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Evidence for an ichnovirus machinery in parasitoids of coleopteran larvae

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ABSTRACT

Bathyplectes spp. are ichneumonid solitary larval parasitoids of the alfalfa weevil which have been classified in the subfamily Campopleginae and which harbor atypical virus particles. Despite the morphological differences between *Bathyplectes* spp. particles and the polydnaviruses carried by a number of related campoplegine species, called ichnoviruses, the process by which they are produced is very similar to that of ichnoviruses. To address the question of the nature and origin of these atypical particles, the *Bathyplectes anurus* ovary transcriptome has been analyzed. We found a number of highly expressed transcripts displaying similarities with genes belonging to the machinery involved in the production of ichnovirus particles. In addition, transcripts with similarities with repeat-element genes, which are characteristic of the packaged campoplegine ichnovirus genome were identified. Altogether, our results provide evidence that *Bathyplectes* particles are related to ichnoviruses.

1. Introduction

Bathyplectes anurus and *B. curculionis* are morphologically similar and closely related ichneumonid wasps that parasitize larvae of the alfalfa weevil, *Hypera postica*, commonly found in alfalfa fields in the south of France. They are solitary parasitoids whose life cycle is synchronized with that of alfalfa weevils. *B. anurus* produces one generation per year and undergoes 2 diapauses, one in the summer at the larval stage and the other following pupation in the winter at the pupal stage. The adult emerges from overwintered cocoons in the spring of the following year to oviposit into hosts. After the larva has consumed the *H. postica* larva, it emerges to spin a white-banded, dark brown cocoon, and enter diapause until the fall. On the other hand, some of the *B. curculionis* pupae issued from spring parasitism may develop until the adult stage leading to a second generation.

Bathyplectes spp. produce unusual particles in their ovaries that accumulate in the oviducts (Hess et al., 1980). Interestingly, *Bathyplectes* spp. are classified in the subfamily Campopleginae that includes thousands of species that carry domesticated endogenous viruses from the *Polydnaviridae* family. Polydnaviruses are characterized by packaged genomes composed of several dsDNA circular molecules. They are reported from thousands of species from the families Ichneumonidae and Braconidae parasitizing lepidopteran larvae. Polydnaviruses associated with ichneumonids and braconids differ in their morphology and genome, so they were classified into the genera Ichnovirus and Bracovirus, respectively. Polydnaviruses illustrate an astonishing example of convergent evolution since the two “polydnavirus genera” result from two independent events of virus domestication. Bracoviruses result from the integration of a nudivirus (Bezier et al., 2009) whereas ichnoviruses derive from the domestication of a still unknown virus (Volkoff et al., 2010). In both cases, polydnaviruses show a similar life cycle and are required for successful parasitism. The virus particles are produced in a specialized tissue from the female called the calyx. They are secreted and stored in the oviduct lumen, then injected into the insect host along with the parasitoid egg upon parasitization. The integrated polydnavirus genomes are dual in nature, and include regions that will be excised, circularized and encapsidated (the “packaged genome” that consists in dsDNA circular molecules or segments), and other regions directly descending from the viral ancestor, that are expressed in the calyx cells, are not encapsidated and are involved in the formation of the virus particles (the “viral machinery”). The viral machinery includes nudiviral genes in the case of bracoviruses, and genes with no similarity to any other known virus group in the case of ichnoviruses (see below). Conversely, the encapsidated segments carry

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almost no genes with homology to known viral genes (e.g. with predicted functions in viral DNA replication, transcription, or virion formation) but instead a number of genes that share homology with genes from insects or other eukaryotes, which are expressed in the parasitized insect host and involved in its physiological manipulation (Chen et al., 2011; Darboux et al., 2019; Desjardins et al., 2008; Espagne et al., 2004; Lapointe et al., 2007; Tanaka et al., 2007; Webb et al., 2006).

Ichnoviruses (IV) have so far been described in two ichneumonid subfamilies, the Campopleginae and the Banchinae. All IV fully described are carried by species of endoparasitoids of lepidopteran larvae. The IV particles reported from these subfamilies are surrounded by two unit membranes but differ in their morphology. Most campoplegine species produce IV particles that consist of a large fusiform nucleocapsid (Krell and Stoltz, 1980; Stoltz, 1981; Volkoff et al., 1995) whereas in the two studied Banchinae, multi-nucleocapsid virions were reported (Djoumad et al., 2013; Lapointe et al., 2007). Nucleocapsids from Banchinae resemble those from Campopleginae, but are smaller in size. A third morphological category of particles associated with campoplegine wasps corresponds to the virus-like particles (VLPs) reported from *Venturia canescens*, that have been proven to not be ichnoviruses but to result from a domestication of a nudivirus that occurred more recently, and functionally replaced the IV originally present in this lineage (Pichon et al., 2015). Lastly a fourth morphological category of virus particles carried by campoplegine wasps were reported in *Bathyplectes anurus* and *B. curculionis* (Hess et al., 1980; Stoltz, 1981). Interestingly, and in contrast to all the other wasp species carrying polydnaviruses that parasitize lepidopteran larvae, these two species parasitize coleopteran larvae. *Bathyplectes* virus particle morphology is quite different from typical campoplegine IV particles. They consist of ovoid membrane particles that contain several small electron dense inclusions (Hess et al., 1980; Stoltz, 1981). As for the other examples, *Bathyplectes* particles are produced in the calyx. However, in absence of any genomic data regarding these species and the particles produced in their ovaries, it was still uncertain till now whether these particles were of viral nature and/or related to ichnoviruses.

Based on the absence of evidence that *Bathyplectes* spp. particles are actually polydnaviruses, we focused in this work on *B. anurus* to address the question of their nature and origin. To identify genes potentially involved in the production of the particles, a transcriptomic analysis of the female reproductive tract, where the particles are produced, has been performed. Our results indicate that a number of IV genes are expressed in *B. anurus* ovaries, demonstrating that *B. anurus* atypical particles are indeed related to the ichnoviruses.

2. Material and methods

2.1. Insect material

Hypera postica larvae were collected in alfalfa fields in different sites around Montpellier. The alfalfa weevil larvae were brought to the lab and kept at room temperature until formation of the *Bathyplectes* cocoons. In the fall, cocoons were maintained outdoors until next spring. At the end of the pupal stage, cocoons were dissected to isolate female pupae that were dissected in PBS to isolate the ovaries.

Alternatively, adult females were directly caught in the fields, then brought to the laboratory and dissected to collect their ovaries. Identity has been confirmed using COI barcoding. Briefly, genomic DNA was extracted from adults using Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer instructions. Genomic DNA was used as a template for PCR amplification using COI specific primers (LCO1490: 5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3' and HC02198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA -3', as described in Folmer et al., 1994). PCR amplification products were sent for sequencing by Eurofins, and annotated subsequent to a Blastn similarity search against NCBI non redundant (nr) database.

2.2. Microscopy

Ovaries were dissected from old pupae and adults and were then fixed with 2% (v/v) glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 for overnight at 4 °C, rinsed 3 times for 10 mn in the same buffer then post-fixed with 2% (v/v) osmium tetroxide for 1 h at room temperature. Samples were dehydrated through an ethanol series and embedded in Embed 812 (EMS products). Semithin sections were stained with methylene blue and observed under a photonic microscope. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under an electron microscope Jeol 1200 EXII at 100 kV (MEA Platform, Université de Montpellier). Images were taken with an EMSIS Olympus Quemesa 11 Mpixels camera and analysed with Image J software (Rasband, 1997-2018; Rasband, 1997).

2.3. RNAseq data generation and de novo transcriptome assembly

To obtain the ovary transcriptome, total RNA was extracted from the ovaries dissected from 60 *B. anurus* female pupae using the Qiagen RNeasy Mini Kit according to the manufacturer's protocol. PolyA-RNA-Seq Library construction and Illumina HiSeq 2000 sequencing (paired-end sequencing 2×100 bp) were performed by GATC Biotech AG company (GATC NG-7281 project).

Upon reception of the sequences, the following analyses were performed: (i) quality assessment using FastQC (available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), (ii) removal of sequencing adapters with Trimmomatic (Bolger et al., 2014); (iii) de novo transcript assembly with the Trinity suite version 2.2.0 (Haas et al., 2013); (iv) calculation of transcript counts and fragments per kilobase per million (FPKM) using RSEM software (Li and Dewey, 2011).

A total of 46,530 assembled transcripts (contigs) were obtained. The transcripts assembly obtained statistics were: a mean size of 990 bp, a median size of 470 bp, a N50 length of 1942 bp, a L50 length of 6588 bp and 4246% of GC content.

For annotation, all 46,530 transcripts were used as a query for a BLASTx search (Altschul et al., 1997) in the 'National Center for Biotechnology Information' (NCBI) non-redundant (nr) database, considering all hits with an e-value < 1E-08. Following BLAST search, we obtained 24,851 transcripts with "no hits" (534%). We also used BUSCO (Simão et al., 2015) to test for the presence of reference genes in this transcriptome. Over the 1658 Total BUSCO groups searched, we obtained 1477 complete BUSCOs (87.6%), 1063 complete and single-copy BUSCOs (64%), 414 complete and duplicated BUSCOs, 101 fragmented BUSCOs and 80 missing BUSCOs.

Finally, after filtering with FPKM > 1 and isoform percentage (isopct) > 15, a total of 41,467 transcripts were kept for further analyses (list in Supplemental Table 1). A selected set of transcripts was manually analyzed to identify the open reading frames, then the predicted protein sequence was used in blastP similarities searches against NCBI nr protein database to assess amino acid identities between the *B. anurus* predicted protein and the best protein match.

3. Results and discussion

3.1. Bathyplectes virion morphogenesis resembles that of ichnoviruses

The *Bathyplectes* female ovary resembles that described in other campoplegine wasps (Fig. 1A–C) with a marked calyx region at the junction between ovarioles and lateral oviducts. The calyx is spherical in shape and is constituted of a unicellular layer of large epithelial cells with a hypertrophied nucleus (Fig. 1D). In the calyx lumen, a number of particles can be observed (Fig. 1E). They are spherical in shape, with a 200 nm diameter and surrounded by two envelopes. In adult females, the particles contain small electron dense inclusions that appear punctiform or slightly elongated (Fig. 1E).

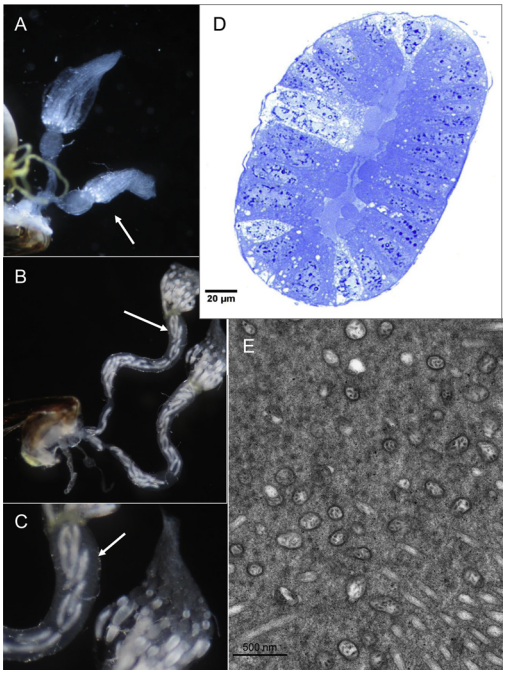

Fig. 1. *Bathyplectes* ovaries and virus particles. A. View of the *B. anurus* ovary at the diapausing pupal stage. B. View of the *B. curculionis* ovary at the adult stage. C. Detail on the *B. curculionis* calyx region at the adult stage. For A, B and C, arrow indicates the calyx. D. Semi-thin transverse section of the calyx region in a diapausing *B. anurus* pupa, stained with methylene blue, showing the layer of calyx cells containing large nuclei. **E.** Transmission electronic microscopy (TEM) observation of a section of the oviduct lumen in a *B. curculionis* adult female. Spherical enveloped particles containing punctiform inclusions (probably nucleocapsids) can be observed.

Morphogenesis of *Bathyplectes* virus (BaV) particles occurs in the calyx cells, and generally corresponds to what was previously described by Hess et al. (1980). Electron dense fibril-like structures are distributed within the calyx cell nuclei where BaV particles are formed. At the vicinity of these structures, probably virogenic stroma, a number of spherical vesicles can be observed (Fig. 2A and B). These vesicles consist of single unit lipid bilayers of still unknown origin. In pupae, the vast majority of the vesicles appear to be empty (Fig. 2A). Conversely, in the adults, they contain punctiform inclusions as described in Hess et al. (1980) and BaV particles can be observed at the vicinity of the virogenic stroma and free in the nucleus (Fig. 2B). The process of BaV particles formation is similar to what has been described for ichnoviruses. Indeed, in ichnoviruses, single enveloped particles are similarly assembled at the vicinity of virogenic stroma (Lapointe et al., 2007; Volkoff et al., 1995). Therefore, and as for the IVs, BaV particles envelopes are neoformed in the calyx cells nuclei and then filled with material probably originating from the fibril like material corresponding to a virogenic stroma. The origin of these envelopes remains unclear, as for IVs and BVs. Possibly they originate from the nuclear envelope as described for another type of enveloped dsDNA insect virus, the baculoviruses that acquire their envelopes from virus-induced intranuclear microvesicles (Crawford and Sheehan, 1985). Unfortunately, except for examples of nuclear envelopes found in the vicinity of the nuclear envelope (Fig. 2C), we did not succeed in clearly observing this event in *Bathyplectes* calyx cells. The enveloped BaV particles then bud through the nuclear envelope and reach the cell cytoplasm (Fig. 2D), similarly to the process that occurs for ichnoviruses. The nuclear envelope remains intact and particles surrounded by two envelopes as can be observed within the cytoplasm (Fig. 2D). As for IVs, this second envelope is lost during the migration of the particles within the cytoplasm towards the plasma membrane. Finally, the particles bud through the cell plasma membrane, which harbors numerous microvilli, and accumulate in the oviduct lumen (Fig. 3A and B). Interestingly, we observed that in *B. anurus* diapausing pupae (Fig. 3C), most of the released enveloped particles appeared empty, suggesting that somehow the calyx cells were already "induced" to produce the virus particles, even though assembly may be incomplete.

Therefore, despite morphology quite different from that of typical campoplegine ichnoviruses, the process by which *Bathyplectes* particles are formed, which includes synthesis of envelopes within the nuclei, migration from the nucleus to the oviduct lumen and acquisition of a second envelope during calyx cell budding, resembles what has been described for ichnoviruses.

3.2. A number of genes similar to IVSPER genes are highly transcribed in B. anurus ovaries

"Ichnovirus Structural Protein Encoding Regions" (IVSPERs) were first described in the campopleginae *H. didymator* (Volkoff et al., 2010) then in the banchine *Glypta fumiferanae* (Beliveau et al., 2015). The IVSPER genes are specifically transcribed in the calyx tissue and 2/3rd of them encode proteins found associated with IV particles by mass spectrometry analyses. IVSPERs remain in the wasp genome and are not packaged within the IV particles. These regions hypothetically correspond to fingerprints of the ancestral ichnovirus which integrated into the ancestral wasp genome. Except for their involvement in the IV particle constitution, the precise function of all the IVSPER genes remains today unknown.

Following Blast search analyses, a total of 119 sequences matched with viruses, 80 (67%) matched with products of IVSPERs, 34 (29%) with products of IV genes, 4 (3%) with picornavirus gene products and 1 (1%) with an entomopoxvirus gene product (list in Supplemental Material 1). After manual curation, homologs corresponding to a total of 28 IVSPER genes were found in the *B. anurus* ovary transcriptome (Table 1; Supplemental Table 1). In *H. didymator* IVSPERs, a total of 24 "unknown" genes (U1 to U24) were described, as well as members of 6 families, named IVSP1 to IVSP4, p53 and p12 (Volkoff et al., 2010). Note that p12 and p53 genes were first described in the campoplegine *Campoletis sonorensis* (Deng et Webb, 1999; Deng et al., 2000). Homologs of 18 out of the 24 unknown *H. didymator* IVSPER genes were identified in *B. anurus*, as well as at least one homolog of IVSP1, IVSP2, IVSP3, IVSP4 (2 genes) and p53 gene families (Fig. 4). However, no p12 gene homolog has been identified in *B. anurus* transcriptome by automatic annotation, maybe because they correspond to short sequences (about 100 aa) quite divergent among them (32 to 40% amino acid identity between the p12 identified in *C. sonorensis*, *H. didymator* and *G. fumiferanae*; data not shown). We also identified in the *B. anurus* transcriptome sequences matching with N-gene family members. In *H. didymator*, the N-gene family includes genes present both in the IVSPERs and in the packaged viral segments. Members of this gene family were also identified in the IVSPER of the banchine *Glypta fumiferanae* (Cusson et al., 2012) although in Banchinae, N-genes are a priori absent from the viral segments. Finally, we also identified a homolog of the *G. fumiferanae* U28, which has for now not being identified in the campoplegine *H. didymator*.

Among all the obtained transcripts, the most highly transcribed one in the *B. anurus* calyx is a gene homolog to the *H. didymator* IVSPER gene U13 (Table 1; Supplemental Table 1). Note that this gene is absent from *G. fumiferana* genome (Darboux et al., 2019). The *B. anurus* U13-like gene encodes a predicted protein which is similar in size with the *H. didymator* protein and with which it shares 44% identity (Table 1, Supplemental Fig. 1). The second most transcribed is a long transcript matching in a short region with *H. didymator* IVSPER gene U20 which is also specific to this species. U20 encodes a very short protein sharing 48% amino acid identity with the *B. anurus* U8L predicted sequence (Table 1, Supplemental Fig. 1). The two other most transcribed sequences in *B. anurus* ovaries with IVSPER similarities correspond to the genes U8 and IVSP4, for which homologs have been detected in both *H.*

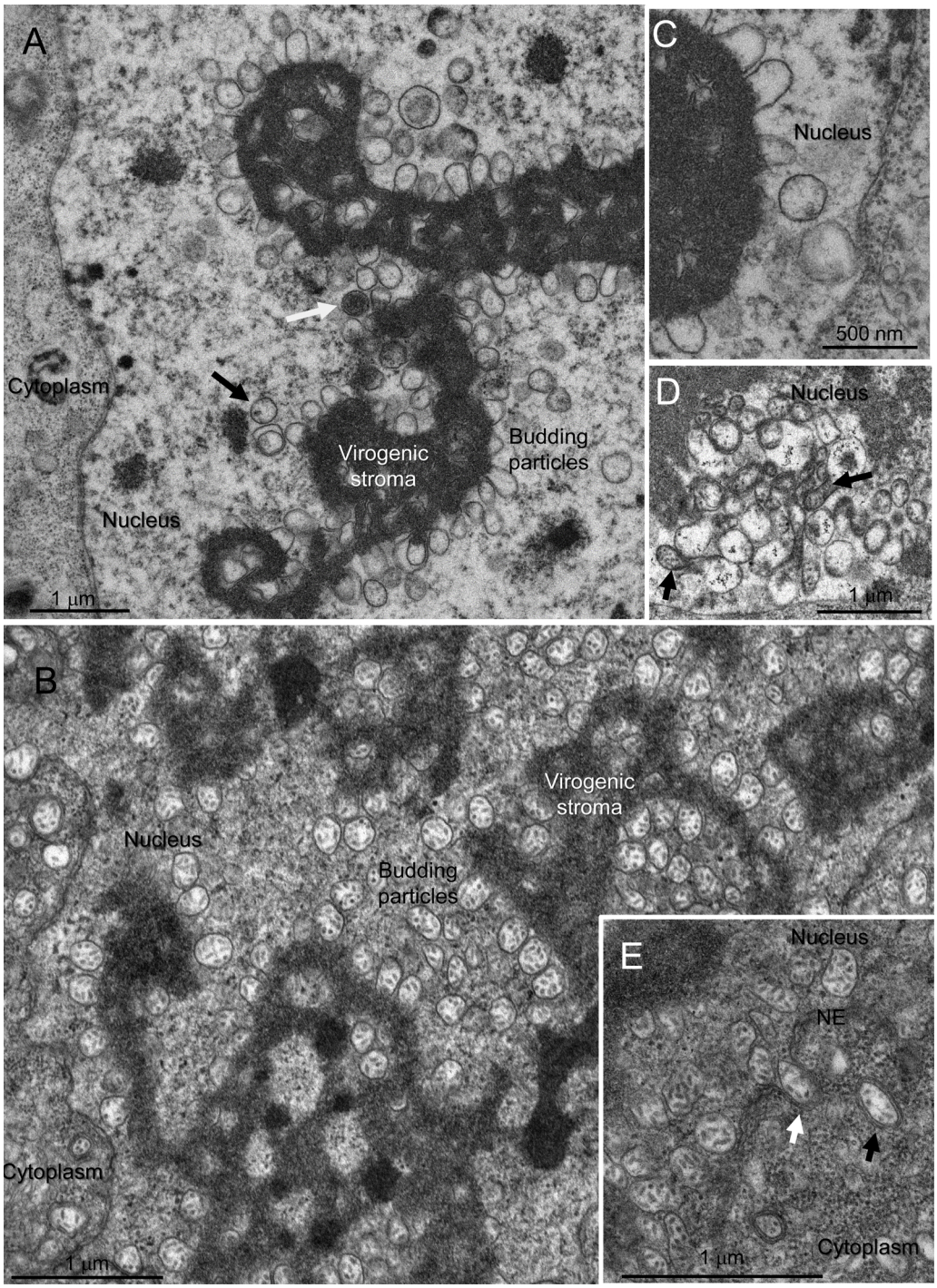

Fig. 2. *Bathyplectes* particles formation within the calyx cells nuclei. A. TEM view of the calyx of *B. anurus* at the diapausing pupal stage. Electron dense fibril-like structure (probably virogenic stroma) is surrounded by spherical envelopes apparently empty (black arrow). Occasionally some contain electron dense material (white arrow). At this stage it is difficult to determine whether the envelopes are migrating towards the virogenic stroma or coming from the virogenic stroma. B. TEM view of the calyx of *B. curculionis* at the adult stage. Close to the virogenic stroma, numerous enveloped particles containing punctiform inclusions (probably nucleocapsids) can be observed. C. Detail of the calyx of *B. anurus* at the diapausing pupal stage showing envelopes at the vicinity of the nuclear envelope (NE). D. Detail showing particles in an older diapausing pupa. Although the majority of them appear empty, some begin to contain the characteristic punctiform inclusions observed in adults (arrows). E. Detail of the calyx of *B. curculionis* at the adult stage showing particles budding from the nucleus to the cytoplasm through the nuclear envelope (NE). One particle is budding (white arrow) whereas another can be observed within the cytoplasm surrounded by two envelopes (black arrow), suggesting that the particles may acquire transiently a second envelope originating from the nuclear envelope.

didymator and *G. fumiferanae*. Actually two genes belonging to the IVSP4 family have been detected in the *B. anurus* transcriptome, one more similar to *H. didymator* IVSP4-1, the other to *H. didymator* IVSP4-2 (Table 1, Supplemental Fig. 1). Overall most of the *B. anurus* genes with similarities with IVSPER genes are highly transcribed in *B. anurus* ovaries, strongly suggesting that they correspond to functional and biologically active genes, therefore probably involved in *B. anurus* particles production.

Interestingly, we found in some cases contigs containing several IVSPER-like genes. This was the case of the contigs DN10347_c3_g1_i1, DN10385_c0_g1_i2, DN10351_c0_g1_i3 and DN10406_c0_g3_i3 which contained U19 L and IVSP4L, IVSP3 L, IVSP1L and U7L, IVSP2L and NgeneL, and U10 L, U11 L and U12 L, respectively. Presence of multiple genes within the same contig suggests that they may have been (mis) assembled due to overlap of genes' UTRs. However, this succession of genes in the contigs matched with the order of their homologs in *H. didymator* IVSPERs (Fig. 4), therefore suggesting some synteny between *B. anurus* IVSPER genes and *H. didymator* IVSPER genes and organization in clusters of the *B. anurus* IVSPER-like genes. A future sequencing of *B. anurus* genome would allow to characterize more precisely the

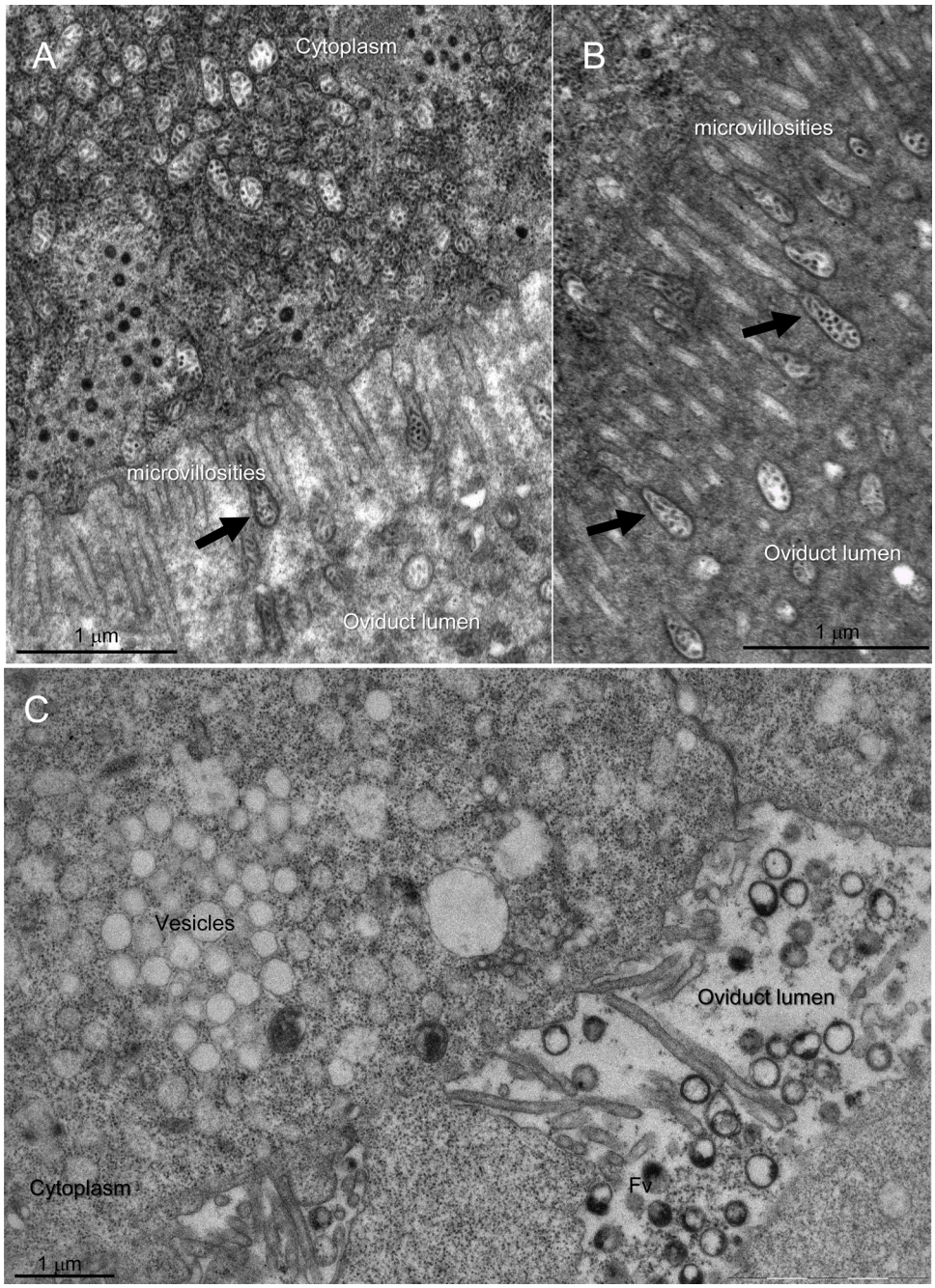

Fig. 3. Exit of *Bathyplectes* particles from the calyx cells. A. TEM view of the *B. curculionis* calyx at the adult stage. Numerous particles are present in the cytoplasm (white arrow) and some can be observed in the microvilli (Mv) of the calyx cell (black arrow), by which they bud towards the oviduct lumen acquiring a second outer envelope. B. TEM view of the *B. curculionis* calyx at the adult stage. BaV particles exit from the calyx cell at the plasma membrane harboring abundant microvilli (black arrows) and accumulate in the oviduct lumen. C. TEM view of the *B. anurus* calyx at the diapausing pupal stage. The cytoplasm is filled with vesicles (Ves). In the cytoplasm as well as in the oviduct lumen, vesicles containing electron dense material can be observed (arrows) that may correspond to "immature" particles. Mv: plasma membrane microvilli.

IVSPER-like genes organization.

Altogether, the large number of IVSPER genes identified in the *B. anurus* ovary transcriptome, their high level of transcription, and the putative conserved organization in clusters indicates that an IV machinery is involved in the production of *B. anurus* particles, that could indeed be considered as IV particles.

3.3. *B. anurus particles probably carry viral genomes with similarities to other campoplegine IVs*

Ichnoviruses carried by Campopleginae and Banchinae have both been sequenced, revealing quite surprising differences between the two. Campoplegine IV genomes include about 50 molecules generally ranging in size between 2 and 10 kb. They encode about 150 genes, including members of 6 gene families, such as the viral ankyrins (Vank), the cysteine-motif (Cys), the viral innexins (Vinx), or the "N-genes". In campoplegine IVs, the most abundant gene family is the "repeat element" (rep) one that may include more than 30 genes (Tanaka et al., 2007; Dorémus et al., 2014). Conversely, banchine IV genomes are more fragmented and contain a completely different set of genes (Djoumad et al., 2013). The most highly represented gene family in banchine IVs corresponds to the protein tyrosine phosphatase (PTP) genes, with 20–30 genes identified in the two sequenced genomes. The other conserved gene families include the NTPase-like gene, the BV-like and the MULE transposase domains encoding genes. The viral ankyrins represent the only gene family shared by both IV lineages (also present

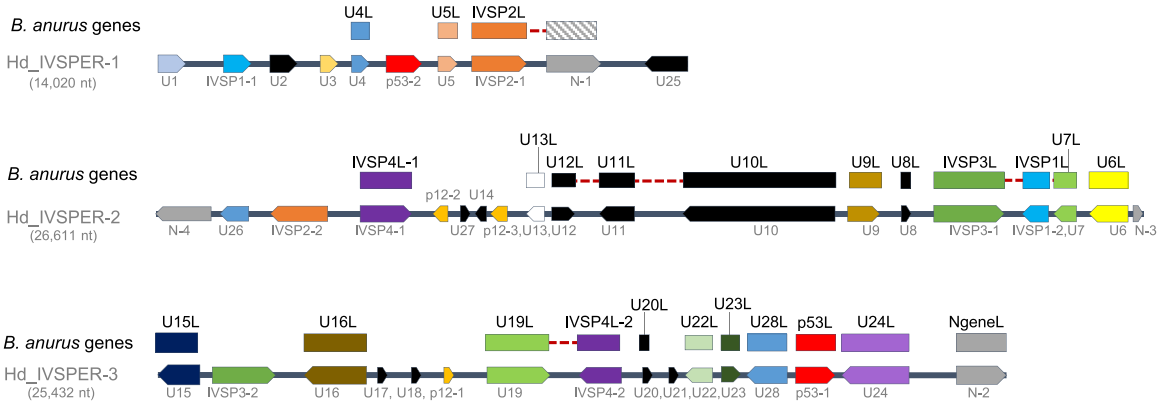

Fig. 4. IVSPER genes in *Bathyplectes anurus* ovary transcriptome. Schematic representation of the IVSPER genes identified in *B. anurus*. They are indicated in the upper part of an alignment with the IVSPERs published for the campoplegine wasp *Hyposoter didymator* (Darboux et al., 2019). Directions for *B. anurus* genes were intentionally not indicated as the orientation of one gene relative to the others in *B. anurus* genome is not known. Dashed red lines indicate clusters of genes that were detected in a single *B. anurus* contig.

in bracoviruses, the polydnaviruses carried by braconid wasps).

Bathyplectes particles morphology is quite atypical. Based on the literature, our observations in *B. anurus* and *B. curculionis*, BaV particles more closely resemble banchine IV than campoplegine IVs. However, the nature of the few genes identified in the *B. anurus* transcriptome with similarities with genes harbored by IV segments, thus putatively carried by packaged segments, strongly suggests that *Bathyplectes* viruses are more closely related to campoplegine than to banchine ichnoviruses. Except for viral ankyrins, no typical banchine IV gene was identified in the *B. anurus* transcriptome: indeed, no PTP or NTPase gene was found. On the other hand, a number of sequences corresponding to repeat element genes, which are characteristic of ichnoviruses associated with campoplegine wasps, were identified (Table 1; Supplemental Fig. 1; Supplemental Table 1).

Regarding the levels of transcription, except for some repeat element genes (for instance contig DN7233_c1_g1_i3, FPKM = 1984; contig DN9406_c3_g1_i3, FPKM = 1103; contig DN9191_c0_g3_i1, FPKM = 1026; Table 1), the *B. anurus* genes matching with IV segment genes were transcribed at lower level compared to those matching with IVSPER genes (Supplemental Table 1). This is expected as viral segment genes are genes transferred via the viral particles to the parasitoid host where they are expressed and involved in host physiology manipulation.

Therefore, based on the presence of one of the most abundant and characteristic gene family of campoplegine IVs, we can conclude that *B. anurus* virus particles probably contain DNA molecules containing genes related to campoplegine IVs.

The genetic resemblances of BaV to campoplegine IVs, despite the host group of the wasps being Coleoptera, brings up the question of whether *Bathyplectes* are truly campoplegines, or something related at some level to camploplegines. Current taxon sampling in molecular phylogenetic and phylogenomic studies of Ichneumonidae do not allow a definitive answer to this question (either not including *Bathyplectes*, or based on only a few genes that conflict in inferred phylogenetic relationships). Classifications based on morphology reveal the following:

Originally, all of the wasps in the "Ophioniformes" (including the current Campopleginae) belonged to the same subfamily, Ophioninae, one of only a half-dozen or so subfamilies of Ichneumonidaeat the time). Townes, in his revisions of ichneumonids of many parts of the world (see especially Townes, 1970), began to recognize many more, so that the classification started to converge to the current number of 39 subfamilies recognized. The definition of the Campopleginae (called Porizontinae by Townes) has been more or less stable for many years now, with only some controversy about whether 3 or 4 odd genera (not Bathyplectes) should be placed there or not.

The relationships within Campopleginae are another matter. Wahl (1991), based on comparative morphology of adults and larvae, recognized 5 "genus groups" within Campopleginae: 1) the *Bathyplectes* group, with 5 genera, was considered the most distant or early-diverging of these, sharing at least one character with the Ctenopelmatinae; 2) a *Gonotypus* group; 3) a *Nemeritis* group; 4) a *Menaka* group; and 5) a *Dusona* group, containing almost all the genera we normally think of (all those except *Bathyplectes* that have been surveyed for PDVs and VLPs). Wahl's classification would be definitely consistent with the idea that *Bathyplectes* might have different-looking PDVs than the others. As remarked above, molecular phylogenetic data neither strongly confirm nor reject the classification at this point, but there is certainly no evidence that *Bathyplectes* are not campoplegines, as the little molecular data (28S rDNA) available place *Bathyplectes* squarely within the Campopleginae (Quicke, 2015).

4. Conclusion

The work conducted on the *B. anurus* pupal transcriptome therefore confirms that an ichnoviral machinery is highly expressed in the ovaries of this parasitoid of a coleopteran host, and therefore most probably involved in the production of BaV particles. Due to the lack of *Bathyplectes* laboratory colonies, we were not able to analyze the particles and their content in proteins and nucleic acids. However, our results suggest that the BaV particles contain proteins encoded by IVSPER genes and DNA molecules encoding genes such as viral ankyrins and repeat-element genes and are therefore related to campoplegine ichnoviruses. Deciphering the function of *Bathyplectes* IVSPER genes, which are involved in particles assembly, will allow us to better understand their atypical morphology whereas deciphering the function of the packaged BaV genes may allow to understand the unusual host range of the parasitoid producing them.

Acknowledgements

We thank the quarantine insect platform (PIQ), member of the Vectopole Sud network, for providing the infrastructure needed for insect maintenance. This work was in part supported by the Agence Nationale de la Recherche (ABC-PaPoGen, ref. ANR-12-ADAP-0001) and by the “Département Santé des Plantes et Environnement” from INRA.

Table 1
List of selected *Bathyplectes anurus* transcripts displaying similarities with ichnovirus genes. For each transcript, are indicated the ID, the length, the obtained FPKM value, the corresponding gene name, the amino acid sequence of the predicted protein, the size of the predicted protein. For each predicted *B. anurus* protein are indicated details concerning the best hit obtained following BlastP similarity search (against nr NCBI database): the e-value, the NCBI ID, whether it is encoded by an IVSPER or a packaged IV segment, its name, the parasitoid species, the protein size and the amino acids identity with the *B. anurus* protein.

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
DN10347_c3_g4_i1	589	**7,646.28**	Ba_IVSP_U13L	> Ba_U13L_aa MSFVSAILNEIFFNGKEKYAGDNP FLDALPIFNCPLYYARMLNEHWNS- GEPSPFKPKT LFTAEGITVSVPEGDNFRPVKRHAS- MPIERPYLL QEIKDVHTYGREIMNAESHGSEDD- AKAEDGE DEDEMLLNSIFGNLDEGMDDVDQS	147	1,E-20	ADI40471.1	IVSPER	U13	*Hyposoter didymator*	152	52/115 (45%)
DN8671_c0_g1_i1	6,494	**4,533.64**	Ba_IVSP_U20L	> Ba_U20L_aa MEERKASRLRVISRRPYNQKN SEAIVQTALKI HARLEAMPIDEPLSPDLRAVTHCD- QCSVR	45 (partial?)	3,E-04	ADI40485.1	IVSPER	U20	*Hyposoter didymator*	70	26/54 (48%)
DN9501_c0_g1_i1	1,290	**4,247.41**	Ba_IVSP_U8L	> Ba_IVSP_U8L_aa MSRDTNTGRRNRR KASSERSKGVGKMLTPIDELLRSLI- AKTINE RQQWADIVLDSVQPVYTSDT FTYINKKTGDEVLYA	79	2,E-14	ADI40466.1	IVSPER	U8	*Hyposoter didymator*	77	37/75 (49%)
DN10347_c3_g1_i1	5,107	**1,100.42**	Ba_IVSP4L-1	> Ba_IVSP4L_aa MTPCSMIRSKQCVSLNTVSVPCVP- LKHPK MIKFSSKTYFDISFVHTGRKVYTVE- EALRTVRTVVSWATASIDKVKEILK- ALSAWKFPTPRKSRNMFDLAFRFG- LQHFYRYLCICEDNKLLYDETYFDD- LYDYLGESGYLSGQVGQLSSEMICE- TLAFIRDHDSSIDATCSANKKEAEQ- ERTKMKEFRERDFVTLFKMKSFID- DDSVAGSDPADPVVTTNPAEAKN- VIVSFISCLLSSESQVNHSVLDFFSK- QWLSEIYKGCYQFTSVEAFCMFFN- NVSVYQFSDRVMFYMPDPREKFT- RRLVIRETCLSGTGDPADCAPEDP- DCPKCDMIRLISGVDIVENGSNLEV- EALASKKLNRKPSMAIRMCPTGDA- IMSIRGNLKTISEILCKNKEQSDGAY- WSDGATVSDDDSASTDVDDIRLSC- RVKKRAAKRRKRGKLI	437	2,E-132	ADI40475.1	IVSPER	IVSP4-1	*Hyposoter didymator*	446	201/396 (51%)
DN10347_c3_g1_i2	6,099	**332.45**	Ba_IVSP_U19L	> Ba_U19L_aa_partial MVHHFVDRIEYRPVTANTSETSNA- TKTNLALMLLNLGISLASLGSFDINI- PLVDSDLLPASPSLVKDVINAFDKM- IQKLRWLVIGSITNVSLFTEWDALT- PLKLPSVSNKNDAQLRATLCNRNE- RLEYYNGPPTAPADLSCAWSKLSEI- ALDFNVCDNGSIILCTVLNRCSFLK- TAKLSNVCSLDDGFLNDERNLCTP-	267 (partial)	3,E-109	ADI40483.1	IVSPER	U19	*Hyposoter didymator*	660	174/281 (62%)

(*continued on next page*)

Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
DN10347_c3_g1_i4	2,423	**2,515.27**	Ba_IVSP4L-2	MLNCLHEKCHVVCVVELLKMLKK-FGIKKLYLTLVADPHDHCQKKMYN-LDKIASYTGEYSEIYSNLLHIT > Ba_IVSP4L-2_aa MTPCSMIRSKQCVSLNTVSVPCVP-LKHPKMIKFSSKTYFDISFVHTGRK-VYTVEEALRTVRTVVSWATASIDK-VKEILKALSAWKFPTPRKSRNMFD-LAFRFGLQHFYRYCCICEGTKLVYD-ETYFDELTEYLNEGSYTSGQLMWL-PPPLICETLLFIKDYNESIDPQPELG-NNNAGNPQSAPGSTLNVTMSYTE-AKRAIVCFIKEVLSLESPVNDSVLDF-FSKQWLSNMYKGCYRFTSREAFCV-FFNNIGAFEFSPHVMFRVPDIHEKF-TERLVIPEITVKNEQIQTTSKVSNS-NMHSGTKGTDDKISDILGQVCGFK-LIENAPTSEINAIAMKKLKRIPSMVI-RMCPNNGETMSADGGQFRTAGEI-LGTLH	373 (partial?)	3,E-164	ADI40484.1	IVSPER	IVSP4-2	*Hyposoter didymator*	431	221/398 (56%)
DN10416_c3_g2_i2	5,617	**2,246.17**	Ba_IVSP_Nge-ne-1	> Ba_IVSP_Ngene-1_aa MNTLDDDDSRGSIGSPIRDEFDHG-SISEFEVYLDEVYNDDAENSEAEND-HAVSPPVVESTSPEPPPVASLKKIP-KRKTPAAKNFPIVKSRKTDDIAQA-VNAGAFEKKKATTVPRKRPTVEPV-AKSDSAELGVETPAAKRKPVSRPK-HPVEGGIHPLPIDESGKKPKTAVRR-SRKLVATATNQPPVAKGRRDVTR-QPDEVVSSAQAMTEVPALTPFVVE-AQVHARPHVNVSRQNVTFKETPA-SSSSVVNSEFADNRFWMGILADDF-KSLDYNALLKNSQFTEQQTYVLQQ-SFYQLCRKLSDICTYSDRFMKTHKC-IECEFSISHQCVSINFTQYVPSSADL-TGVTTITSQSTTMCICQFSFFHSHA-TQTRVKLVNNLSLRGSSRMLEHNR-SVSVSCPRCHMNVVMKNRNHDV-CCDFTNWNSLEGANRRQFYGSLE-NRLQTAKMSKPYLNELYACSKQCC-HLFHRCVENAIANNTIPMRPQ	479	0.0	ADI40491.1	IVSPER	IVSP N-2 gene	*Hyposoter didymator*	492	314/492 (64%)
			Ba_IVSP2L-1	> Ba_IVSP2L-1_aa_Cterm DNYTCYEITQGLSRSKGHFHFMPP-MLRHGYLILKMPYIRAMVTSKSTD-YLFKLNAKMMMVEKIEHIAPPLCF-DIRSGKVVDDTEAIFMSDGCSDVD-WADGVENFISLPSTRDLSKLTAAAS-KLLAISSTSHGPSQNSDDADSKAVA-DVSEDDDGNITIGESLEQYKTLSRA-IHDFIKTIEDEQNWSPSVVDLLEFV-NRFIRVMKRKKPSLLKMRSGVVIQ-NMSKVPELSIAGSEDDQTSAAFED-	308 (partial)	3,E-121	ADI40459.1	IVSPER	IVSP2-1	*Hyposoter didymator*	509	191/308 (62%)

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Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				LVKKSYNMFILHRDADEIMRSCWN-EIFSSVEEAPGLSGLRALNKRTRSST-SLVSNDVKHRKIKR							
DN10416_c3_g1_i1	1,780	**1,659.95**	Ba_IVSP2L-2	> Ba_IVSP2L-2_aa_Nterm MATRANLAAYQEESAAFEDRSVTL-KSIQQLRQNVIGQLDAFNELFGLSN-PLIPLSYSLHSKSKYEFVDFGYKLHR-EHEVLIGPIDMYGLCTLTGDNRSN-GKVEADVNQRNERFMMFIPNCDN-MSNAPSGSLLSLILRDMANKLELVL-RNEEVVNKITSRYPKFNGSAFAKN-KESIFQKYITGKESDARGLIRLRVD-VAPTRGETYNCYEIAQGFCRSKGH-FHFMPPTLRHGYLVLKIPLIRAMVT-SKSTDYLFKLNAKMMMVEKIEH	267 (partial)	9,E-123	ADI40459.1	IVSPER	IVSP2-1	*Hyposoter didymator*	509	175/267 (66%)
			Ba_IVSP_U5L	> Ba_U5L_aa MLLTVAATASISYAISYFISSLGTSK-RCDELREQMRAVEVKYYRRNGGN-SVCILWADQQCAMWHNQKLYIYD-YWNSKNEAGASTFNIANLYLRSSE-MVLVYDFHERRKYMQKHFPNEY-MLREMFEVNDFPIPEEIMMDFRTS-RDDNFPMLVKRLYTVTDELNIISVI-YSALEEKILT	177	9,E-73	ADI40458.1	IVSPER	U5	*Hyposoter didymator*	176	111/182 (61%)
DN7233_c1_g1_i3	587	**1,983.84**	Ba_repeat-element-gene-1	> Ba_repeat-element-gene-1_aa_Cterm LYMSKFLDFVDYRNFIRSFCPNYEK-SDILRAKLWRLSTHEVTIPFLNGKLI-QIEYNYDPSRTEEERVLINVDSMLP-IFGGWVSPAMDGFRSASKLHNFIT-MHIHLNRCSGFQYACCPCHLPNV-NASSARAFERPSVTA	138 (partial)	1,E-50	BAF45626.1	Segment	f3.2	*Tranosema rostrale*	237	77/136 (57%)
DN10385_c0_g1_i2	5,252	**1,342.13**	Ba_IVSP3 L	> Ba_IVSP3 L_aa MSLLRKILGSWVYTYISNRLDDDV-DI EQYRVDLAVPGPGDEMYRFYTFLL-KTEIIMRDEVHGGRF EQWLKEGLHFKIPTEEFGKPITWK-YLKCLCRL LHSFNTLKNDEKFISVLKKHVSDVV-RNLDTLEKTEPVYASLLFALQLNG-NLRNTVSVFNSLDELMHRLLATDD-PLVNGLFKLTKGQSYVYVNSVLDS-ALILFEPILQYLPADENKSLTIINLVS-ERLPEPLLQQTLWKNLATSPLNEV-GSSDVRRVRSSKQEQGSLCWEFIT-GLTSQEYYDDVLTDKIFEKVDFERL-RSPVKATLAHDDGNEHDHANTVD-KQQVFKLSMYSMSTLLLLSSKTMV-HCHCASKTIQFISLIFSTCDEMKLR-AQSLPNSKVLVFAAHELAKFPMVR-PSERFHFRNIFITYRQADIFERPTGT-	629	0.0	ADI40465.1	IVSPER	IVSP3-1	*Hyposoter didymator*	630	405/626 (65%)

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Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				IFQHKYLNELNFVRILETSGLEPTH-ETLLHLLLMLKNDSSVVLPDFLHSQ-LVRAIEQSFGLGRITSDFNNPEVIE-MYRTMSEKNLLLRLHGTGVYDAIN-IFCLSYVHKMAYFAQGVVINATHLI-PLLHLIDLNTRNKEYPITWAMLQM-YIRQMPGRHINAVDLTSPSVDNQQ-VETRTMFKYLSLVVNVMIMEQTK-GEYTMVDLIEHAGGITLY							
			Ba_IVSP1L	> Ba_IVSP1L_aa MHDNTATNSKPIEVVSAVVLDSDD-IPQNKLAYFTKDRNRRSRLSDALN-TIHKIATEPQVLAWVAAILFFAGSV-SDLFLDFTYITNHSLLVAGSIFWMF-HALAKKEYPMFLQHVVMSSISVYV-FTKQLNVGKFPYLTAALLCTSCAV-YRHENVMSTDLGPCPNATNANLD-NTDPMPWSPRPPICELETINIALDE-KVNIVRKLEEILVDIKGNRGEDGPL-DNSEYHHVYIAARAQMNDILHSLE-TKLAQC	249	7,E-71	ADI40464.1	IVSPER	IVSP1-2	*Hyposoter didymator*	196	104/194 (54%)
			Ba_IVSP_U7L	> Ba_U7L_aa MPHTSSKQSINDILGERVFLLNTSG-TLENQQLESHSNHNDNLRPPTTAE-QFFQPAQNISGSVQHIDQPVIFKEQ-YKVCNSVEKMFSCNGNSANLVNT-TDSLRKKHTRTLWSYDNLFKILMLL LLFAIYVEVRKSDGSMVDGQSFDS-FNNSFITSAVGYGSDTFMEKIADAC-TYGRQTYDTMYQNAKLIYHRIKKA-LSLSAS	201	8,E-70	ADI40463.1	IVSPER	U7	*Hyposoter didymator*	212	129/203 (64%)
DN9406_c3_g1_i3	1,849	**1,102.93**	Ba_repeat-element-gene-2	> Ba_repeat-element-gene-2_aa MSQSPSTSQLPTSEPIVLPFDIIRYM-SKFLDFV DYRNFILSFCPNYEKNVDLRKEVW-RMSTHRVTVPFINGKMIDMEYNY DPSRTEEDRVLINVDSCLAIFGGWI-SPAMDKFT TLSKLRHYIKKYVHLNICSGLQYAC-CPCHFPNVDDPSVQAFERPSVTTC-KYGHYHHYCWRHVVAWIVQVHF-FVEELENKLPLAEEYARLDIRVLDK-AVFFPGK	216	4,E-61	BAF45626.1	Segment	f3.2	*Tranosema rostrale*	237	92/181 (51%)
DN9191_c0_g3_i1	478	1026**.46**	Ba_repeat-element-gene-3	> Ba_repeat-element-gene-3_aa_Nterm MSQAPSTLPLPTSEPIVLPLDIILYM-SKFLSFVDYRNFIQSICPNYDESDIF-RPTLWRLSTHKITTKFLNGELMQIE-YNYDPSREDRVLINLDSLLPVFGGF-VLPAMDKFTSPSKLRNFIAMHVHL-NMCSDRRYACCPCHLPNVDHHSA-IAFERP	155 (partial)	4,E-65	BAF45610.1	Segment	d5.1	*Tranosema rostrale*	229	105/ 156(67%)
DN10332_c3_g1_i2	2,637	**768.56**	Ba_IVSP_U22L		270	1,E-54	ADI40487.1	IVSPER	U22		257	

(*continued on next page*)

Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				> Ba_U22L_aa MNKKYVKLVTKKLMVRVATGLES- KAAKKIFSRVLSVALKKIAARIAVK- AAAKFAVRYAAAAILACTGPVGLA- IDVIFVILDVISFISDLIDVNNYALAY- TRETVQEMQNSLEDEWKKETLEKL- GSGIMDPEYPVEVNYLGVSSTQEW- TIDTDEVTAKSLSYSNTLLLNLNVN- SLGEVIDLKALGDSGADTDDRLLT- HPPSWNECVQQSIQSIVKSNERAM- IIQHELDGECKQINALASISWITFLT- VGCTIALTALKRTTFTRHATNKPK					*Hyposoter didymator*		94/251 (37%)
DN10351_c0_g1_i3	4,957	**755.81**	Ba_IVSP2L-3	> Ba_IVSP2L-3_aa_Cterm TLRHGYLVLKIPLIRAMVTSKSTDY- LFKLNAKMIMVEKIENTAPPYCFNI- RTGKIVDDTEDTFMLDECSDDDCA- DNVESVITIPSTRELSKLTTATSEPP- SILPASHGPLQDFNDDDSKSGPDA- FEDDDADITTDVSVDRYKTLSRTIN- DFIKSIQNEGNWSPSIDNLIEFVNR- FIRALKRQKASLLGTNSGTVIVNVN- KVHELSVAGGEGNQTLASVEDLVV- QSYEKFRLNCDADEIMRLCWNETL- SHIERTLELTKVQTVNKRKRSSTPT- INNATKTRKIKH	284 (partial)	1,E-114	ADI40459.1	IVSPER	IVSP2-1	*Hyposoter didymator*	509	180/282 (64%)
			Ba_IVSP_Ngene-2	> Ba_IVSP_Ngene-2_aa_Nterm MHAHDDDDSRGSVGSPTQGQFVR- GSVPECEDDSEEEYNDDAGNSGVE- IDHAVSSDDEFDSTVVIPQPLPVVE- SISPELPTVPSLKKITKRKTAAAKNA- PLAKTRKSDNNAQAANAGGSEKK- KAIIVPKNRSIVEPVAKFDSVEFAK- RSPATKKKPVSKAKDPEEERSHML- PIDETRNKPKVVVKKSCKLVATAT- DEPPVKKGRRDFKHQPEDVSSPQV- TNEVLSTSFSVEAEVHASPQANVV- RKHKTSSKTSAKNFSVLNSESADR- RFWMETLAGDFKSSGYNALLKNS- QFTEQQTNVLQHSFYQLYR	308 (partial)	9,E-116	ADI40491.1	IVSPER	IVSP N-2 gene	*Hyposoter didymator*	492	193/309 (62%)
DN10165_c1_g1_i1	1,941	**497.28**	Ba_IVSP_U15L	> Ba_U15L_aa MESFIFMASGTLAMTKIIQKCLDVS- TAGTAASLTREPPLILSNDDSERHF- QTRTYFNIDKTHYPPPVIDVHELQ- TASCNNTDECTVRFGDGFSCILIAR- PVQLTITIDGVDKKIVYDADINSNV- ASSNQSLDCKRCVLTGQSRRLLKC- NALTSVAVGSAVYSPQTGRFNAH- WVCESLYPDMFSKDSESGDITISHA- CGAAENRGQLVHEETGLSWDEHY- KRYGRYDPADSLVCECYPFPFKPE- DVPANRSLYSDNIIVLPSTSTCRRST- CAPGRPHLSEPESCECPRGYVSCPV-	367	1,E-142	ADI40477.1	IVSPER	U15	*Hyposoter didymator*	410	198/384 (52%)

(*continued on next page*)

Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				IGLNTNEQFHRRLCWGSYVRNHE-GHPMPTCVRDPCLPRGTLDASSG-MCVSNKYEEVKAFNEIHVLPKVTW-SIL							
DN8699_c0_g2_i1	483	**418.96**	Ba_IVd7.3 L	> Ba_IVd7.3 L_aa FFFYYSFFPPVLRLAPITGSARVSSR-SPPGCLVRISNETPMRPHPTSPSVA-HLPWHSIPWARGNCGKPEPLQPV-PGFASRSRSYVTSC	88 (partial)	6,E-13	BAF45618.1	Segment	d7.3	*Tranosema rostrale*	117	32/43 (74%)
DN10436_c0_g1_i1	3,628	**345.61**	Ba_IVSP_U4L	> Ba_IVSP_U4L_aa MVRNPPKRRRRHNESNRRETPTT-VLFLKFLSTTCSTMLAFVIGRQFW-HTRPAKWTMEHDALLRYRKTRLV-KFCHGFGFGEQREKYLLRLLESSVT-NNDETSLIMHILFPYLSITEEFAPSD-NALGN	126	2,E-34	ADI40456.1	IVSPER	U4	*Hyposoter didymator*	131	58/115 (50%)
DN10332_c6_g1_i2	5,354	**318.96**	Ba_IVSP_U23 L	> Ba_IVSP_U23 L MVDYKQTSWCANTTIQKNDPVLS-DAYLIKIIRTFFELDDLTISCNDMLT-KILSMFRNAIARGGLQRILDGFMLF-LCGKKENSHSHIPRIVTYWDLKILI-AGLIRRFSNDKTLKDVLELDNGNG-TTDIYSLRLNGNMLSTMATEAHAG-ISLLPPRKIIVLDPKQAAVLVFLFQA-YYSNNSVIDDSSVHNFMRELERIDE-NIHGSIVVKESELRAVKSCTRESLL-KTYCGTSLVVQRYIMGQYSYIFSM-LNECFLRFDFLNTDRLPETDPRRQ-AKSLMLNYLTVFIGWNTSPPEWSV-WSKNINEVGVMTNLYDMLNSGAI-SNDFLSYVLLKSLLFTEPGGDGEQA-SYGDQFSANQREVDSLLQKGQYK-NINPYIEGVTRKDPLGINKEKLKVL-KKISEALLRLRGGTTTSE	409	2,E-155	ADI40488.1	IVSPER	U23	*Hyposoter didymator*	415	229/406 (56%)
DN6598_c0_g1_i2	3,083	**288.46**	Ba_IVSP_U24L	> Ba_IVSP_U24L_aa MKYFAKMDIAPYHLQVERVFSRLA-DLVLNWEDDEPGDSDGEPMVFYL-FMIENRNQRKNLAVESFCDVGMN-QISSIEKDSAEHALIENHKGHTDST-SCAIGFCQSQSNFVPMEAHESCNH-GKADAGMVSNRGIFRSVNQNEVE-VVLTMAHYYPWLLGDIGSVFATLP-DVWQLRVEILFILGELMFGYLSKVE-SFLNVNTSRASLQHDKQSCNDAA-QTARMYFQLLDAVCARASKGKKCI-ADLIKQSLASHGHQSVKLILKSKIM-HSYLKNRMNCPVRDKKFNGQPFS-RLTNVFPSDCDLALIFSNVHTWRSF-VGFLQKMYDLNVELFAAYTMCPD-QNDLLIVLFDDINTAQQSAANRFG-STPWNCNYGYLDEPLLQRALRKTH-ITQSYMYGFLHDISKDVTCTARLFR-	529	0.0	ADI40490.1	IVSPER	U24	*Hyposoter didymator*	563	372/564 (66%)

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Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				NLTTINGNMETIVKDLCGTVRSTM-LQGMPNDLLLGALHITRLAENFGI-ASSCGFMEYMLRNESVKMSFRNV-VDTFLNSIRKNAKHAIIDEYVFLHR-MCHVRSALCEFNVFANVIVSQTL-PKE							
DN10406_c0_g3_i3	9,174	**235.02**	Ba_IVSP_U10L	> Ba_IVSP_U10L_aa MEPTAIKKCFSSEIQPLASGFEELSD-NGRVVKRR HKVTTGVNLKYYEFILTLEKICSNIA-SVPENAYSSVIQEL FRTLANYRVLICDPMANMLVDAL-QRLSELNIIYVLKQTELIVMNILLPE-TVKDSDAIHMFTISLRL LVLNMFVCGLARRDLHNAISFSTR-ERSTLDYELDVQCAAMHTQSN HCCLCIAENYPKRDIDERGADLILD-DVRKEFVNYSRITTCKCIAN SNLCDQCLCSLRESLYLTYIFYKFED-EKKSKSMRDKNA ELRIFKAKSLAKREARLRATTEKIKL-QAAGTNTPPVLN KRVRCNLTKELLETPVNTDIDHQK-SQKIVYEDSDLQLRVRKS GAIGFKRKSANGYDEIVILSERKDP-PKTAHKLMSM GLPSGKFRRGRLPERSNTVSRTFIN-KLINDLNPQTRKRSEEI PRAQSSILCAKKPGLAQRFVKVIST-ALLNNERLLT ESVIAELCKVVLKLRGSVLEMTRCN-CEECKNTVSDALSTEDMRPIILALF-LHNEILNSNFLMDICNDKMFMSLQ-MPRRSAYKMYFNIVDNRKSITKDK-LAEARTRIHNITSSFMLYDVSKGVA-KPISLCFAIRKYGTQLHDMYRLDLN-AGLRNPENNLVWLKNCTKNDAAL-FLYKSEQESSLIGQATLHYNTAYHT-YHASKPEDNKTSFSEMFNPWSSAA-GTSILRQGPAVTSRTLNRYNKMRN-RPKYAAISGVAVKSVKR DFRVNGDGINRKRRSDVHRGKEAI-LCCQANESELEATILQMFDSMLLG-HLLR VYCNILSNQIECHDTDYRGFYDQF-HYLNRTRSTERYES DIFVLIADRTFKATEKVMIEGNVLL-RNKGPLSMKNIPRIVKYYARSTLD-AFTKFEFNGTLIVLFRPTGNNYFVI-QFENDRSIIVQHIAKCILVELVSKDP-LDADAPLTSTERYFENVDNALFSR-NEVVAIQNFLNTDNMLPKADSSYS-	1350	0.0	ADI40468.1	IVSPER	U10	*Hyposoter didymator*	1355	1120/1355 (83%)

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Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				TLMFLVGGNHNDESWDEAIHDCE-RIAEVVMKVKTVAIQDRCASIVFN-KLFEAAWNLQPVSYTRQIDERTLA-CIPAYDADEICTWTREEHITLCTAA-KYAVVFVGQLLVMSDYNVLDMRL-LDCFRRRISVLKCSSRAGVVIPLNRI-PNCEKEVALFLKNGINHIENYRCIH-ENIPRHMLVHDPFAVMYTATTTA-LHLSLDSMRAIRRKNLNGMFHGR-HSNTGFLTTSANRLLLAIYAATVLI-ANTYHREYFTLKSSLLLLSEHGLNE-VNFARLRYIVQKIVLADGYIVSKES-QSYDYKKLISLLLVGEQAVLLMHQI-PLNNDRVLKSVGHYHRCLHVLYV-YRKLIDYFTSQYSDMPMECDPTVF-MLNIVSNAKKFILHFEKRTVIRVPL-RYK							
			Ba_IVSP_U11L	> Ba_IVSP_U11L_aa MEYKNSSMEFSETLLDVLNFIDGY-DLSTMDVINGHSPIPLDNLAMLIDE-DSHEEKLHHMYSNQNMTFKRPNII-LYGEDEVSVQKDDVYQSTKGVLVL-QAEAVKSWSGLIESSDLYKRHCDL-LIHEEVSAVTLDYTAMIPINTFIDTV-LIEEVTTKSQAFLKKFVLPVKELVL-HLHRCNLKLNHPFMSSIYVCKKCN-GFIATDIQHTNFRMMRELKKSNQE-NVRMMINAVLDVFTYPAILPWGIR-ENRSTVQSAVLDGKISSAYYRSLSI-RDRTSFMDFLEVFVEYYRAIALQY-RGFNISTAYYELQNLFSFGT	313	0.0	ADI40469.1	IVSPER	U11	*Hyposoter didymator*	316	245/316 (78%)
			Ba_IVSP_U12L	> Ba_IVSP_U12L_aa MTSFNLSNDMLVKRKKVTSWIIIV-MDNFDRKHLKHATCATLRLFDRV-VTNEVPTDLLQSVAVGVMYIACKA-TNIDVDIDTLLYYSGGGCSIDSLLD-VAPLMKVLHEEDLRVELLPTYYITD-LMQLTRLQGPLQPVVRWIIHLMSN-SRYLSVETTPRMTAAIALLAYNKYL-SSRSSKLLAPVLLRDFSLVIKTRRYK-RLERRAQ	203	2,E-132	ADI40470.1	IVSPER	U12	*Hyposoter didymator*	203	184/203 (91%)
DN10047_c0_g1_i1	5,425	**257.48**	Ba_IVSP_Gf-U28L	> Ba_IVSP_GfU28L_aa MYDFSHLISNNYFRSDDNGDVFYI-LDDILESKQCCKSLAKLYPKVSQRFI-DDHVKVLRSYKQ DNYFYRVIGHHFFNRIRESYCYMIR-GVTDKRKTIQKISRDPPGRG GVNILTKFELYAENRPDLNDSTLSIL-NYPPDRLRSVMNLWNTPRH ECGFSTLFDQNTHKNILWCRIEQN-HTSLVMNRQNVPRHALTSDLRTLI-TYDKFLAMTSKDRYATSTSDPGWL-	1247	0.0	AKD28060.1	IVSPER	GfU28	*Glypta fumiferanae*	1322	598/1329 (45%)

(*continued on next page*)

Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				VDKQKLRTYITAESSFGRSSYNNSA- KHAVFDAVDDVEMFSDEMSTATT- ADARLIHRECPYTMTPHSLYNTLQ- SRKDASYHVHNPQMKFSFDVRSL- VETTTLCRALSVFQDRFSGTRIMD- NGDIVAFAYDPETNDFAVEFVNGK- IYFTRMNYDDNARCGSVIRPVIKTY- PLPTADEINGARLKQAFSQQLKNC- SISRYCVRVSSRSISVILGNRSKFIIEI- ARDRLFVVYEIDMEDQDASYLRES- LVKAHEISSKVVETAKNMAEKKGR- RQRSTRNIKDRDIKVTLYAMIHGIH- DTNLLNLARHAYIPTMLCDRDVPF- FTALHKSLNNMKEFQRRIRLEYKA- VCAYSTQPCLVSIDVTGRKILSCAA- TTKLQDVEPPTALNSKTDRSHSAA- DVHAKYKFTKKSLHKRKTTYSLGE- QAESIKRYLNMGN RLVYKLSGTVEEFIDYDLTRLLYTN- RDRRVAFVVIRWDS ESTQYDIVSSGRLNPIWNRNFSDIN- MMREKQLINACDDG TADRCIAKHPELEHYDRDTGRSER- VRFLESEKISFPMNAEVAKLLSIAN- NRVNEFQVVLRQPRTKFRKSSFLR- RYGIASCQAIDICERIDSNTERGIYT- LYYYVCSLNSRFWGLGLLFYETGH- LSNIPEVDFNVCETPYTIVIRGSVAC- NGSSKSFTYVYTCNLDMWVDMVQ- LDRNCEFALIYNDNPIIINVAKYCTS- IARLFNDPRTPFYNLATLFADFYM- DQCFSSQSEECKTLVHWATISRSLC- LDMHTEYEFDIDCWTAARKIRCSN- LTVLLTVRFLLVHCSRGKVCKRTYS- EESNIYARRAMFSLTTRYLDAAMK- YNIYKELEWHLNEEGMLDSLKSVS- FNAFDFSEHVYRYIFNGTETCLRSA- KLRKIEEFDHTIFKLMLKRAMSEVF- VELAAARTYSTFVEWFKRGVCSTP- TTCVNNRPVVGGWTYQLTNIMRY- VERIIKPHYSCTEVVIKRSCETMELP- LVIERQENVDKIYRNGFAWAYERE- TRNKIKPNKTAVRSRRFPNIMMNI- EHA							
DN9620_c0_g1_i3	2,489	**243.73**	Ba_IVSP_U9L	> Ba_IVSP_U9L_aa MDIPSTVRLLGKLNVSAIKTASIIAN- LNDANVFTRQLKNISGASTSLTEYI- PFLCNVQVTDLCVVDGSGIRKTVY- SLHNNTDDERDYSALVVENIILWV- CSRKSDRIRNFFSMFFTTSTNKDVD- CDNLDFLEHVVEDFCPDMKILTES- KYCSEATVCLLVDGKSIDLRDWRS-	269	1,E-114	ADI40467.1	IVSPER	U9	*Hyposoter didymator*	274	169/268 (63%)

(*continued on next page*)

Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				TMIRVMEHWKTMFKLNRVDVTTT-KDSKINLLYDDGSKKSTLPDISQLD-NECIRDIELAFSYITPVSAPLENDGA-SSTLCRKRRASDDVSNIDCKKAN							
DN9251_c0_g1_i1	4,092	**203.54**	Ba_IVSP_U6L	> Ba_IVSP_U6L_aa MDSCHIVTLTINIQDNAFVNWAPI-FESVFHAAKSYEYCLANFNSQQPA-YQVLCTIIIKTTMSTYDFTILDSEVN-KKMWIAGKIYGYSKFAIFHLAHTID-DPLNLNSVHEICGSSGFPQSVMME-YVKKLSVSASMDYFSTAATSTFSLK-QANVTIHNAFAFCCEDNNIACHRS-VREVAGVRLIFNGRRAQFLNKLRS-LLPREYITALANSASNVQFFLNVSQ-ALIDRINSECRAFVNNHKICRELVF-YCLCCMTSHLQITSGEIDEVDDSEP-CEKQPRIQGNPVPFCQLPIEILPHNI-FAEHISSPPFLSSLLHFDIHVNCFYE-HEVENIENIRSNLLVKTHSLNTM-YNT	349	0.0	ADI40462.1	IVSPER	U6	*Hyposoter didymator*	349	310/349 (89%)
DN9992_c1_g1_i4	2,435	**94.7**	Ba_IVSP_U16L	> Ba_IVSP_U16L_aa MIKSIGGFTFQGCNEKDQPSSHLFP-RKCMVCTPNASGYNALDLFAASYK-PQIGKLK CFQEKNGCAPERGYPYRIVVDLDS-NDENLLESLLCEIRKLLRELLP QAEASDFKLIICILRKQQTGRFHIHL-LNVVSNDLITYKNFLMVLHE KIKAVDRGAGVNYFMVFGAIKAYK-QKVDPTTPVADQCYLPWKMCSAK-INEIKLNNFIDLPPFTGGSSDEYCN-DIQNYFNKYFQRSSCGTLFHLLSLH-RRYNPDFDVVLPLCQESIVRSSRKR-TSDDSDSEGKKKIRTAKSDPINRAK-ESFFENVLFKLPKNYYEEYDSWIGI-GKIIAFVKQTDGLNLFHKFSSQSRD-KYDAERVATTYEGLLEAMKVNQS-DKAAKPVIHMTSALRALLLGSNSII-DQIERKMFYKWKGDTHAIVGAVT-CCIQQMSPMLTFPHPLNANYLFAI-EFSRVDGAYRISHDTFIQSFGGQRA-TIDAGVERHEHYERILECFIYLAIKY-FNDNHRFWRMYSAHSILDSTPFKL-KQHLHAKNNVEDMRSSQHSFYRR-AVVQKLNKLKKRWLLAQSNGATIS-SKKKNKRLWSKLYTQYEFQRALDS-ARKCGRYPQSVFDKAVPRSDASS-CSR	612	0.0	ADI40479.1	IVSPER	U16	*Hyposoter didymator*	612	487/605 (80%)
DN2005_c0_g1_i1	3,05	**79.4**	Ba_IVSP_p53 L	> Ba_IVSP_p53 L_aa MPTSTIHGGGSAPTQEAARQPPGT-AQGVKPFPVQNEQSEDSNRNTKTS-ILEETDNPKTETQRIVNTETDIIGD-	397	3,E-90	CAR31590.1	IVSPER	p53-1	*Hyposoter didymator*	395	180/407 (44%)

(*continued on next page*)

Table 1 (*continued*)

Bathyplectes anurus transcript_id	length (nt)	FPKM	Gene name	Predicted protein	Predicted protein size (aa)	BlastP e-value	BlastP_ID	IVSPER/ segment	Name	Species	Protein size (aa)	Amino acids identity
				NEDADAGMLEATIDEELVAEFKVN-								
				CNEGMRLLKILKYPSTARHDEQSSI-								
				LSHVLVKERDSKRIRVGAIVVNDA-								
				AVKGKGKITRWDVLDKCPEYKRAL-								
				LSALENSSPNSVHAFHATLRYPND-								
				FVLAVVASPNDDRIPDGAHVDGKT-								
				DENLLKYNIVVRRIKHHQTGSAPS-								
				KKNLFSRWFKRSNSKSISEGRSQTS-								
				SEPVSHRIEIKKSEIRPPSNGNKKPT-								
				PNEYVPLGGIDDSQRPIQTSAQHT-								
				KDVGLDSEKRVHFDFQDGQQSSA-								
				FNEAQPTHRRRKRALTECQIIAPNS-								
				TNFALKAVLQIGSICGLSVGITYLLV-								
				KKSKLA								
DN10161_c0_g2_i8	1,803	**71.18**	Ba_VankyrinL	> Ba_VankyrinL_aa	156	1,E-27	AFH35119.1	Segment	vankyrin 5	*Hyposoter didymator*	168	62/150 (41%)
				MSLRNLLSERGTSGGTVFHDMVEL-								
				GLVGLLKRIQAAIGKSHTDILQMTD-								
				DHGDYAIHLAAKRHRGHKAMELI-								
				EVLVGMGADINAADKRYGLTVLH-								
				CAVEMGDSTLVELLCQQPRINLDA-								
				RIGVEQTPYQKTRKDRNKKNGILE-								
				RNGADNQELEWSD								

The *B. anurus* transcriptomic dataset is available in the Sequence Read Archive (SRA) database under the accession number PRJNA508369. This Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GHFB00000000. The version described in this paper is the first version, GHFB01000000.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2019.02.001.

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