phylogenetic resolution was improved by cell cycle gene ftsZ (Werren et al., 1995). But Braig et al. (1998) has cloned and sequenced a major surface protein of Wolbachia (wsp) from a number of Wolbachia strains and showed that this gene has evolved at a much faster rate. Scott O’Neil and his team (Zhou et al., 1998) also proved that wsp gene sequences could divide and classify Wolbachia into a number of subgroups.

Outer membrane proteins of Gram negative bacteria are of great importance in bacteria-host interaction. Outer membrane proteins (OMP) thus have been the subject of extensive studies to unravel the biology of host bacteria dynamics and also of their rapid evolution (Baldo et al., 2010). The highly variable OMPs are among the fastest evolving microbial proteins and involve in diverse functions including nutrient transport, bacterial invasion, defense, adhesion and signaling pathways (Zheng et al., 2004). OMPs of human pathogens such as Ehrlichia, Rickettsia, Haemophilus influenza and Neisseria meningitides, function as antigens and are the targets for vaccine development (Ohashi et al., 2003). In silico prediction of OMPs show a characteristic transmembrane β-barrel structure with even number of anti parallel
sheets, connected to the loops of variable length at the extracellular side and to short
turns containing both N termini and C termini at the periplasmic side. Residues in the
β-barrel are highly conserved where as domains of extracellular loop are highly
variable (Callaghan et al., 2008; Hobbs et al., 1994). There is more information
available for OMPs of vertebrate pathogens while little is known about OMPs of
bacteria that infect invertebrates.

Wolbachia Surface Protein (WSP) is an OMP whose molecular function
remains unknown. But experimental studies suggests that WSP can activate host
innate immune response in humans and also can trigger inflammatory response in
humans and canine filariasis (Brattig et al., 2004; Porksakorn et al., 2007). Recent
studies also showed a delay in apoptosis in human Polymorphonuclear cells (PMNs)
which is involved in innate immune response against microbial pathogens (Bazzocchi
et al., 2007). It is also proved in studies that, WSP proteins have localized to various
host tissues in Brugia malayi, and interacting with key enzymes involved in host
glycolytic pathway and cytoskeletal proteins (Melnikow et al., 2013). In Anopheles
gambiae, elevated levels of transcription of antimicrobial peptide genes was shown in
the presence of WSP (Pinto et al., 2012).

The need for horizontal transfer of Wolbachia bacterium between invertebrate
host hinges on the ability to adapt to new intracellular environments (McMeniman et
al., 2008). This is because of the species specificity of Wolbachia. Though the
functions of Wolbachia remain unknown there are several evidences of its role in
host-symbiont interaction. Experimental studies suggest that wsp can induce host
immune responses and recent hypothesis predicts that wsp is an important player in
the establishment and persistence of symbiosis via apoptosis inhibition (Siozios et al.,
2008). Pintureau et al. (2002) has classified the Wolbachia reported in Trichogramma
in to two super groups A and B based on the reproductive modifications it induces on
its host. The structure, microevolution and population genetics of wsp was analyzed
from 515 unique alleles reported in 831 Wolbachia isolates. Wsp showed an eight
stranded transmembrane β-barrel with four extracellular loops containing hyper
variable regions (Baldo et al., 2010).

This study concentrates on the detection of Wolbachia in Trichogramma
populations and characterizing the wsp genes reported in Trichogramma spp. to find
the structural difference. This would give insights to the selection of bacterium for horizontal transfer and provide knowledge on the species specificity of Wolbachia.

3.2. Materials and methods

3.2.1. DNA extraction from Trichogramma

Adult *T. pretiosum* three days after emergence were used for DNA extraction by Chelex method (Ciociola JR et al., 2001).

3.2.2. Wolbachia detection by wsp and PCR

Fifty nano grams of *T. pretiosum* DNA isolated by above method was used for detecting the presence of Wolbachia in all the populations. *T. embryophagum* which naturally harbors Wolbachia is considered as a positive control for the PCR based detection. Specific Wolbachia Surface Protein (wsp) primers (Forward- 81F 5’TGGTCCAATAAGTGATGAAGAAAC-3’, Reverse- 691R 5’AAAAATTAAACGCTACTCCA-3’) were used for the detection and confirmation. The PCR reaction was carried out in a 25µl reaction volume with 1x PCR buffer, 2.5mM dNTPs, 10 pM of forward and reverse primer and 1U of *Taq* polymerase (NEB, England). The amplification conditions for wsp was 3 mins of initial denaturation at 94°C followed by 35 cycles of 1min denaturation at 94°C, 1min annealing at 53°C and 1min elongation at 72°C. The PCR amplicon was excised from the gel using Qiaex II Gel Extraction Kit (Qiagen) following manufacturers instruction. The extracted product was then sequenced at Chromous Biotech, India by Sanger’s method.

3.2.4. Sequence collection

The Wolbachia Surface Protein sequences reported in *Trichogramma* sp. were collected from the primary PubMLST site hosted at The Department of Zoology, University of Oxford, UK (http://pubmlst.org/Wolbachia).

3.2.5. Evolutionary analysis of Wolbachia surface protein

All the 33 sequences were aligned in ClustalW 2 and are exported to http://www.phylogeny.fr/ for phylogenetic tree construction with default parameters.
A representative sequence was selected from every similar group and was used for structure predictions and comparisons.

### 3.2.6. Protein structure prediction and comparison

The secondary structure of the protein sequences were predicted by SOPMA tool (www.expasy.org). The structural variations at α-Helix, Extended strand, β-turn and Random coils were estimated for all the representative sequences.

### 3.2.7. Homology modeling and antigenic region determination

The three-dimensional structure prediction based on homology modeling of representative sequences was predicted using Phyre² (Protein Homology/analogY Recognition Engine V 2.0) online search engine. The antigenic regions were predicted using a protein domain search at Pfam (http://pfam.sanger.ac.uk) and at http://imed.med.ucm.es. The ligand binding site was also predicted using 3DLigandSite-Ligand binding site prediction Server (http://www.sbg.bio.ic.ac.uk/3dligandsite).

### 3.2.8. Trans-membrane structure and ligand prediction

PRED-TMBB (http://bioinformatics.biol.uoa.gr/PRED-TMBB/) is online software that helps to predict trans-membrane beta-strands of the Gram-negative bacteria outer membrane proteins. The selected representative sequences were predicted to understand their amino acid positions in the lipid bilayer of bacterial membrane (Bagos et al., 2004). Ligand binding prediction is done at 3DLigandStie, an automated method for the prediction of ligand binding sites (http://www.sbg.bio.ic.ac.uk/3dligandsite) (Wass et al., 2010).

### 3.3. Results

#### 3.3.1. DNA extraction from Trichogramma and Wolbachia detection by wsp PCR

DNA extracted from 14 populations of Trichogramma was of good quality (Fig. 1). The 14 populations of Trichogramma subjected for the detection of Wolbachia turned to be negative except one population from Andhra Pradesh which is identified as T. pretiosum.
As in the above Figure 2 amplified PCR product was of 600bp size. The nucleotide sequences of wsp region obtained by Sanger’s method were then searched for sequence similarity at NCBI database. The wsp nucleotide sequence of *T. embryophagum* and *T. pretiosum* matched to their corresponding sequences in Genbank as determined using BLAST (Version 2.7). The *wsp* nucleotide of *T. pretiosum* was submitted in the Genbank.

### 3.3.2. Sequence mining

There were 831 wsp sequences found in different insect species, 31 sequences were of *Trichogramma*. Repetitive reports of same species were eliminated but different wsp sequences reported for same species were considered for comparison. *Wsp* reported for *Drosophila simulans* and *Nasonia vitripennis* were also included to have a comparison as *Nasonia* being the closest organism to *Trichogramma* and *Drosophila* being the model organism making the sequences to 33 in number (*Supporting information 1).

### 3.3.3. Evolutionary analysis of *Wolbachia* surface protein

The evolutionary analysis of *wsp* showed high variability in distribution of amino acids among *Trichogramma sp* (Fig. 3). The 33 different sequences were
majorly bifurcated into two groups one with 15 species and other with 18 species (Fig. 4). It was noted that similar wsp sequences reported in different species of *Trichogramma* were grouped together and wsp of *D. simulans* and *N. vitripennis* have separately fallen in different clusters. *T. pretiosum* was found clustered with *T. kayaki*, *T. deion*, *T. oleae* and *T. nubilale*.

### 3.3.4. Protein structure prediction and comparison

The secondary structure was predicted and the α-Helix, Extended strand, β-turn and Random coils were presented in Table. 1. The variability of amino acids in all the species varies depending on the molecular weight, hydrophathycity and aliphatic index, indicating small evolutionary changes present in protein sequences (*Supporting information 2).

### 3.3.5. Homology modeling and antigenic region determination

Homology modeling was done for a representative species of every separate cluster formed in the evolutionary analysis (*Supporting information 3). The sequence identity of all wsp was identical to the outer membrane protein NspA from *Neisseria meningitides*. NspA is a homolog to the Opa proteins which mediates adhesion to the host cells. A total of 11 structures were predicted and structural variations were observed among the species (Fig. 5-15). The biophysical properties of the structures were calculated (Table 2). The predicted ligand binding sites were located mostly on the extra cellular loops, *T. pretiosum* has 8 amino acids at the binding site making it the largest binding site among wsp of *Trichogramma* populations (Fig. 16-26), and binding residues are tabulated (Table 3). The antigenic region determined by pfam online tool predicted the antigenicity from 23rd to 43rd amino acid position highlighted in red (Fig. 27). A cladogram (Fig. 28) constructed for the antigenic region grouped *T. semblidis* and *N. vitripennis* separately suggesting the variability in their antigenic region.

### 3.3.6. Trans-membrane structure

The transmembrane protein prediction based on the hidden Markov model arranged the amino acids in the lipid bilayer and represented as 2D image (Fig. 29-38). The hydrophilic and hydrophobic pattern positioned in the lipid bilayer.
confirmed the typical eight β-barrel structure of wsp with four extracellular loops and periplasmic turns connected to β-barrel core

**Fig. 3:** Amino acid sequences aligned for evolutionary analysis showing conserved sequences
Fig. 4: Phylogenetic tree constructed based on wsp sequences
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Fig. 5: \textit{D. simulans} _15 

Fig. 6: \textit{N. vitripennis} _25 

Fig. 7: \textit{T. embryophagum} _312 

Fig. 8: \textit{T. cordubensis} _324 

Fig. 9: \textit{T. brassicae} _310 

Fig. 10: \textit{T. dendrolimi} _10
Fig. 11:  *T. dendrolimi_314*  

Fig. 12:  *T. evanescens_427*  

Fig. 13:  *T. ostriniae_411*  

Fig. 14:  *T. pretiosum_325*  

Fig. 15:  *T. sembiidis_326*
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Fig. 16: *D. simulans_15*

Fig. 17: *N. vitripennis_25*

Fig. 18: *T. embryophagum_312*

Fig. 19: *T. cordubensis_324*
Fig. 27: Antigenic region determination of wsp protein structure
Fig. 28: Cladogram of predicted antigenic region of \textit{wsp} protein
Fig. 29-38: Transmembrane structure prediction of wsp sequences

**Fig. 29**

**Fig. 30**

**Fig. 31**

**Fig. 32**

**Fig. 33**

**Fig. 34**
3.4. Discussion

*Wolbachia* plays an inevitable role in manipulating the host reproduction. As the number of females in *Trichogramma* determines the success of biological control, it is very necessary to detect the presence of *Wolbachia* in a given population. Good qualities of DNA extracted from fourteen populations of *Trichogramma* were used to amplify the *Wolbachia* surface protein. DNA extraction by Chelex resin method yielded good quality DNA from less number of insects whereas conventional methods required large quantity of insects.
Use of the specific primers (wsp) was successful in detecting Wolbachia in Trichogramma samples. Wolbachia was detected in T. pretiosum collected from Andhra Pradesh. This is the first incidence of detecting the presence of Wolbachia in Indian Trichogramma population. Though the presence of Wolbachia was reported in T. chilonis (ENA|ACS68804|ACS68804.1) population, the presence of bacterium was not detected in any of the T. chilonis sample as this species is most abundant in Indian vegetative ecosystem. Hence, horizontal transfer of Wolbachia into non harboring T. chilonis will improve the efficacy of biocontrol program in India.

Wolbachia is very species specific and reduces the success rate of intra species horizontal transfer. Wsp region is antigenic that triggers host immune response (Siozios et al., 2008) and also has a crucial role in bacterial interaction with the host. Wsp is one of the outer membrane protein (OMPs) that are highly variable and among the fastest evolving protein. It shows four distinct hyper variable regions (HVR) and the primary source of amino acid diversity at HVR remains unknown and unclear whether this genetic diversity is adaptive; because arthropod Wolbachia does not infect vertebrate hosts.

The phylogenetic estimate of wsp sequences majorly clustered into two groups each with 18 and 15 individual sequences. Wsp of D. simulans and N. vitripennis were clustered separately suggesting the specificity of wsp across species. The sequences differ in their amino acid content and length and this variability is largely associated with the HVR.

The secondary structure prediction of wsp amino acid indicates random distribution of alpha helix, extended strands, beta turns and more distribution of random coils across the species. The online server based prediction of tertiary structure of wsp shows homology to NspA protein. Wsp tertiary structure is an eight β-barrel structure with four extracellular loops. The structure contains porins and act as ion channels which help in transfer of molecules. The diversity of structure was mainly in the extracellular loops. The biophysical properties of wsp structure are diverse further confirming the species specificity of the protein. This variation may be the effect of mutation and recombination during the course of evolution. Further, the transmembrane protein prediction based on hidden Markov model using the online PRED-TMBB server confirmed that wsp shows eight β-barrel structures with four
hydrophilic extracellular loops and periplasmic turns connected to a β-barrel core containing predominantly the neutral or polar residues, valine, alanine, glycine and tyrosine, in accordance to the typical composition of β-barrel proteins.

A cladogram constructed for the antigenic region of wsp determined by pfam server, classified them in to three different clusters. The formation of cladogram is in consonance with the formation of genetic phylogeny. The antigenic region predicted is extended between 23rd to 43rd amino acid positions which are embedded with the lipid bilayer. This could be a possible reason that Wolbachia invades the host system without triggering the host immune response. The ligand binding also varies in their binding amino acid regions as predicted. T. pretiosum has eight residues in their binding region making it the largest binding site.

3.5. Conclusion

Though Wolbachia was detected in Indian population of T. pretiosum, T. chilonis is the most extensively used biocontrol agent in the country. The intra species horizontal transfer of Wolbachia reduces its ability to induce reproductive manipulation in the new host. The phylogenetic relationship of wsp reported for all Trichogramma was constructed and the species specificity of the Wolbachia was confirmed with respect to the wsp protein which has predictable role in antigenicity and bacterial interaction with its host. Here, the structure of Wolbachia detected in all Trichogramma sp. was predicted and compared with the Wolbachia reported in D. simulans and N. vitripennis. The variability in the structure was calculated. The antigenic region and ligand binding region was also determined. This study enlightens the species specificity of the wsp region which further helps in selection of Wolbachia for horizontal transfer in closely related species.

3.6. References


*Supporting information 1 (wsp sequences for analysis)*

> 1. *T. brassicae* (302)
TKVDGITYKGDNSYSLKASFLAGGGAFGYKMDDIRVDVEGVYSLKNKNVDKFTPDTI
ADSVTAISGLNVYDDIAEDMPITPYVGVGYATYISTPLKAVNDQKSKFAGGQVKAGVS
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> 2. *T. dendrolimi* (10) - China
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ANSVAFSGLNVYDDIAEDMPITPYVGVGYAAAYISNPSEASAVKDQKGFAYQAKAGVS
YDVATPEIKLFAGARYFGYSYGANFDKSGSKEKDKGGHTV

> 3. *T. evanescens* (310)
TKVDGITYKGDNSYSLKASFLAGGGAFGYKMDDIRVDVEGVYSLKNKNVDKFTPDTI
ADSVTAISGLNVYDDIAEDMPITPYVGVGYATYISTPLKAVNDQKSKFAGGQVKAGVS
YDVATPEKLYAGARYFGYSYGANFDKSGSKEKDKGGHTV

> 4. *T. dendrolimi* (314) - Japan
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ADSVTAISGLNVYDDIAEDMPITPYVGVGYATYISTPLKAVNDQKSKFAGGQVKAGVS
YDVATPEKLYAGARYFGYSYGANFDKSGSKEKDKGGHTV

> 5. *T. dendrolimi* (385) - China
TRIDGIEYKGTVDPLASFMAGGAFFGKMDDIRVDVEGVLGYSQLKNVDKGATFTPTTV
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> 6. *T. brassicae* (10)
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> 7. *T. ostriniae* (10)
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> 8. *T. dendrolimi* (289)
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> 9. *T. cacoeciae* (310)
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> 10. T. brassicae (310)
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> 11. T. embryophagum (312)
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> 12. T. evanescens (314)
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> 13. T. evanescens (315)
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> 14. T. evanescens (316)
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> 15. T. cordubensis (324)
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> 16. T. embryophagum (324)
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> 17. T. evanescens (324)
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> 18. T. kaykai (325)
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> 29. *T. evanescens* (427)
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> 31. *T. deion* (509)
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SYYVAPEIKLFAGARYFGSYGASFDKAAKGDGIKN

> 32. *D. simulans* (15)
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ANSVAAFSGLVNYYDDIAIEDMPITPYVGVGAAAYISNPSEASAVKDQKEFGFAYQAKGV
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> 33. *N. vitripennis* (25)
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*Supporting information 2 (Secondary structure prediction)*

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Alpha helix (Hh) : 31 is 19.14%
3_10 helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 42 is 25.93%
Beta turn (Tt) : 8 is 4.94%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 81 is 50.00%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 4. T. dendrolimi (314) - Japan

Sequence length : 160
SOPMA :
Alpha helix (Hh) : 33 is 20.62%
3_10 helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 38 is 23.75%
Beta turn (Tt) : 11 is 6.88%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 78 is 48.75%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 5. T. dendrolimi (385) - China

Sequence length : 161
SOPMA :
Alpha helix (Hh) : 46 is 28.57%
3_10 helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 32 is 19.88%
Beta turn (Tt) : 8 is 4.97%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 75 is 46.58%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 6. T. brassicae (10)

TRIDGIEYKGGTEVHDPKASFMAGGAAGFYKMDIRVDVEGLYSQNKNDVGATFTPTTVANSVAAPS

Please purchase PDF Split-Merge on www.verypdf.com to remove this watermark.
Sequence length : 161
SOPMA :

Alpha helix (Hh) : 41 is 25.47%
3_10 helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 34 is 21.12%
Beta turn (Tt) : 5 is 3.11%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 81 is 50.31%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 7. T. ostriniae (10)

10 | 20 | 30 | 40 | 50 | 60 | 70
---|----|----|----|----|----|----
TRIDGIEYKGETVHDKLASFMAGGAAFGYKMDDIRVDFGTYLQNLKNDVGATFTPTTVANSVAFS
heeeeccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc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Other states : 0 is 0.00%

> 9. T. cacoeciae (310)

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12. *T. evanescens* (314)

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Sequence length: 160

SOPMA:

- Alpha helix (Hh): 33 is 20.62%
- 3_10 helix (Gg): 0 is 0.00%
- Pi helix (Ii): 0 is 0.00%
- Beta bridge (Bb): 0 is 0.00%
- Extended strand (Ee): 38 is 23.75%
- Beta turn (Tt): 11 is 6.88%
- Bend region (Ss): 0 is 0.00%
- Random coil (Cc): 78 is 48.75%
- Ambiguous states (?): 0 is 0.00%
- Other states: 0 is 0.00%

13. *T. evanescens* (315)

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Sequence length: 171

SOPMA:

- Alpha helix (Hh): 31 is 18.13%
- 3_10 helix (Gg): 0 is 0.00%
- Pi helix (Ii): 0 is 0.00%
- Beta bridge (Bb): 0 is 0.00%
- Extended strand (Ee): 46 is 26.90%
- Beta turn (Tt): 9 is 5.26%
- Bend region (Ss): 0 is 0.00%
- Random coil (Cc): 85 is 49.71%
- Ambiguous states (?): 0 is 0.00%
- Other states: 0 is 0.00%

14. *T. evanescens* (316)

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Sequence length: 171

SOPMA:

- Alpha helix (Hh): 31 is 18.13%
- 3_10 helix (Gg): 0 is 0.00%
- Pi helix (Ii): 0 is 0.00%
- Beta bridge (Bb): 0 is 0.00%
- Extended strand (Ee): 46 is 26.90%
- Beta turn (Tt): 9 is 5.26%
- Bend region (Ss): 0 is 0.00%
- Random coil (Cc): 85 is 49.71%
- Ambiguous states (?): 0 is 0.00%
- Other states: 0 is 0.00%
T. cordubensis (324)

> 15. T. cordubensis (324)

| TKVDGIKNVTSGEDSPLKRSFIAGVVAFGYKMDIRVDVEGLYRSLAKNAVIDASEANVADSLTAFSG |
| eeeeeeccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc |
| NVYVIDIVIEDMPITFYVGVGAAYISNPSNAADVQDRRFPGFYQAQAGVSYDVAPEIKLFAGARYF |
| hhhheeeccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc |

Sequence length : 160
SOPMA :
Alpha helix   (Hh) : 37 is 23.12%
3_10 helix   (Gg) : 0 is 0.00%
Pi helix      (Ii) : 0 is 0.00%
Beta bridge   (Bb) : 0 is 0.00%
Extended strand (Ee) : 42 is 26.25%
Beta turn     (Tt) : 9 is 5.62%
Bend region   (Ss) : 0 is 0.00%
Random coil   (Cc) : 72 is 45.00%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 16. T. embryophagum (324)

| TKVDGIKNVTSGEDSPLKRSFIAGVVAFGYKMDIRVDVEGLYRSLAKNAVIDASEANVADSLTAFSG |
| eeeeeeccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc |
| NVYVIDIVIEDMPITFYVGVGAAYISNPSNAADVQDRRFPGFYQAQAGVSYDVAPEIKLFAGARYF |
| hhhheeeccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc |

Sequence length : 160
SOPMA :
Alpha helix   (Hh) : 37 is 23.12%
3_10 helix   (Gg) : 0 is 0.00%
Pi helix      (Ii) : 0 is 0.00%
Beta bridge   (Bb) : 0 is 0.00%
Extended strand (Ee) : 42 is 26.25%
Beta turn     (Tt) : 9 is 5.62%
Bend region   (Ss) : 0 is 0.00%
Random coil   (Cc) : 72 is 45.00%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%
17. *T. evanesens* (324)

```
10  20  30  40  50  60  70
|   |   |   |   |   |   |   |
|TKVDGIKNVTSKGDSPLKRSFIAGGVAFGYKMDDIRVDEVEGLYSRLAKNAVIDASEANVADSLTAFG|
|eeeeeecccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc|
LVNVVYDIVIEDMPITFYVGVGAAAYISNPSNAADVKDQRRFGFAYQAKAGVSYDVAPFIEKLFAARYF|
|hhhhheeecccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc|
```

Sequence length : 160

SOPMA :
- Alpha helix (Hh) : 37 is 23.12%
- 3_10 helix (Gg) : 0 is 0.00%
- Pi helix (Ii) : 0 is 0.00%
- Beta bridge (Bb) : 0 is 0.00%
- Extended strand (Ee) : 42 is 26.25%
- Beta turn (Tt) : 9 is 5.62%
- Bend region (Ss) : 0 is 0.00%
- Random coil (Cc) : 72 is 45.00%
- Ambiguous states (?) : 0 is 0.00%
- Other states : 0 is 0.00%

18. *T. kaykai* (325)

```
10  20  30  40  50  60  70
|   |   |   |   |   |   |   |
|TKVDGIKNATSKEKDSPLRSFIAGGVAFGYKMDDIRVDEVEGLYSRLAKNAVIDASEANVADSLTAFG|
|ceeecccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc|
LVNVVYDIVIEDMPITFYVGVGAAAYISNPSNAADVKDQRRFGFAYQAKAGVSYDVAPFIEKLFAARYF|
|hhhhheeehtcccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc|
```

Sequence length : 160

SOPMA :
- Alpha helix (Hh) : 28 is 17.50%
- 3_10 helix (Gg) : 0 is 0.00%
- Pi helix (Ii) : 0 is 0.00%
- Beta bridge (Bb) : 0 is 0.00%
- Extended strand (Ee) : 40 is 25.00%
- Beta turn (Tt) : 8 is 5.00%
- Bend region (Ss) : 0 is 0.00%
- Random coil (Cc) : 84 is 52.50%
- Ambiguous states (?) : 0 is 0.00%
- Other states : 0 is 0.00%

19. *T. pretiosum* (325)

```
10  20  30  40  50  60  70
|   |   |   |   |   |   |   |
|TKVDGIKNATSKEKDSPLRSFIAGGVAFGYKMDDIRVDEVEGLYSRLAKNAVIDASEANVADSLTAFG|
|ceeecccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc|
LVNVVYDIVIEDMPITFYVGVGAAAYISNPSNAADVKDQRRFGFAYQAKAGVSYDVAPFIEKLFAARYF|
|hhhhheeehtcccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc|
```

Sequence length : 160

SOPMA :
- Alpha helix (Hh) : 28 is 17.50%
- 3_10 helix (Gg) : 0 is 0.00%
- Pi helix (Ii) : 0 is 0.00%
- Beta bridge (Bb) : 0 is 0.00%
- Extended strand (Ee) : 40 is 25.00%
## Beta turn (Tt)
- **T. oleae** (325): 8 is 5.00%
- **T. semblidis** (326): 12 is 7.50%
- **T. chiloni** (410): 8 is 5.00%

## Bend region (Ss)
- **T. oleae** (325): 0 is 0.00%
- **T. semblidis** (326): 0 is 0.00%
- **T. chiloni** (410): 0 is 0.00%

## Random coil (Cc)
- **T. oleae** (325): 84 is 52.50%
- **T. semblidis** (326): 77 is 48.12%
- **T. chiloni** (410): 45 is 26.60%

## Ambiguous states (?)
- **T. oleae** (325): 0 is 0.00%
- **T. semblidis** (326): 0 is 0.00%
- **T. chiloni** (410): 0 is 0.00%

## Other states
- **T. oleae** (325): 0 is 0.00%
- **T. semblidis** (326): 0 is 0.00%
- **T. chiloni** (410): 0 is 0.00%

### 20. *T. oleae* (325)

- Sequence length: 160
- SOPMA:
  - **Alpha helix** (Hh): 28 is 17.50%
  - **3_1 helix** (Gg): 0 is 0.00%
  - **Pi helix** (Ii): 0 is 0.00%
  - **Beta bridge** (Bb): 0 is 0.00%
  - **Extended strand** (Ee): 40 is 25.00%
  - **Beta turn** (Tt): 8 is 5.00%
  - **Bend region** (Ss): 0 is 0.00%
  - **Random coil** (Cc): 84 is 52.50%
  - **Ambiguous states** (?) : 0 is 0.00%
  - **Other states** : 0 is 0.00%

### 21. *T. semblidis* (326)

- Sequence length: 160
- SOPMA:
  - **Alpha helix** (Hh): 26 is 16.25%
  - **3_1 helix** (Gg): 0 is 0.00%
  - **Pi helix** (Ii): 0 is 0.00%
  - **Beta bridge** (Bb): 0 is 0.00%
  - **Extended strand** (Ee): 45 is 28.12%
  - **Beta turn** (Tt): 12 is 7.50%
  - **Bend region** (Ss): 0 is 0.00%
  - **Random coil** (Cc): 77 is 48.12%
  - **Ambiguous states** (?) : 0 is 0.00%
  - **Other states** : 0 is 0.00%

### 22. *T. chiloni* (410)

- Sequence length: 171
- SOPMA:
  - **Alpha helix** (Hh): 26 is 15.23%
  - **3_1 helix** (Gg): 0 is 0.00%
  - **Pi helix** (Ii): 0 is 0.00%
  - **Beta bridge** (Bb): 0 is 0.00%
  - **Extended strand** (Ee): 45 is 26.41%
  - **Beta turn** (Tt): 12 is 7.05%
  - **Bend region** (Ss): 0 is 0.00%
  - **Random coil** (Cc): 77 is 45.23%
  - **Ambiguous states** (?) : 0 is 0.00%
  - **Other states** : 0 is 0.00%
SOPMA:

Alpha helix (Hh) : 36 is 21.05%
3₁₀ helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 42 is 24.56%
Beta turn (Tt) : 9 is 5.26%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 84 is 49.12%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 23. T. ostriniae (411)

Sequence length : 162

SOPMA:

Alpha helix (Hh) : 30 is 18.52%
3₁₀ helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 37 is 22.84%
Beta turn (Tt) : 7 is 4.32%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 88 is 54.32%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

> 24. T. chiloni (411)

Sequence length : 162

SOPMA:

Alpha helix (Hh) : 30 is 18.52%
3₁₀ helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 37 is 22.84%
Beta turn (Tt) : 7 is 4.32%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 88 is 54.32%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%
> 25. *T. dendrolimi* (412)

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Extended strand (Ee) : 39 is 22.81%
Beta turn (Tt) : 6 is 3.51%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 90 is 52.63%
Ambiguous states (?) : 0 is 0.00%
Other states : 0 is 0.00%
ccc

Sequence length : 160
SOPMA :

| Alpha helix (Hh) | 27 | 16.88% |
| Pi helix (Ii)    | 0  | 0.00%  |
| Beta bridge (Bb) | 0  | 0.00%  |
| Extended strand (Ee) | 36 | 22.50% |
| Beta turn (Tt)   | 7  | 4.38%  |
| Random coil (Cc) | 90 | 56.25% |
| Other states     | 0  | 0.00%  |

> 31. T. deion (509)

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| TKVDGIKNATSKEKDSPLKRSFIAGGVAFYKMDDIRVDEVGLYSRLAKNKAVIDASEANVADSLSAFGS | cccccc | cccccc | cccccc | cccccccccc | cccccccccc | cccccccc | cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc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**Supporting information 3 (Representative sequences for homology modeling)**

> **2. T. dendrolimi (10) China**

TRIDGIEYKKGTEVHDPLKASFMAGGAAFGYKMDIRVVDVEGLYSQNLKNDVSGATFTPTTTV
ANSVAASGLNVYDYIAEDMPITYPYGVGVAAYISNPEASAVKDKQGFAGYQAKAGV
SYDVTPFIEKLFAGARYFGSYGASFNKEAVSATKEINV

> **4. T. dendrolimi (314) - Japan**

TKVDGIKNKSKEEEDSPFLKRSFAAGGAFYKMDIRVVDVEGLYSRLAKNAVIDASCHAVA
DSLTAASGLNVYDYIVEMIPITYPYGVGVAAYISNPSNAAETVKDFQRFAGYQAKGV
SYDVPEIKLFAGARYFGSYGASFDKAAKGGDKIKKN

> **10. T. brassicae (310)**

TKVDGITYKGDSDYPLKASFLAGGGAAGFYKMDIRVVDVEGLYSNLKNNVDTAKFTPDTI
ADSVTASGLNVYDYIAEDMPITYPYGVGVAAYISTPKDAVNDQKSKKGFAQVQKAGV
SYDVTPFVLYAGARYFGSYGANSFDKSGKGGKGGHTV

> **11. T. embryophagum (312)**

TKVDGITYKGDSDYPLKASFLAGGGAAGFYKMDIRVVDVEGLYSNLKNNVDTAKFTPDTI
ADSVTASGLNVYDYIAEDMPITYPYGVGVAAYISTPKDAVNDQKSKKGFAQVQKAGV
SYDVTPFVLYAGARYFGSYGANSFDKSGKGGKGGHTV

> **15. T. cordubensis (324)**

TKVDGITYKSGEDSPFLKRSFIAGVYAFYKMDIRVVDVEGLYSRKNAVIDASEANVA
DSLTAASGLNVYDYIVEMIPITYPYGVGVAAYISNPSNAADVQRRFGFAGYQAKGV
SYDVPEIKLFAGARYFGSYGASFDKAAKGGDKIKKN
> 19. T. pretiosum (325)
TKVDGIKNATSKEKDSPLRSFIAGGVAFGYKMDDIRVDVEGLYSRLAKNAVIDASEANVADSLTAFSGLVNYDYDIEDMPITPYVGVSAYISNPNAADVKDQRRFGFAYQAKAGVS YDVAPEIKLFAGARYFGSYGASFDKAACKDDGIIKNI

> 21. T. semblidis (326)
TKVDGIKNSGREGSPLTRSFAGSAFGYKMDDIRVDIEGLYSRLAKDTAVIDASEASLA DSVTAISSGLVNYDYDYAEEMMPITPYVGVSAYISNLASADVKNQKRFGFAYQAKAGVS YDVTPFIEIKLFGASYGAGDFDKENGINIKNV

> 23. T. ostriniae (411)
TKVDGIYKKDNSYDPLKSFLAGSPAAGAFGYKMDDIRVDVEGYSYLKNKTVTDATPKPDTI ADSVTASTGLVNYDYDYAEEMMPITPYVGVSAYISNLASADVKNQKRFGFAYQAKAGVS SYDVTPFIEIKLFGASYGAGDFDKENGINIKNV

> 29. T. evanescens (427)
TKVDGIYKKDNSYDPLKSFLAGSPAAGAFGYKMDDIRVDVEGYSYLKNKTVTDATPKPDTI ADSVTASTGLVNYDYDYAEEMMPITPYVGVSAYISNLASADVKNQKRFGFAYQAKAGVS SYDVTPFIEIKLFGASYGAGDFDKENGINIKNV

>gi|113714147|gb|ABI36797.1| surface protein [Wolbachia endosymbiont of Nasonia vitripennis]
TKVDGIYKKDNSYDPLKSFLAGSPAAGAFGYKMDDIRVDVEGYSYLKNKTVTDATPKPDTI ADSVTASTGLVNYDYDYAEEMMPITPYVGVSAYISNLASADVKNQKRFGFAYQAKAGVS SYDVTPFIEIKLFGASYGAGDFDKENGINIKNV

>gi|113714127|gb|ABI36787.1| surface protein [Wolbachia endosymbiont of Drosophila simulans]
TRIDGIYKKGEVHDPLKAFMAGGAAGFGYKMDDIRVDVEGLYSQLNKNKDVGATFTPTTV ANSVAAPSGLVNYDYDYAEEMMPITPYVGVSAYISNLASAVKDQKEFGFAYQAKAGVS SYDVTPFIEIKLFGASYGAGDFDKENGINIKNV