A PCR detection method for mutations in receptor-protein genes from *Busseola fusca* potentially involved in Bt-resistance

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ABSTRACT

Genetically modified (GM) crops attracted interest globally when use of these crops resulted in significant increases in yield and production. These increases were due to protection of crops from pests, weeds and diseases. However, evolution of resistance by pests threatens the continued efficacy of GM crops. One such example is the resistance to Cry1Ac toxin in *Helicoverpa armigera* (Lepidoptera: Noctuidae). Resistance in this pest was due to a mutation in the *aminopeptidase N1* (*APN*) Cry receptor gene, encoding the receptor for Cry1Ac. Laboratory studies have indicated that species in families Noctuidae, Pyralidae and Plutellidae can develop resistance to Bt-toxins. To date, field-evolved resistance has only been reported in *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the south-eastern United States, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Puerto Rico, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in India, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in northern China and *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in The Philippines and Hawaii. Resistance development in lepidopteran species is thus a common phenomenon. The stem borer *B. fusca* is a major insect pest to Bt-maize in the Vaalharts irrigation scheme (South Africa). The first official report of *B. fusca* resistance to Cry1Ab toxin was recorded in 2007, although farmers observed increased damage to Bt-maize from stem borers as early as 2004. A second report of resistance in an area nearby followed in 2009. No study has yet been done to determine the molecular mechanism of *B. fusca* resistance to Cry1Ab. As mentioned, a mutation in the *APN* receptor gene is responsible for *H. armigera* resistance to Cry1Ac. Although *B. fusca* has developed resistance to the *B. thuringiensis* Cry1Ab toxin, the binding-patterns and -sites of Cry1Ac and Cry1Ab are similar. Thus a similar mutation may be responsible for *B. fusca* resistance to Cry1Ab. Aminopeptidase, cadherin and alkaline phosphatase are the major Cry toxin receptors that have been identified in lepidopteran species. The present study was concerned with the investigation of mutations in these receptor genes. However, in order to study mutations, sequence data of receptor genes are essential. Degenerate primers were designed based on conserved regions observed in multiple protein sequence alignments of *aminopeptidase N* (isogenes 1 to 6), *cadherin* and *alkaline phosphatase* of several lepidopteran species. Primers were degenerate to take into consideration the

variant regions in receptor gene sequences among lepidopteran species. These primers were used to amplify genomic DNA (gDNA) from susceptible and resistant larvae by using PCR. Sequences of PCR amplicons were determined through Sanger sequencing reactions and subjected to BLAST searches. Results of the BLAST searches showed some similarities to the respective receptor genes. These sequences were also used in phylogenetic analysis. This analysis intended to determine the phylogenetic relationship of the respective receptor genes between *B. fusca* and other lepidopteran species. Mutations could not be identified in the present study, due to a lack in receptor gene sequence data for *B. fusca*. Thus a goal of the present study was to generate sequence data for *B. fusca.* In addition to the proposed objectives, *cytochrome b* gene sequences of *B. fusca* were used to determine the phylogenetic relationship between *B. fusca* and other lepidopteran species. Genome sequencing of *B. fusca* is recommended, as this will provide a platform for genomic, transcriptomic and proteomic studies on this species. These studies will provide much needed information, which can be used to formulate strategies to prevent resistance development in and spread of resistance to other *B. fusca* populations in sub-Saharan Africa.

***Keywords*:** *Busseola fusca*, resistance, PCR, mutations, receptor genes

DEDICATION

This dissertation is dedicated to my father, James Charles Venter, who passed away on Christmas day 2010. You have always wanted me to achieve great things in life, and I have always wanted to make you proud. There have been times when I thought that this was not worth doing anymore, but disappointing you was one thing I could not do.

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When I stand before God at the end of my life, I would hope that I would not have a single bit of talent left, and could say, 'I used everything you gave me'. – Erma Bombeck

DECLARATION

I declare that the dissertation for the degree of *Master of Environmental Science* at the North-West University (Potchefstroom Campus) hereby submitted, has not been submitted by me for a degree at this or another University, that it is my own work in design and execution, and that all material contained herein has been duly acknowledged.

..... **.....**

Bianca Venter **Date**

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CHAPTER 1

INTRODUCTION

1.1. General introduction and problem statement

Genetically modified (GM) crops commercially introduced in 1996 in the US, Argentina, Canada, China, Australia and Mexico had several advantages over conventional crops (James, 2006). These included reduced input and maintenance costs (Ismael *et al*., 2001), improved tolerance to environmental stresses (such as drought, increased rainfall and high salinity) (Kfir *et al*., 2002; Lewis *et al*., 2010), increased yield due to protection of crops from pests, weeds and diseases (Gouse *et al*., 2005; James, 2009a) and effective defence against burrowing pests that are difficult to reach with insecticides (Ranjekar *et al*., 2003). Bt-crops are a type of GM crop that contain the *cry* genes from *Bacillus thuringiensis* (Tabashnik, 2008). These *cry* genes produce crystal proteins that are insecticidal (Zhang *et al*., 2009). This crop technology was adopted rapidly. There was a 94-fold increase in use of GM crops between 1996 and 2011 (James, 2011).

During the first generation GM crops (1996-2005), yield and production significantly increased due to the protection of crops from pests, weeds and diseases (James, 2009a). From the onset, development of resistance in target pests to toxins produced by the GM plants was a concern. To prevent this from happening, refuge systems were proposed and introduced (Gould, 2000). This system allowed for the selection of sufficient sensitive individuals in the pest population. Studies were conducted with laboratory populations of target pests in which individuals developed resistance to Bt-toxins (Tabashnik *et al.*, 2003). It was not expected that field evolved resistance of these species will emerge during the first generation of GM crops (Kruger *et al*., 2009). However, in the Northern Cape and North-West maize producing area of South Africa, the first report of a resistant stem borer species (*Busseola fusca*) was mentioned in 2004 (Van den Berg, 2010). In 2007, the first official report was published and since then more has followed (Kruger *et al.*, 2009; Van Rensburg, 2007).

Bt-maize has been commercially cultivated in the Vaalharts irrigation scheme since 1996, primarily to target *Chilo partellus* (Lepidoptera: Crambidae) and *B. fusca* (Lepidoptera: Noctuidae). These are two major stem borer pests of maize in this region

(Kruger, 2010). In 2004, however, *C. partellus* and *B. fusca* were already identified as the most dominant pests with the most likely resistance risk for Bt-maize (Fitt *et al*., 2004).

Evolution of resistance by pests to GM crops is a great threat to the continued efficacy of these crops. Many mechanisms of resistance are proposed. One mechanism proposes bacterial involvement, where it is hypothesized that indigenous midgut bacteria are essential for Bt-toxicity (Broderick *et al*., 2006). This theory is controversial and several studies have been done to prove and disprove it. Another mechanism proposes that resistance to Bt-toxins may be caused by mutations (insertions or deletions) of Cry receptor genes, resulting in altered Cry toxin-binding sites (Ferré & Van Rie, 2002; Heckel *et al*., 2007; Khajuria *et al*., 2011). In the present study, Cry toxin receptor(s), Cry receptor gene(s) and receptor protein gene(s) are used in an interchangeable manner, and refer to the genes which encode for Cry toxin receptors (Ogunnariwo & Schryvers, 1996; Tabashnik *et al*., 2009; Tsuda *et al*., 2003). Another more recently proposed mechanism suggests that reduced or lack of expression of Cry receptor genes may also confer resistance to Cry toxins (Jurat-Fuentes *et al*., 2011).

Selection experiments with species in Noctuidae, Pyralidae and Plutellidae families (order Lepidoptera) under laboratory conditions and in the field have demonstrated that mutations in certain genes is the most common mechanism that confers resistance (Tabashnik *et al*., 2003). *Helicoverpa armigera* (Lepidoptera: Noctuidae) developed resistance to the Cry1Ac toxin produced in Bt-cotton. This resistance is due to a mutation in the *aminopeptidase N1* (*APN1*) gene, which codes for the Cry1Ac receptor (Zhang *et al*., 2009). *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) developed resistance to the Cry1Ac toxin produced in Bt-cotton due to mutations in three *cadherin* alleles (Morin *et al*., 2003). The development of resistance in pest insects to Bt-crops is thus not a straightforward mechanism.

The development of field evolved resistance in *B. fusca* to the Cry1Ab toxin that is produced by Bt-maize is a real problem for the farmers in the Vaalharts irrigation scheme of South Africa, and now also further north. Studies to determine the mechanisms by which resistance is evolved are thus important and will allow for the development of an Integrated Pest Management (IPM) plan.

1.2. Research aim and objectives

The aim of this study was to investigate possible mutations in receptor protein genes from *Busseola fusca* potentially involved in Bt-resistance by using a PCR method.

The specific objectives were to:

i. isolate genomic DNA (gDNA) from Bt-resistant and -susceptible stem borer larvae;
ii. design degenerate primers that will amplify the regions of interest;
iii. use PCR for the amplification of Cry receptor genes from gDNA;
iv. determine the DNA sequences of the respective PCR products for analyses of potential mutations; and
v. investigate for potential mutations by bio-informatics methods.

The approach of this study was based on the mechanism of *H. armigera* resistance to Cry1Ac. This resistance was due to a mutation in a Cry receptor gene (*APN1*) which encodes for the Cry1Ac receptor. Even though *B. fusca* has developed resistance to Cry1Ab, the binding-patterns and ï sites of Cry1Ab and Cry1Ac are very similar (Ferré & Van Rie, 2002; Pigott & Ellar, 2007). It was thus proposed that this study should assess whether a similar mutation, as the one observed in *H. armigera*, or different mutations are responsible for *B. fusca* resistance to the Cry1Ab toxin.

CHAPTER 2

LITERATURE REVIEW

2.1. Overview of GM crops

Genetically modified (GM) crops have been altered with genes that confer certain properties, such as insecticidal properties, herbicide- or drought-tolerance, which make these crops extremely important in agriculture (Yang *et al*., 2007). These crops were first commercialized in 1996 with only 6 countries growing these crops then. This increased to 29 countries in 2011, of which 19 are developing countries and 10 are industrial countries. The global hectarage of these crops increased from 1.7 million hectares in 1996 to 160 million in 2011. There was also a 94-fold increase in use of GM crops between 1996 and 2011. It is thus evident that GM technology is rapidly adopted where it has been introduced (James, 2011).

Yield and production significantly increased in the first generation GM crops (1996-2005) due to the protection of crops from pests, weeds and diseases (James, 2009a). James (2009b) stated that the ISAAA (International Service for the Acquisition of Agri-biotech Applications) predicted that 1.6 billion accumulated hectares will have been planted by the end of the second decade (2006-2015) of commercialization of GM crops.

GM crops may offer many potential benefits such as protection of crops against pests, weeds, diseases and environmental stresses (James, 2009a; Kfir *et al*., 2002; Lewis *et al*., 2010); reduced insecticide use and subsequently, minimized impacts of these chemicals on non-target pests (Barton & Dracup, 2000; Kruger, 2010). Other benefits include reduced labour and maintenance costs (Ismael *et al*., 2001) and improved nutritional quality of food crops (De Groote *et al*., 2004). Transgenic crops can thus be used to increase food production to aid the continuous need for food through minimizing crop losses, especially losses caused by insect pests (Mugo *et al*., 2011). Although many benefits are gained from use of these crops, potential problems should not be overlooked.

It is important to consider potential effects that GM crops may have on the environment, ecosystem and non-target insects prior to commercial release of these crops (Bale *et al*., 2008; Dale *et al*., 2002). Some environmental effects of GM crops include transfer of herbicide-tolerant genes to other plants through cross pollination (Chilcutt & Tabashnik, 2004). This may result in super weeds (Vinay & Jadav, 2010). There is also the development of resistance to GM crops among pests (Baxter *et al*., 2008; Bravo & Soberón, 2008; Yang *et al*., 2007). According to Altieri and Nicholls (2005), resistance to conventional insecticides has been observed in more than 500 species. This implied that pests may have the ability to also become resistant to the Bt-toxins in GM crops. However, by then this was only demonstrated in laboratory strains of insects (Tabashnik *et al*., 2003).

Increased cultivation of GM crops and subsequent changes in farm management practices may result in a decrease in perennial species (Hails, 2000). This is due to colonization of pests associated with GM crops as well as a decline in plant, invertebrate and bird diversity affecting the ecosystem. Non-target impacts can include toxic effects caused by the transgenic products which were produced to target only certain pests (Sanvido *et al*., 2006). Carpenter (2011) reviewed the effects of Bt and non-Bt crops on target and non-target species in several hundred studies. The overall conclusion among these studies was that Bt-crops do not have direct toxic effects or significant adverse effects on non-target pests. Negative effects may, however, occur if the non-target pest is related to the target pest.

Impacts of GM crops on non-target pests are difficult to assess due to a lack of data regarding the species present in agro-ecosystems (Van Wyk *et al*., 2007). If all potential risks of GM crops are taken into account, safety measures can be established to prevent or delay resistance development and non-target effects. In this way, the benefits of GM crops will outweigh the risks and consequently promote the advanced use of these crops to benefit people all over the world.

2.2. Transgenic crops in Africa

South Africa was the first country in Africa to produce transgenic crops commercially in 1997 (Gouse *et al*., 2005; Van Wyk *et al*., 2008). Burkina Faso and Egypt are now also producing transgenic crops commercially, while Nigeria, Ghana, Uganda, Kenya,

Tanzania, Malawi and Zimbabwe are conducting field trials (Figure 2.1) (James, 2011; Nordling, 2010). Approximately 6.5 billion metric tons of crops, including maize, cotton and soybean are produced in South Africa annually (James, 2009a). From 2000 to 2006, the South African Bt-maize production increased from 77 000 ha (2.8% of total area under maize) to 943 000 ha (34.9% of total area under maize) (James, 2006; Van Rensburg, 2007). According to James (2011), South Africa was the ninth biggest producer of transgenic crops in the world in 2011.

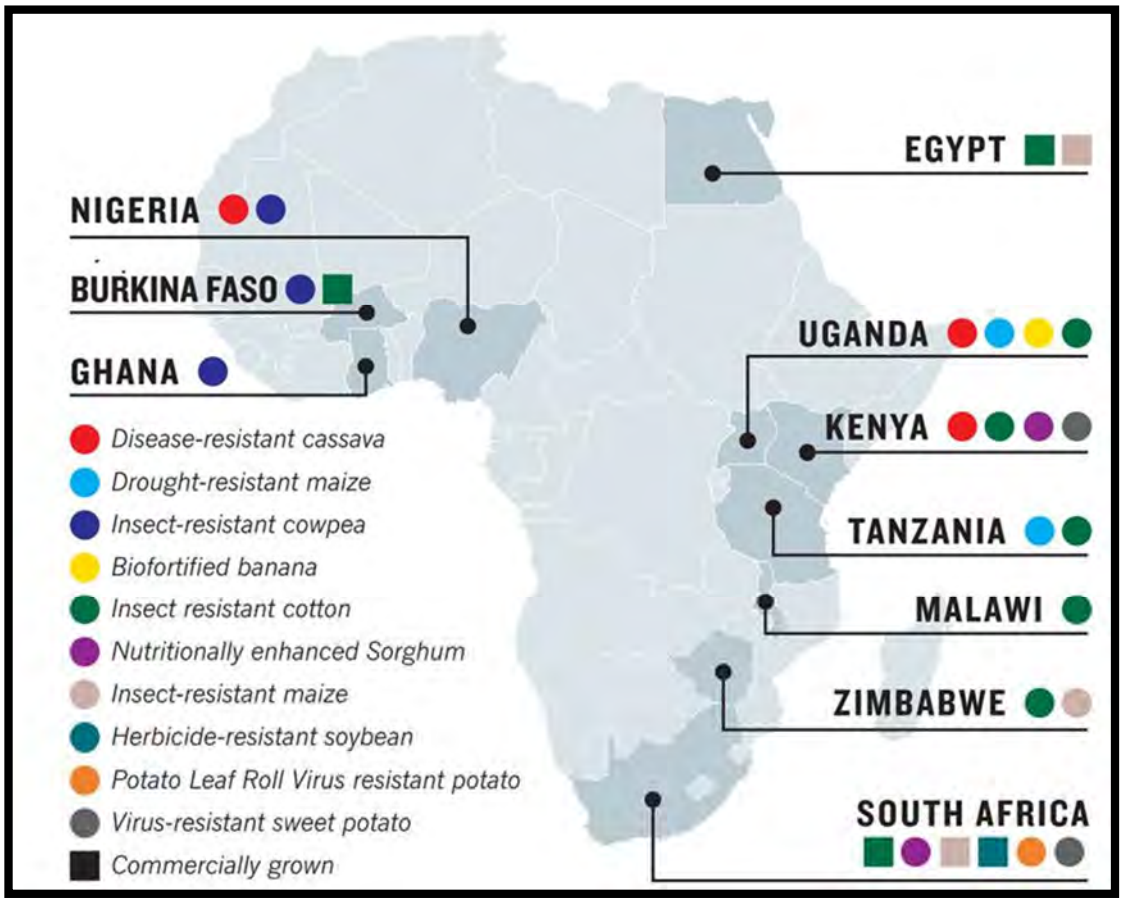


Figure 2.1: A map indicating countries in Africa growing GM crops commercially (squares) and conducting field trials (circles). The different crop types are indicated by different colours (Nordling, 2010).

There seems to be controversial viewpoints regarding the benefits and suspected potential risks associated with GM crops. This is illustrated by the disputes between the United States of America (USA) and the European Union (EU) over GM adoption (Adenle, 2011). European legislation adopts the precautionary principle, which causes most European countries to ban GM crops. Adenle (2011), however, ascribes these problems to lack of awareness and education regarding modern biotechnology. In contrast, standard tests on allergenicity, digestivity and toxicity are adequate to support

the commercial release of GM products in the USA. These disputes place Africa in a misfortunate position, having to choose between adopting GM technology to fight poverty, malnutrition, hunger and food insecurity and losing its trade relationship with the EU, thus affecting commercial export sales (Cooke & Downie, 2010). South Africa's economy has benefited greatly from the commercialization of GM crops (Adenle, 2011), but many factors restrain further research into and expansion of GM crop production. These factors include lack of infrastructures, funding shortages, inadequate human resource capacity, poor education, biosafety regulation and intellectual property rights (Cooke & Downie, 2010).

Benefits of GM crops seem to be documented properly, whereas only a few cases of potential health effects (Ho, 2002) or economic drawbacks (Glover, 2009) to GM crops are documented. Effects of GM crops on non-target species have been reported to be similar to those of conventional crops (FAO, 2004), yet people still express their disapproval of GM crops based on unverified sources. The World Health Organisation (WHO), the Food and Agriculture Organisation of United Nations (FAO) and several other international regulatory bodies have concluded that human health effects or environmental problems supposedly caused by GM crops have not been validated with any scientific evidence (Paarlberg, 2010).

B. fusca (Fuller) (Lepidoptera: Noctuidae) and *C. partellus* (Swinhoe) (Lepidoptera: Crambidae) are the target pests of Bt-maize in South Africa. In the Vaalharts irrigation scheme (South Africa) *B. fusca* has become a major pest, causing extensive crop damage and yield losses ranging between 10% and 100% (Gouse *et al*., 2005; Kfir *et al*., 2002). Increase in damage to Bt-maize from stem borers have been observed as early as 2004 (Van den Berg, 2010), with damage becoming more extensive in subsequent growing seasons (Kruger, 2010; Tabashnik *et al*., 2009).

Resistance development to GM crops among pests was probably due to the selection pressure exerted on these pests where GM crops were extensively cultivated (Kruger *et al*., 2011a). When GM crops were introduced into main stream agriculture, the U.S. Environmental Protection Agency (USEPA) mandated a resistance management plan (Gould, 2000). This required farmers to plant refuges (conventional cultivars) when transgenic crops are grown (Gahan *et al*., 2007; Monsanto, 2011). This strategy was enforced to prevent or delay resistance development among pests by promoting

survival of susceptible pest insects, thereby decreasing the selection of Bt-resistance alleles (Bourguet, 2004). The importance of refugia is discussed in more detail in Section 2.3.

Data regarding species that are present in agro-ecosystems are lacking, resulting in difficulties in resistance monitoring and assessments of non-target effects. According to Van Wyk *et al*. (2008) a total of fifteen species of Lepidoptera have been recorded on maize in South Africa. Six of these species feed on Bt-maize. In order to monitor resistance development in target pests and unintended effects on non-target species, studies are needed to compile a list of all species (target and non-target) present in agro-ecosystems. Only then can effective management strategies be devised for each specific target pest and non-target effects be prevented or minimized.

2.3. Refuge requirements

The resistance management plan mandated by USEPA requires farmers to grow a certain amount of refuges (conventional cultivars) where transgenic crops are grown (Gahan *et al*., 2007; Monsanto, 2011). This resistance management plan declares that refugia can be either 20% conventional cultivars that may not be sprayed with insecticides or 5% conventional cultivars that may be sprayed with insecticides (Monsanto, 2008).

The purpose of refugia is thus to employ a high-dose/refuge strategy, which requires the transgenic crops (that produce high doses of toxin) and the refugia (conventional crops) to be planted in close proximity (Kruger *et al*., 2009). Many individuals of the target pest will be killed by the high dose of toxin, whereas some individuals will survive on the refugia (Gould, 2000; Tabashnik *et al*., 2003; Van Rensburg, 2007). Individuals that become resistant to the transgenic crops will ultimately mate with some of the susceptible individuals that survived on the refugia (Gould, 2000), and thus giving rise to progeny with lower resistance to the transgenic crops (Kruger *et al*., 2009). The progeny will not be able to survive on the transgenic crops with the high doses of toxin (Gould, 2000) and therefore the development of resistant populations will be unlikely.

Farmers in the Vaalharts irrigation scheme seem to prefer the 5% refuge option where cultivars may be sprayed with insecticides (Kruger *et al*., 2009). Treatment of refuges

with insecticides is only allowed when the level of pest pressure meets or exceeds the economic threshold (10% of infested crops) for control (Monsanto, 2008). Once this threshold is reached, the common refuge may be treated with a non-Bt insecticide to control the pest.

To ensure compliance with refuge requirements, stewardship programmes have been instituted in South Africa. These include grower education programmes, signing of contracts, on-farm inspections and instituted punitive measures for farmers that do not comply (GMO Act 15 of 1997; Kruger *et al*., 2009, 2011b). In certain areas, seed deliveries to farmers include a consignment of conventional cultivar seed to plant a 5% refuge area as part of the stewardship programme (Kruger *et al*., 2011b). Farmers that are non-compliant two years in a row are not allowed to purchase GM seed of the relevant crops (maize, cotton and soybeans) for the following year (Bourguet *et al*., 2005).

Four refuge layout options, namely perimeter-, block-, strip- and separate field refuge (Figure 2.2), are prescribed by Monsanto (Monsanto, 2011). The farmers in the Vaalharts area that planted refugia made use of these prescribed layout options. However, 8% of farmers did not plant a refuge field for each Bt-maize field in 2008 (Kruger *et al*., 2009). According to Kruger *et al.* (2009), most farmers in the Vaalharts area made use of the separate field refuges, but not in accordance with prescribed designs. These authors also doubted the use of prescribed refuge layouts prior to 2005.

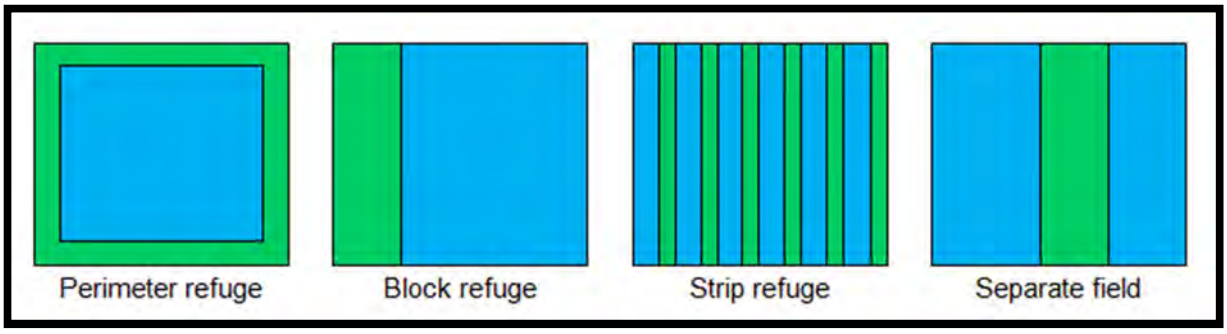


Figure 2.2: Illustration of refuge layout options, as prescribed by Monsanto. Blue areas indicate transgenic cultivars and green areas indicate refuges (conventional cultivars) (adapted from Monsanto, 2011).

The purpose of refugia is to employ a high-dose/refuge strategy to delay resistance development among pests. Although this strategy seems realistic in theory, it is

undermined by the variable toxin production in different plant parts as well as pollen-mediated gene flow from transgenic crops to refuge plants. The latter was observed in the U.S. where DNA sequences from transgenic maize, soybean, and canola were found in the seed supply of the same, respective conventional crops (Chilcutt & Tabashnik, 2004; Mellon & Rissler, 2004).

Pest resistance to Bt-crops could be accelerated as a consequence of transgene movement when susceptible larvae are killed by the toxin being produced by refuge plants. This will reduce the amount of susceptible larvae available to mate with resistant larvae. Conversely, intermediate toxin levels produced by refuge plants may allow heterozygotes to survive, thus increasing the functional dominance of resistance (Chilcutt & Tabashnik, 2004; Gould, 1998). In order to promote susceptibility of pests, and thus decrease occurrence of resistance, the high-dose/refuge strategy should be enhanced with additional methods or features. One such example is toxin stacking. In this case multiple toxins are co-expressed in the same crop to target multiple pests or single pests that have already developed resistance to one of the toxins (Griffitts & Aroian, 2005).

2.4. Cry toxins as biopesticides

Cry genes from *B. thuringiensis* produce crystal proteins that are insecticidal (Tabashnik, 2008; Zhang *et al*., 2009). These insecticidal crystal proteins offer commercial advantages and are thus considered as environmentally friendly alternatives to conventional insecticides (Bravo *et al*., 2007; Morin *et al*., 2003).

Protein crystals produced by these genes contain entomocidal protein protoxins that are activated upon ingestion. There are a number of different protoxins, of which Cry proteins are one type. Insects in the orders Lepidoptera (butterflies and moths), Diptera (flies and mosquitos) and Coleoptera (beetles and weevils) (Bravo *et al*., 2007; Rosi-Marshall *et al*., 2007; Xu *et al*., 2009) are targeted by these Cry proteins. Bt-crops thus proved to be an effective control strategy for pests.

GM food crops, such as rice or maize, are becoming very important in food security. Safety assessments of Cry toxins that are expressed by these crops are thus crucial (Xu *et al*., 2009). These toxins are expected to be innocuous to most other organisms

(humans, non-target pests, vertebrates and plants) (Bravo *et al*., 2007; Luo *et al*., 2006), but viewpoints on the safety of GM crops are controversial (Adenle, 2011). Separate Cry toxins (e.g. Cry1Ab and Cry1Ac) may not be toxic, but fusion toxins (e.g. Cry1Ab-Ac protein encoded by the fused Cry1Ab-Ac gene) have novel sequences (Xu *et al*., 2009). Changes in the primary and secondary toxin structures, and thus also the protein digestion characteristics and thermal stability of these proteins may elicit allergenic or toxic effects on non-target organisms (Xu *et al*., 2009).

New cultivars contain stacked toxins, which entail that the same crop co-expresses multiple toxins (Griffitts & Aroian, 2005). Even if safety assessments have been done on the separate toxins, new tests need to be performed to determine whether these combined products will have allergenic or toxic effects (Xu *et al*., 2009). It is thus important to look at the diversity, structure and function of Cry toxins.

2.5. Cry toxin diversity, structure and function

Crystal (Cry) toxins, also called ŭ-endotoxins, are classified into 67 types (Cry1-Cry67) and many sub-types (e.g. Cry1Aa or Cry1Ba), based on primary sequence similarity (Bravo & Soberón, 2008; Zúñiga-Navarrete *et al*., 2012). A total of 567 Cry toxins have been classified on the basis of amino acid sequence similarity (Crickmore *et al*., 1998; Dhurua & Gujar, 2010). Based on amino acid sequences and insecticidal activity, Cry toxins are broadly divided into five groups. A single group of Cry toxins can target species in more than one phylogenetic order (Dhurua & Gujar, 2010).

Cry toxins differ considerably in their amino acid sequences and insect specificity, although highly similar three domain structures are present in all these toxins, namely domain I, II and III (Pigott & Ellar, 2007). The functions of the various domains were determined through genetic and electrophysiological studies. This illustrated that domain II is mainly involved in receptor recognition and -binding (Karim & Dean, 2000; Zúñiga-Navarrete *et al*., 2012). Domain III has a role in structural, ion conductance and receptor binding (Karim & Dean, 2000; Wolfersberger *et al*., 1996). Domain I is considered to be involved in membrane insertion, toxin oligomerization and ion channel formation (Zúñiga-Navarrete *et al*., 2012). Domain II and III also show structural similarities with carbohydrate-binding proteins (Bravo *et al*., 2007; De Maagd *et al*.,

2003), which suggest that carbohydrate moieties may have an important role in the mode of action of three-domain Cry toxins.

Domain III exchange among toxins may occur (De Maagd *et al*., 2001), which gives rise to toxins with dual specificity (e.g. toxins that target both coleopteran and lepidopteran pests). Thus toxins with a similar mode of action, but very different specificities may be generated by domain III swapping (Bravo *et al*., 2007). The high degree of structural conservation among Cry toxins, however, suggests that they possess a fundamental mechanism of action (Griffitts & Aroian, 2005). Furthermore, the remarkable variety of known Cry proteins is explained by the high degree of plasticity of the Cry toxins (Pigott & Ellar, 2007).

Whilst many *cry* genes are associated with transposable elements, most *cry* genes are found on plasmids. Transposable elements, which are mobile sequences, have the potential to produce a wide range of changes in their hostsôgenomes (Kidwell & Lisch, 2000; Yang *et al*., 2007). Thus new toxins may also arise during gene amplification (De Maagd *et al*., 2001; Pigott & Ellar, 2007) or through horizontal transfer by conjugation (Thomas *et al*., 2001). Changes in toxin specificity may be caused by differences in proteolytic activity between target insects (Bradley *et al*., 1995; De Maagd *et al*., 2001), e.g. the main digestive proteases of Lepidoptera and Diptera are serine proteases, whereas those of Coleoptera are mainly cysteine and aspartic proteases (Oppert *et al*., 2006).

Many different toxins with variable specificities may arise during different processes (such as domain III swapping, gene amplification or horizontal gene transfer through conjugation) (De Maagd *et al.*, 2001; Thomas *et al*., 2001). However, sequence alignments show that five blocks of amino acids seem to be conserved among all ŭ-endotoxins, which results in similar three-domain tertiary structures. Some toxins may only contain some, and not all, of the blocks. According to Pigott and Ellar (2007), these regions may be of importance for toxin stability or function.

Pore-forming toxins (PFT) are a class of bacterial toxins, to which Cry Bt-toxins belong. These toxins are secreted as water-soluble proteins that undergo conformational changes to facilitate insertion into, or translocation across, cell membranes of their host (Bravo *et al*., 2007). Prior to toxin insertion, host proteases activate the toxin and

receptor binding occurs. Cry1Ac toxins bind to receptors by means of domain III of the toxin that recognizes N-acetylgalactosamine (GalNAc) epitopes on the receptors. Cry1Aa and Cry1Ab toxins do not show GalNAc-binding capacities (Bravo *et al*., 2007; Zúñiga-Navarrete *et al*., 2012). Toxin-receptor-binding then induces the formation of an oligomeric structure that is insertion-competent.

An additional processing step has been observed in some species, where proteolytic cleavage in the *N*-terminal end of Cry1Aa and Cry1Ab toxins (helix Ǔ-1) facilitated the formation of pre-pore oligomeric structures prior to insertion into the membrane (Bravo *et al*., 2007; Gómez *et al*., 2002). Formation of Cry oligomeric structures has also been demonstrated for Cry1Ca, Cry1Da, Cry1Ea, Cry1Fa and Cry3 toxins (Muñoz-Garay *et al*., 2006; Rausell *et al*., 2004). According to Parker and Feil (2005), a decrease in pH triggers membrane insertion, whereby a molten globule state of the protein is induced. Bravo *et al*. (2007) do not, however, share this view, and state that the molten globule state of the pre-pore complex is induced by an alkaline pH.

2.6. Cry toxin mechanism of action

A variety of insects are targeted by Cry toxins, including Lepidoptera (moths), Coleoptera (beetles), Diptera (mosquitoes and flies), Hymenoptera (wasps and bees), and nematodes (De Maagd *et al*., 2001; Griffitts & Aroian, 2005). Single Cry toxins may affect a broad class of organisms, but very distantly related toxins, such as Cry1Aa and Cry2Aa, can be active against similar organisms. A two-phase mechanism of Cry toxin action have been proposed, namely (i) solubilization and proteolytic activation in the midgut and (ii) specific binding to protein receptors and cytolytic pore formation (Griffitts & Aroian, 2005; Schnepf *et al*., 1998).

The target site of Cry toxins is the apical membrane of midgut columnar epithelial cells (Braun & Keddie, 1997; Chen *et al*., 2005), on which lepidopteran protein-receptors are present (Aimanova *et al*., 2006; Hara *et al*., 2003; Midboe *et al*., 2003). These Cry toxins induce changes in the physiological status of the intestines of larvae (Vázquez-Padrón *et al*., 2000; Xu *et al*., 2009), which results in the death of these pests. Thus the larval midgut serves as the site of action (Heckel *et al*., 2007).

Bt-toxins, i.e. Cry toxins, have a very complex mode of action, which makes them highly specific (Heckel *et al*., 2007; Zhang *et al*., 2009). Pigott and Ellar (2007) have proposed three contrasting models of Cry1A toxin mode of action (Figure 2.3), namely the Bravo model, Zhang and Jurat-Fuentes model. The initial steps of all the models are identical (Khajuria *et al*., 2011).

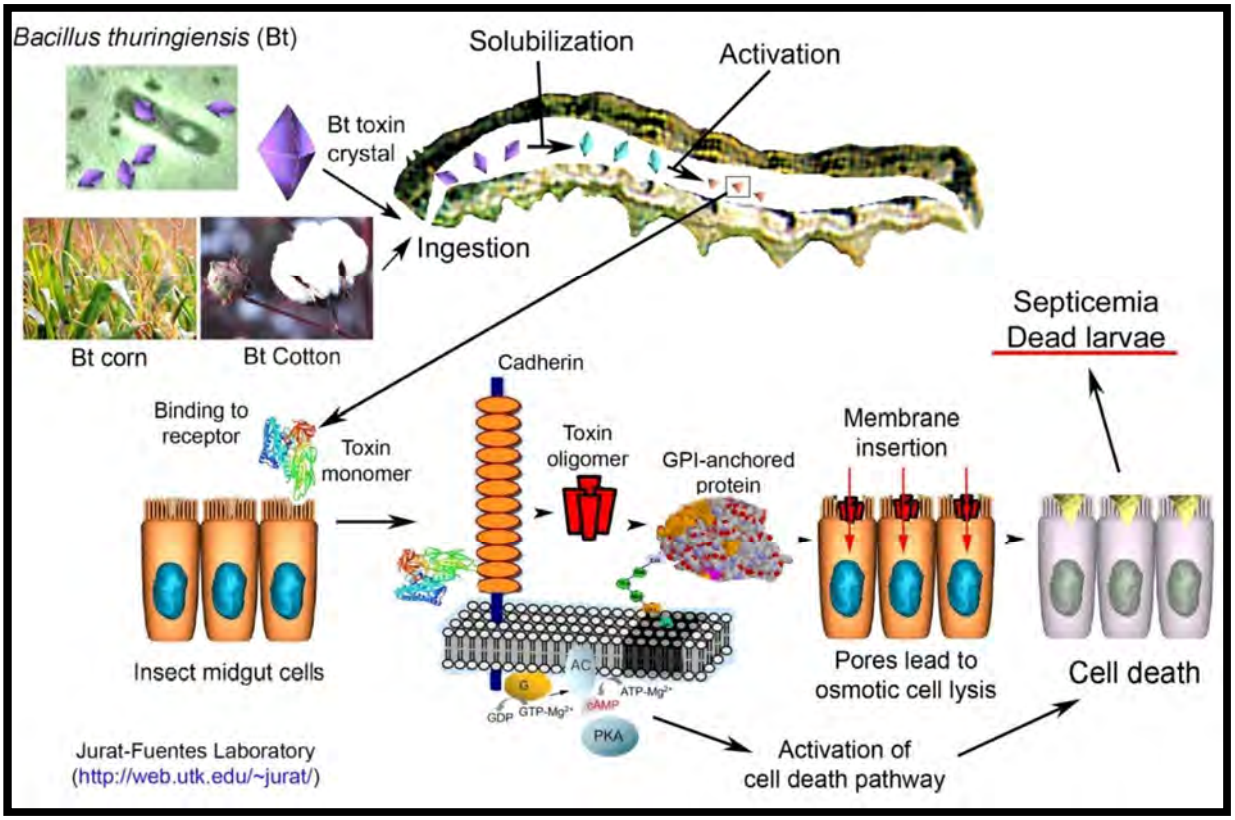


Figure 2.3: Schematic outline that illustrates the Bravo and Zhang model of Cry1A mode of action in susceptible larvae (Jurat-Fuentes, 2010).

According to these models, a proteinaceous parasporal crystalline inclusion contains the toxin (Heckel *et al*., 2007). When susceptible insect larvae ingest these ŭ-endotoxins (130-140 kDa) (Karim & Dean, 2000), the protein crystal is solubilised in the lumen of the midgut and the protoxin is released. Host digestive proteases then remove 500 amino acid residues from the C-terminus of the protoxin (Gahan *et al*., 2010; Gómez *et al*., 2002; Pigott & Ellar, 2007) to give rise to an active protease-resistant toxin (60-65 kDa) (Heckel *et al*., 2007; Karim & Dean, 2000). The monomeric toxin is then translocated through the peritrophic matrix to the brush border membrane (Krishnamoorthy *et al*., 2007) where cadherin, the primary protein-receptor on the surface of the midgut epithelial cells (Khajuria *et al*., 2011; Soberón *et al*., 2009), binds these activated toxins (Figure 2.3). The activated toxin monomers then undergo

additional proteolytic activation where helix Ŭ-1 (Domain 1) from the N-terminus is cleaved (Bravo *et al*., 2004; Gómez *et al*., 2007) and hydrophobic residues are exposed.

The Bravo model proposes that sequential, reversible receptor binding occurs (Bravo & Soberón, 2008; Gahan *et al*., 2010) to form an oligomeric Cry toxin that is insertion-competent (Figure 2.3) (Bravo *et al*., 2004; Gómez *et al*., 2002). The oligomeric Cry toxin then binds to secondary glycosylphosphatidylinositol (GPI)-anchored receptors (such as aminopeptidases (APN) or alkaline phosphatase (ALP)) (Khajuria *et al*., 2011; Upadhyay & Singh, 2011). According to Gómez *et al*. (2002), oligomer formation is required for proper toxin insertion into membranes. Toxins are then irriversibly inserted into the membrane to form pores (Gahan *et al*., 2010; Pigott & Ellar, 2007) in the bilayer lipid membrane (Heckel *et al*., 2007).

The bilayer lipid membrane is a detergent-resistant membrane (DRM) enriched in glycosphingolipids, cholesterol and GPI-anchored proteins (Bravo *et al*., 2007; Gómez *et al*., 2007; Munro, 2003). These lipid rafts are involved in signal transduction (Bravo *et al*., 2004; Schroeder *et al*., 1998), membrane and protein sorting (Bravo *et al*., 2007; Simons and Toomre, 2000) and also function as pathogen portals for viruses, bacteria and toxins (Cabiaux *et al*., 1997; Rosenberger *et al*., 2000).

Formation of pores subsequently disrupts the membrane integrity (Gill & Ellar, 2002; Heckel *et al*., 2007). This causes very rapid changes in membrane potential, equilibration of ions across the membrane, influx of water, cell swelling and eventual lysis of the midgut epithelial cells (Karim & Dean, 2000; Khajuria *et al*., 2011). Insect mortality is then due to starvation (Pigott & Ellar, 2007; Zhang *et al*., 2009) and septicemia (Upadhyay & Singh, 2011; Zhang *et al*., 2005). This lytic pore-formation model has, however, been challenged (Broderick *et al*., 2006; Pigott & Ellar, 2007; Zhang *et al*., 2006).

Starvation and septicemia were the assumed mechanisms of insect killing for decades, until a study by Broderick *et al*. (2006) had shown that larvae of the gypsy moth (*Lymantria dispar*) are not killed by Bt-toxins in the absence of indigenous midgut bacteria. In that study, *B. thuringiensis* insecticidal activity was abolished when the gut microbial community was eliminated by antibiotics. *B. thuringiensis*-mediated killing was

restored after the midgut microbial community was re-established. According to Broderick *et al*. (2006) *Enterobacter* sp. seems to be mostly responsible for causing septicemia in gypsy moth larvae when Bt-toxins were ingested by these larvae. Mortality is, however, not induced by the enteric bacteria alone, but the Bt-toxins cause permeability of the gut epithelium which enables the bacteria to reach the hemocoel (Broderick *et al*., 2006).

Spores may also gain access to the hemocoel when cells are lysed. In the more favourable environment of the hemocoel, the spores germinate and reproduce. The vegetative cells cause septicemia and this leads to insect mortality (Broderick *et al*., 2006; Schnepf *et al*., 1998). This alternative mechanism of killing has been proposed due to inconsistent experimental observations found with the starvation model, where it takes larvae 7-10 days to die from starvation, compared to only 2-5 days when Bt-toxins are consumed. The septicemia model has, however, also been challenged when mortality of larvae was still induced by the toxin in the absence of bacterial cells (Broderick *et al*., 2006; Schnepf *et al*., 1998). Broderick *et al*. (2009) concluded that *Bacillus thuringiensis*-induced mortality due to contributions of gut bacteria vary across a range of Lepidoptera.

The Zhang model proposes an alternative mode of action where cell death (apoptosis) (Krishnamoorthy *et al*., 2007; Zhang *et al*., 2006) is promoted by a Mg^{2+}-dependent adenylyl cyclase/PKA signalling cascade (Jurat-Fuentes & Adang, 2006; Xu & Wu, 2008). This cascade is induced when monomeric Cry toxins bind to cadherin (Figure 2.3). Jurat-Fuentes and Adang (2006) and Lilien and Balsamo (2005) proposed that actin interacts with the cytosolic domain of cadherin proteins. This is done by means of tyrosine phosphatases, catenin and actinin. The activation of intracellular pathways in response to extracellular signals follows. Additional work suggested that the cytoskeleton and ion channels are destabilized when G protein and adenylyl cyclase (AC) causes cyclic AMP (cAMP) levels to increase. This increase in cAMP leads to the activation of protein kinase A (PKA). Knowles and Farndale (1988) did a study on the increase in intracellular cAMP and argued that the increase was due to a secondary effect of the toxinôs interaction with the membrane. In order to strengthen the Zhang model, more evidence is needed to establish the connection between cytotoxicity and the rise in cAMP.

The Jurat-Fuentes model, a combination of the Bravo and Zhang models, proposes that the combined effect of osmotic lysis caused by toxin pore formation and cell signalling leads to cytotoxicity (Jurat-Fuentes & Adang, 2006; Pigott & Ellar, 2007). This model suggests that an intracellular signalling pathway is activated after active monomeric Cry toxins have bound to receptors (Bravo & Soberón, 2008; Heckel *et al*., 2007; Zhang *et al*., 2006). Although various models are used to describe the mode of action of Cry toxins, these are not absolute and may vary between species.

The mechanism of how the toxins bind to the receptors in brush border membranes, in the first step of the toxin action, is also not fully understood. To explain this, models are also proposed. The most common model in this case proposed that three sites exist (Luo *et al*., 1997; Upadhyay & Singh, 2011). This model is diagrammatically demonstrated in Figure 2.4 and suggests that: (i) receptor A (cadherin-like protein or APN) binds Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa and Cry1Ja toxins (Banks *et al*., 2001; Jurat-Fuentes & Adang, 2001); (ii) receptor B binds Cry1Ab and Cry1Ac toxins but not Cry1Aa and (iii) receptor C binds only Cry1Ac toxin (Upadhyay & Singh, 2011). According to Lee *et al*. (1995) and Luo *et al*. (1997) a *H. virescens* strain showed resistance to Cry1Ac due to an absence of Cry1Aa binding sites, suggesting that not all binding sites are equally effective in mediating toxin function. It has, however, been shown by Smedley *et al*. (1997) that Cry toxins, in the absence of protein-receptors, can insert and form pores in bilayer lipid membranes. Further studies are required to support this observation.

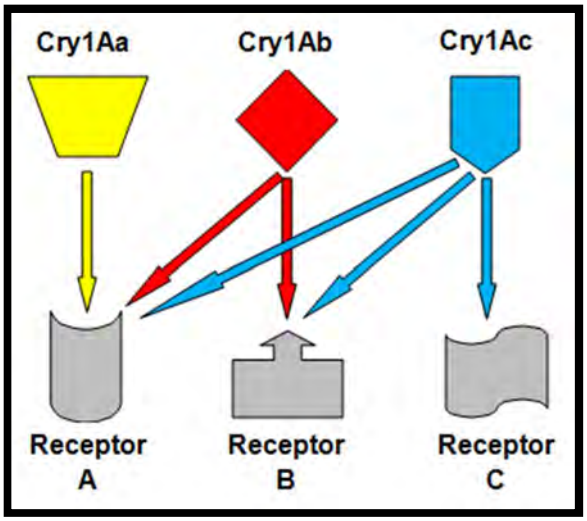


Figure 2.4: Three-site binding model proposed for Cry1A toxin binding to protein receptors (adapted from Luo *et al*., 2006).

There have been some cases where a resistance allele has caused resistance to all three Cry1A toxins, although binding of the toxin to one or two of the receptors still occurred, but incorrectly (Griffitts & Aroian, 2005). This incorrect binding does not result in the conformational changes that usually occur prior to pore-formation. Thus the ability of the toxin to insert into the membrane and form functional pores is different from its ability to recognize and bind to receptors on the membrane (Griffitts & Aroian, 2005). An alternative co-receptor model has been proposed, wherein both receptor and co-receptor are required for toxicity, otherwise incorrect pore formation will occur if either the receptor or co-receptor is lost (Luo *et al*., 1997).

An understanding of toxin-receptor-binding and toxin mechanisms of action is essential for the sustained use of GM crops. Although several models exist to describe these concepts, there is no single model to describe the mode of action of Cry toxins in all sensitive species. Therefore specific experimental data are required to explain the effects of Cry toxins in a specific target pest. Thus models should be developed case-by-case, which will then help to elucidate the mechanistic and genetic basis of resistance development to Cry toxins among pests.

2.7. Insect pest resistance

Pests have the evolutionary capacity to adapt to insecticidal traits in crops (Gunning *et al*., 2005; Tabashnik *et al*., 2003; Yang *et al*., 2007). Selection experiments under laboratory conditions have indicated that species in families Noctuidae, Pyralidae and Plutellidae can develop resistance when exposed to Bt-toxins (Kruger *et al*., 2011a) suggesting that resistance development among Lepidoptera is a common phenomenon. Field-evolved resistance to date has only been detected in *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa (Van Rensburg, 2007), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the south-eastern United States (Luttrell *et al*., 2004), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Puerto Rico (Dhurua & Gujar, 2010), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in India (Dhurua & Gujar, 2010), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in northern China (Liu *et al*., 2010) and *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in The Philippines and Hawaii (Griffitts & Aroian, 2005). Evolution of resistance in pests to GM biopesticides is a major threat to the continued success of GM crops (Yang *et al*., 2007).

Many other species have developed resistance to Bt-toxins in laboratory selection experiments, implicating that these species are also likely to develop resistance to these toxins in the field. *P. xylostella* was the first lepidopteran pest to develop resistance to Bt-insecticides in the field (Dhurua & Gujar, 2010). The presumption that resistance was unlikely to develop in the field was led on by a lack of reports of resistance as well as inaccurate reflections of potential for resistance developed in laboratory selection experiments compared to actual resistance development in the field (Tabashnik *et al*., 1990). In other words, laboratory-selected resistant strains may have different mutant alleles than field-evolved resistant strains (Yang *et al*., 2007). Thus farmers did not foresee that resistance development in the field would happen over a short period (less than a decade) (Kruger *et al*., 2009).

Resistance can be caused by variations in any one of the steps of the Cry toxin mode of action (Jurat-Fuentes *et al*., 2004). It includes: (i) incomplete crystal solubilization (Ferré & Van Rie, 2002); (ii) incomplete protoxin processing due to deficient proteolytic activation (Griffitts & Aroian, 2005); (iii) toxin degradation by protease (Shao *et al*., 1998); (iv) prevention of toxin binding due to modified receptors (Oppert *et al*., 1997); (v) interference with pore formation (Shai, 2001) and (vi) plugging of pores due to rapid repair of cell damage (Khajuria *et al*., 2011).

Insect resistance by means of internal signalling is still unfamiliar (Pigott & Ellar, 2007). Recent literature suggests that resistance may be caused by retrotransposon insertion (Fabrick *et al*., 2011; Gahan *et al*., 2007) or down-regulation of genes (Jurat-Fuentes *et al*., 2011). According to Griffitts and Aroian (2005), decreased glycosylation has also correlated with resistance to Cry1A toxins. Ning *et al*. (2010) supported this by demonstrating that there was a link between genes involved in glycosylation pathways and resistance to Cry toxins in the nematode *Caenorhabditis elegans*.

Candas *et al*. (2003) and McNall and Adang (2003) proposed that resistance to Cry toxins could also be linked to increased levels of specific proteins (glutathione transferase, cytochrome c oxidase subunit I, and NADH dehydrogenase subunit 5). This is based on observations in the resistant strain of laboratory-reared Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). These increased levels were associated with alterations in oxidative metabolism (Candas *et al*., 2003). In that case there was

also a decrease in chymotrypsin activity, which may have affected toxin and/or protein processing (McNall & Adang, 2003).

The role of epithelial regenerative mechanisms in resistance to Cry toxins are also not yet understood, but an increase in differentiating cells was observed when mature cells were damaged by Cry toxins (Castagnola & Jurat-Fuentes, 2009; Loeb *et al*., 2001). Several authors (Castagnola & Jurat-Fuentes, 2009; Forcada *et al*., 1999; Martinez-Ramirez *et al*., 1999) found a direct correlation between midgut stem cell-mediated regeneration and resistance to Cry1Ac in *H. virescens* larvae. This suggests that larvae may recover completely after intoxication.

Toxin sequestration by esterase has been proposed as another resistance mechanism in which Cry toxins are sequestered by nonspecific esterases in the insect gut (Gunning *et al*., 2005). It renders the toxins harmless before it reaches the receptors. This resistance mechanism has been observed in a resistant *H. armigera* strain, where both Cry1Ac protoxin and activated toxin were bound by esterase, contrary to susceptible *H. armigera* where esterase did not bind to Cry1Ac (Gunning *et al*., 2005). The inheritance of the ability of esterase to bind to Cry1Ac toxin is semi-dominant. According to Gunning *et al*. (2005), esterase-based resistance mechanisms in insects are not uncommon.

It has also been demonstrated that resistance in *H. virescens* occurred when *alkaline phosphatase* was up- or down-regulated due to mutations (Bravo *et al*., 2007; Jurat-Fuentes & Adang, 2004). Reduced *ALP* activity correlated with Cry toxin resistance in some cases. In one case, there was no reduced Cry1Ac binding when ALP activity was reduced (Jurat-Fuentes & Adang, 2006). This family of phosphatases activate intracellular pathways via lipid rafts in response to extracellular stimuli (Eyster, 2007), which supports the Zhang model for Cry1A toxin mode of action (Section 2.6).

Another important resistance mechanism involves mutations generated by the insertion of mobile DNA or transposable elements (TEs) (Yang *et al*., 2007). TEs are divided into two major classes: RNA (Class I) and DNA (Class II) transposons (Fabrick *et al*., 2011; Pritham, 2009). Class I TEs are retrotransposons that move through reverse transcription of RNA intermediates (Chen & Li, 2008). Class II TEs directly transpose DNA to DNA using a ócut-and-pasteô mechanism. The latter mechanism has been described in *P. gossypiella* as the r3 mutation (Morin *et al*., 2004).

Resistance to Cry1Ac in the pink bollworm (*P. gossypiella*) was linked with three alleles (*r1*, *r2* and *r3*), each carrying a different mutation which codes for an incomplete cadherin protein (Fabrick *et al*., 2011; Morin *et al*., 2004). The *r1* mutation has a deletion in an exon and the *r2* mutation a deletion spanning an intron-exon splice site that codes for a premature stop codon. The *r3* mutation results from the insertion of a large DNA fragment which leads to the loss of an exon.

Thus far Cry1Ac resistance caused by TE insertions have been observed in eight different *cadherin* alleles in three lepidopteran species: the *r3* allele in *Pectinophora gossypiella*, one allele in *Heliothis virescens* (Gahan *et al*., 2001) and six alleles in *H. armigera* (Zhao *et al*., 2010). Disruptions of lepidopteran *cadherins* by TEs conferring resistance to Cry1Ac seem to occur frequently (Fabrick *et al*., 2011).

Each insect pest can develop resistance through any of the mentioned mechanisms, as well as others not discussed here. This implies that studies of resistance should be done case-by-case (Griffitts & Aroian, 2005). The important mechanism of resistance development seems to be mutations in Cry receptor genes. Resistance might be accompanied by a loss in toxin binding in some insects, while others may develop resistance with toxin binding still taking place (Li *et al*., 2004; Pigott & Ellar, 2007).

2.8. Receptors involved in Cry toxin binding

Four main Cry1A toxin-binding proteins have been described in different lepidopteran insects, namely a cadherin-like protein, aminopeptidase N, alkaline phosphatase and a glycoconjugate (Heckel *et al*., 2007; Valaitis *et al*., 2001). The latter three are glycosylphosphatidyl-inositol (GPI)-anchored glycosylated proteins that have been identified in lipid rafts associated with the epithelial membrane in insect midguts (Gahan *et al*., 2010).

Aminopeptidases have been perceived as the most important binding sites for Cry1A toxins (Knight *et al*., 1995; Sangadala *et al*., 1994), but evidence suggests that cadherin-like proteins are the primary functional receptors (Jurat-Fuentes & Adang, 2006). It is not yet clear whether APNs, ALPs, glycolipids or an unknown receptor mediates specificity for these Cry toxins. Other Cry toxin receptors that have also been reported include GPI-ADAM metalloprotease, glycolipids, glyco-conjugates, V-ATP

synthase subunits and actin (Krishnamoorthy *et al*., 2007; Valaitis *et al*., 2001). Even though toxins may bind to any of these receptors, it does not necessarily implicate that these receptors have a functional insecticidal role.

Cry toxin resistance have been observed in several insects that could be linked to mutations in Cry receptor genes such as *cadherin* and *APN*. Cry1Ac resistance in *H. virescens* is caused by a single mutation in *cadherin*. In *P. gossypiella* and *H. armigera* Cry1Ac resistance is caused by different mutated *cadherin* alleles, although a mutation of the *APN* gene in *H. armigera* also seems to be associated with Cry1Ac resistance. According to Gahan *et al*. (2010) Cry1Ac toxin resistance in *H. virescens* is also due to a mutation in the *ABC* transporter gene (*ABCC2*). The pore formation model proposes two binding steps, namely binding to cadherin protein and binding to GPI-anchored protein. Khajuria *et al*. (2011), however, suggest that there is an additional binding step where Cry toxins bind to the open configuration of the ABC transporter protein, which facilitates subsequent membrane insertion. Thus mutations in *ABC* transporter genes may also cause resistance development.

In order to investigate the mode of toxin action and molecular mechanisms of insect resistance to Cry toxins, it is necessary to identify toxin-binding proteins in lepidopteran insects. In order to prevent or delay resistance development, multiple toxins are used to target different binding proteins to maintain susceptibility (Peferoen, 1997). Therefore the identification of these proteins is crucial for resistance management. Not all of these binding proteins, however, function as actual receptors in the intoxication process (Xu & Wu, 2008). Toxin binding also does not necessarily implicate that an organism is susceptible (Banks *et al*., 2003). Jurat-Fuentes and Adang (2006) suggest that by comparing midgut epithelium proteins from susceptible larvae to those from resistant larvae, one can reveal additional receptor alterations involved in resistance. Of the most important Cry toxin receptors are aminopeptidase, cadherin and alkaline phosphatase. Resistance to Cry toxins could thus be due to structural and physiological processes involving these receptors. It is thus important to discuss these.

2.8.1. Aminopeptidase

Two types of aminopeptidases have been identified. These are aminopeptidase N (APN) and aminopeptidase P (APP). Only the former will be discussed.

Aminopeptidase (APN) is an exopeptidase (Banks *et al*., 2001) that has been identified as a Bt-toxin binding protein in midguts of several lepidopteran insects (Bravo *et al*., 2007; Crava *et al*., 2010; Nakanishi *et al*., 1999). However, Upadhyay and Singh (2011) dispute that APN is not an important receptor for insecticidal activity. The number of *APN* genes for each lepidopteran species is still uncertain, although it is suggested that at least seven classes of APNs occur (Crava *et al*., 2010). Similar *APN* genes have been observed in two species from two families, Plutellidae and Sphingidae, rather than within a single species. Chang *et al*. (1999) proposed that this may be due to an *APN* gene duplication event in the ancestral Plutellidae and Sphingidae lineage. Banks *et al*. (2001) suggested that a similar phenomenon in Noctuidae (i.e. a gene duplication event in the ancestral Noctuidae lineage) may explain the high sequence homology between *APN* from *H. virescens* and *APN2* from *H. punctigera*.

Common motifs have been identified in homologous positions in APN proteins of lepidopteran species (Figure 2.5) by *in vitro* and *in silico* analysis. These include: a signal peptide (N-terminus) (Angelucci *et al*., 2008), glycosylphosphatidylinositol (GPI)-anchor sequence (C-terminus) (Banks *et al*., 2003), zinc-binding motif $HEXXH(X)_{18}E$ (Hooper, 1994) and the GAMENWG gluzincin aminopeptidase sequence (Banks *et al*., 2003), which are essential for their enzymatic activity. The $HEXXH(X)_{18}E$ and GAMENWG motifs are demonstrated for *APN* in Appendix A. Angelucci *et al*. (2008) and Chang *et al*. (1999) proposed that *APNs* are derived from multiple gene duplications from a common lepidopteran ancestor, as has been also suggested by Crava *et al*. (2010).

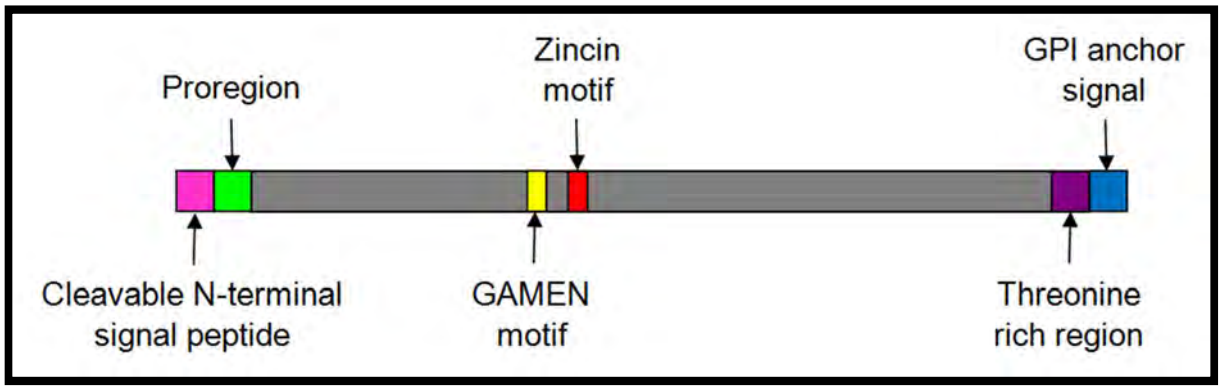


Figure 2.5: Schematic illustration of a typical lepidopteran APN protein. The proregion (green) and threonine-rich region (purple) have only been reported in some APNs (adapted from Pigott & Ellar, 2007).

The GPI-anchor anchors secondary receptors, such as APN, to the membrane (Gill & Ellar, 2002; Sangadala *et al*., 1994), but it is unclear whether the anchor has any role in toxin binding (Angelucci *et al*., 2008). GPI-anchors in midgut APN molecules have been found to be heterogenous, which suggests differences in the functional roles of APN (Luo *et al*., 1997). Upadhyay and Singh (2011) suggest that the GPI-anchor may possibly play some critical role in the toxicity of ŭ-endotoxins.

Different patterns of toxin-receptor-binding can occur (Luo *et al*., 1997; Rajagopal *et al*., 2003): (i) some APNs may bind multiple Cry toxins; (ii) some Cry toxins may bind to multiple APNs or (iii) toxin-APN pairs may be unique. Pigott and Ellar (2007) observed differences in toxin binding to native and recombinant forms of APN. These authors suggest that these differences may be due to differences in posttranslational modification, such as glycosylation. Glycosylation of cadherin proteins does not seem to be essential for toxin binding, whereas glycosylation of APNs is critical for binding in some cases (Pigott & Ellar, 2007).

Previously it was demonstrated by Lee *et al*. (1996) that all APNs contain N-acetylgalactosamine (GalNAc) residues to which Cry1Ac toxins bound. These authors (Lee *et al*., 1996), however, uncovered evidence of maintained toxicity of a Cry1Ac mutant toxin with an altered GalNAc binding pocket in *H. virescens*. Banks *et al*. (2001) confirmed that GalNAc is not required for Cry1Ac binding to APN in *H. virescens* and that Cry1Ac-induced membrane permeation can proceed by both GalNAc-dependent and -independent mechanisms.

Gill and Ellar (2002) and Griffitts and Aroian (2005) have demonstrated that Cry toxin susceptibility can be conferred to a normally resistant insect via an *APN* transgene. This proved that *APN* could confer susceptibility *in vivo*, which signifies that APN is an important receptor. After the pre-pore oligomer has bound to the APN, it drives the oligomer into lipid rafts where pore formation takes place (Gómez *et al*., 2007). According to Gómez *et al*. (2002) oligomer formation is a prerequisite for membrane insertion.

2.8.2. Cadherin

Cadherins represent a large family of glycoproteins and have been identified as Bt-toxin binding proteins in midguts of several lepidopteran insects (Fabrick & Tabashnik, 2007; Gahan *et al*., 2001). These proteins are involved in several cellular processes. Cellular processes include: recognition, signaling and communication between cells, morphogenesis and maintenance of cell structure (Angst *et al*., 2001). For lepidopteran species the physiological functions of these proteins are, however, not yet clear (Bel & Escriche, 2006). Cadherin is a transmembrane protein with a cytoplasmic domain and an extracellular ectodomain in which several cadherin repeats occur (Bel & Escriche, 2006; Gómez *et al*., 2007). A typical lepidopteran cadherin protein is illustrated in Figure 2.6.

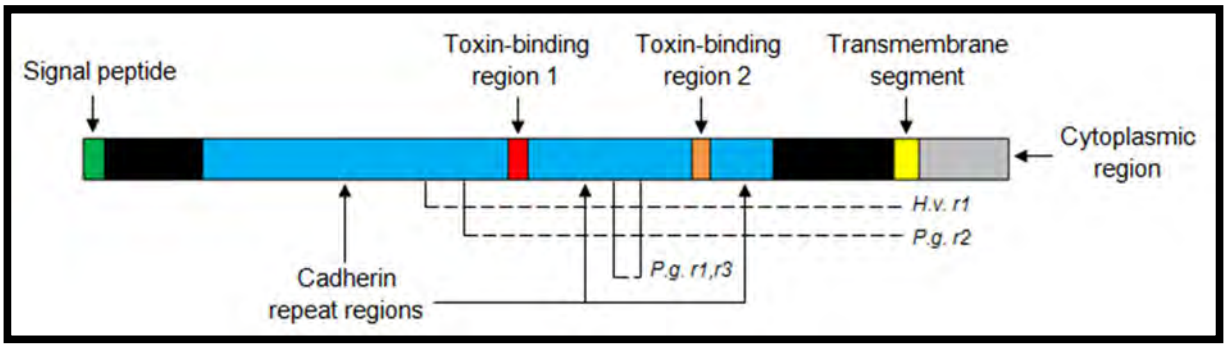


Figure 2.6: Schematic illustration of a typical lepidopteran cadherin protein. Regions of the protein that are expected to have been deleted in mutant alleles from *H. virescens* (*H.v. r1*) and *P. gossypiella* (*P.g. r1, r2, r3*) are indicated by dashed lines (adapted from Griffitts & Aroian, 2005).

Intron-exon patterns are highly conserved in *cadherin* genes (Bel & Escriche, 2006). *Cadherin*-like genes from *O. nubilalis*, *H. armigera* and *B. mori* were compared and a common structure, consisting of 35 exons joined by 34 introns, was observed in these genes. Sizes of exons within the genes were almost completely conserved, whereas introns varied in size among the three insect species (Bel & Escriche, 2006). Intron insertion positions were also found to be conserved in the three genes. Bel and Escriche (2006) observed the same intron insertion pattern in partial sequences of *cadherin*-like genes from *P. gossypiella* and *P. xylostella*. However, introns were generally larger in *B. mori* with respect to other lepidopteran species. Approximately 30% of *B. mori* genes were derived from transposable elements (Xia *et al*., 2004). Wang

et al. (2006) verified that this was also the case for other lepidopteran genes. Overall, these data suggest that *cadherin*-like genes are highly conserved.

The spectrum of toxicity is different for each type of Cry1A toxin, despite the high degree of homology among these proteins (Karim & Dean, 2000). While Cry1Aa is most toxic for *B. mori* (Van Frankenhuyzen *et al*., 1991), Cry1Ab and Cry1Ac are most toxic to *O. nubilalis* (Bel & Escriche, 2006) and *H. armigera* (Avilla *et al*., 2005), respectively. Differences in Cry toxin susceptibilities cannot be affirmed by differences in the Cry toxin protein sequences, as these differences in susceptibilities can be caused by other specific physiological differences in these three insect species (Bel & Escriche, 2006). According to Bel and Escriche (2006) recognition between cadherins and Cry1A toxins is highly specific only in Lepidoptera. These authors (Bel & Escriche, 2006) suggest that all the Bt-related *cadherins* are from a single origin.

According to Griffitts and Aroian (2005), different mutations in *cadherin* are responsible for Cry1 toxin resistance in *H. virescens* and *P. gossypiella*, respectively. Cry toxin resistance in *H. virescens* and *P. xylostella* have in each species been mapped to a single major resistance locus, designated *BtR-4* (Gahan *et al*., 2001) and *BtR-1* (Heckel *et al*., 1999), respectively. The *H. virescens BtR-4* locus corresponds to a *cadherin* gene that is interrupted by a retrotransposon (Gahan *et al*., 2001; Gómez *et al*., 2007), expected to eliminate the toxin-binding regions on the truncated protein product. The *P. gossypiella* (pink bollworm) *BtR-1* locus revealed three mutated *cadherin* alleles that are associated with Cry toxin resistance (Morin *et al*., 2003). Cry1Ac resistance was also observed in *H. armigera* as a result of a mutated *cadherin*-like gene (Xu *et al*., 2005).

2.8.3. Alkaline phosphatase (ALP)

Alkaline phosphatase has been identified as a Bt-toxin binding protein in midguts of several lepidopteran insects (Jurat-Fuentes & Adang, 2004; McNall & Adang, 2003). It is a secondary receptor that is also GPI-anchored (Bravo *et al*., 2007; Jurat-Fuentes & Adang, 2006), glycosylated and enriched in lipid rafts (Arenas *et al*., 2010; Gahan *et al*., 2010). Multiple ALP isoforms exist, and some protein regions (GFFLFVEGGR) are conserved among insect membrane-bound ALPs (Perera *et al*., 2009). The predicted structure of a typical alkaline phosphatase protein is illustrated in Figure 2.7. According to Perera *et al*. (2009), the signal peptide plays a role in the expression of this protein

on the surface of epithelial cells. The GFFLFVEGGR conserved motif is illustrated in Appendix A. The O- and N-glycosylation sites, phosphatase domain and GPI-anchor site are only putative at this stage (Perera *et al*., 2009).

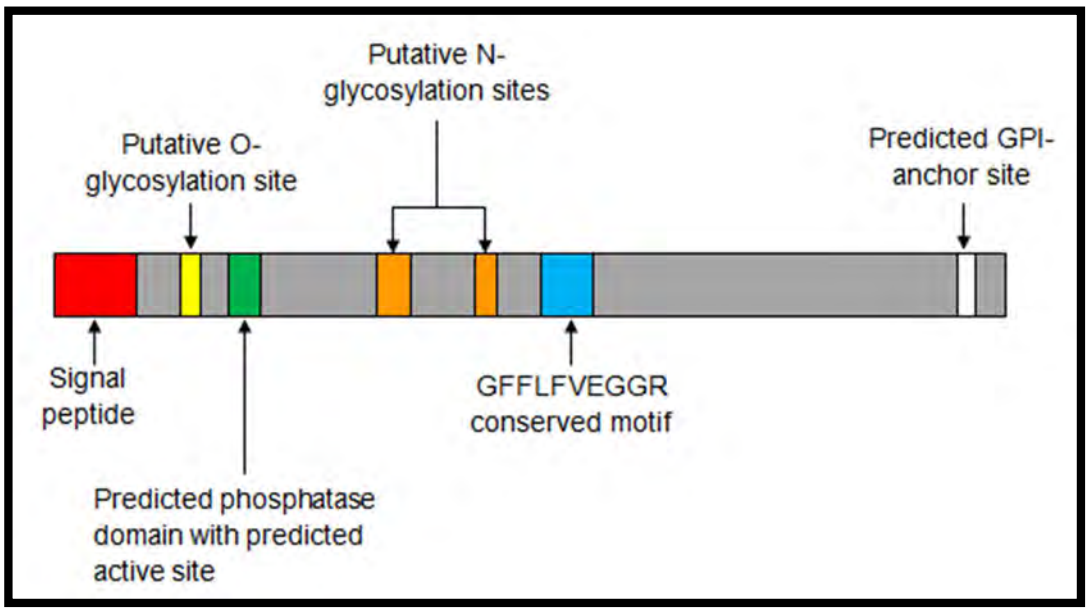


Figure 2.7: Schematic illustration of the predicted structure of a typical lepidopteran alkaline phosphatase protein (adapted from Perera *et al*., 2009).

Two isoforms of *ALP* isolated from *H. armigera* showed more than 94% sequence homology, which indicated that these may be allelic variants of a single gene (Ning *et al*., 2010). However, the existence of multiple *ALP* genes in insects was confirmed by Itoh *et al*. (2003) and Perera *et al*. (2009). Gómez *et al*. (2007) and McNall and Adang (2003) demonstrated that ALP of *Manduca sexta* (Lepidoptera: Sphingidae) was able to bind Cry1Ac toxin. It was also demonstrated that reduced levels of ALP in *H. virescens* larvae correlated with Cry1Ac resistance (Gómez *et al*., 2007; Jurat-Fuentes *et al*., 2004). These observations suggested that ALP has a functional role in toxin action (Jurat-Fuentes & Adang, 2006).

According to Arenas *et al*. (2010) Cry toxin binding to ALP is more important for insecticidal action. These authors (Arenas *et al*., 2010) demonstrated that the affinity between a mutant Cry toxin and APN was retained, whereas affinity to ALP as well as insecticidal property was lost for this mutant Cry toxin. Arenas *et al*. (2010) as well as Upadhyay and Singh (2011) reported that ALP plays a predominant role in the insecticidal action of Cry1Ab and Cry1Ac to *M. sexta* and *H. armigera*, respectively.

Jurat-Fuentes *et al*. (2011) stated that reduced expression of *ALP* seemed to be a common phenomenon in Bt-resistant lepidopteran larvae. No direct correlation between reduced levels of *ALP* expression and resistance has, however, been reported to date (Jurat-Fuentes *et al*., 2011). Previously, Rodrigo-Simón *et al*. (2008) hypothesized that changes in glycosylation of receptors may cause reduced Cry1A toxin binding, lack of pore formation and resistance. This hypothesis was confirmed by Ning *et al*. (2010). These authors (Ning *et al*., 2010) observed that changes in glycosylation or the presence of GalNAc residues inhibited Cry1Ac toxin binding to *H. armigera* brush border membrane vesicles. Jurat-Fuentes and Adang (2004) also reported that the amount of ALP inversely correlated with resistance development in *H. virescens* against Cry1Ac.

Jurat-Fuentes *et al*. (2011) proposed that the reduced expression of *ALP* may be used as a potential biomarker for resistance to diverse Cry toxins. Further research is, however, needed to determine if the reduced ALP levels cause a direct decrease in functional Cry toxin receptors and/or compensatory alterations in resistant larvae. In order to characterize this protein and its physiological role in lepidopteran larvae, further research is needed to understand the genetic alteration responsible for the lower levels of ALP in resistant larvae (Jurat-Fuentes & Adang, 2006).

2.8.4. Other receptors

Various glycolipids/proteins have also been identified as Bt-toxin binding sites in midguts of several lepidopteran insects (Hossain *et al*., 2004; Xu & Wu, 2008). Griffitts and Aroian (2005) reported that a mixture of Cry1A toxins can bind to insect glycosphingolipids. In that *in vitro* study it was demonstrated that Cry1Aa, Cry1Ab, and Cry1Ac each bound to *M. sexta* glycolipids. Jurat-Fuentes *et al*. (2002) and Upadhyay and Singh (2011) found that *H. virescens* developed resistance against Cry1Ac when glycosylation patterns of glycoproteins were altered. This suggests that the glycans on the receptors play a very important role in insecticidal activity. BTR-270 and P252 are glycosylated GPI-anchored glycoproteins (Pandian *et al*., 2008) that are enriched in lipid rafts associated with the membrane, and have also been identified as Cry toxin receptors (Pigott & Ellar, 2007).

ABC transporters are proteins integrated with the membrane, and play a role in the export of toxic molecules from the cell. ABC transporters cycle between closed and open configurations during transportation of molecules, and involve interactions of the oligomeric toxin pre-pore structure in the final binding step (Gahan *et al*., 2010). Gahan *et al*. (2010) hypothesized that membrane integration of the toxin pore is facilitated when the oligomers (Cry1Ab and Cry1Ac toxins) bind to the open configuration of ABCC2. A reduction in toxin binding was observed when a mutation inactivated ABCC2. This inactivating mutation was genetically linked to Cry1Ac resistance and correlated with loss of Cry1Ac binding to membrane vesicles. ABC transporters have, however, not been implicated in the mode of action of Bt-toxins before, but this was due to failure to detect or isolate them (Gahan *et al*., 2010).

Gahan *et al*. (2010) and Jurat-Fuentes *et al*. (2011) more recently demonstrated that resistance to Cry1Ac was conferred to *H. virescens* when expression of an ABC transporter protein failed, resulting in lack of Cry1Ac binding. Gahan *et al*. (2010) propose that *H. zea*, *S. frugiperda* and *B. fusca* should be examined for ABC transporter mutations, since their genetic basis of field-evolved resistance to Bt-crops has not yet been identified in these strains. Isolation of these proteins is thus essential in order to characterize their toxin-binding properties and determine their role in the mode of action of Bt-toxins.

2.9. *Busseola fusca* resistance to Cry1Ab

According to Kruger *et al*. (2009), 99.8% of the farmers in the Vaalharts area that planted refugia did not allow any spatial separation between the Bt-maize field and the adjacent refugia. This enables *B. fusca* larvae to migrate to adjacent plants approximately 14 days after egg hatch and since certain plant parts are less concentrated with the Bt-toxin, larger larvae may be exposed to sub-lethal doses of the Bt-toxin (Siegfried *et al*., 2001). Larvae are also able to feed on leaves after flowering. This is due to decreases in toxin concentration in the leaves over time (Van Wyk *et al*., 2008). These larvae will survive on Bt-maize after they have grown on conventional maize (refugia) to the third or fourth instar. This will ultimately contribute to resistance development (Kruger *et al*., 2009). Kruger *et al*. (2011a) demonstrated that larvae found in refugia were also resistant to Bt-maize. Thus the high-dose/refuge strategy was

unsuccessful for the control of *B. fusca* and particularly for the management of resistance development.

The rapid evolution of resistance to Cry1Ab by *B. fusca* can thus be attributed to non-recessive inheritance, failure to achieve the high-dose standard and inadequate refuges (Tabashnik, 2008; Van Rensburg, 2007). According to field data, Cry1Ab maize in South Africa does not kill 99.99% of *B. fusca* larvae (Van Rensburg, 2007). This is below the USEPA standard for a high dose (USEPA, 1998). No study has yet been done to determine the molecular mechanism of *B. fusca* resistance to Cry1Ab. However, such studies have been conducted on several other lepidopterans. One such example is the cotton bollworm, *H. armigera* (Bravo & Soberón, 2008; Zhang *et al*., 2009).

H. armigera, a major insect pest in cotton-growing regions in China, has developed resistance to the Bt-protein toxin Cry1Ac. A mutation in the *APN* receptor gene, a receptor for Cry1Ac, has been identified as one of the main mechanisms of resistance in *H. armigera* (Zhang *et al*., 2009). Even though *B. fusca* has developed resistance to the Cry1Ab toxin, the binding patterns and binding sites of Cry1Ac and Cry1Ab are similar (Ferré & Van Rie, 2002; Li *et al*., 2004). According to Aimanova *et al*. (2006), Cry1Ab and Cry1Ac toxins are more than 80% identical. The main question posed here is thus whether similar mutations, as observed in *H. armigera*, are responsible for *B. fusca* resistance to Cry1Ab toxin.

In the case of *H. armigera* an allele-specific PCR was developed to detect the mutation in the *APN* gene, designated *HaAPN1*. The wild-type (susceptible) and the mutant (resistant) allele of *HaAPN1* were amplified and discriminated by using a pair of specific primers, which yielded genomic DNA fragments of 270 bp and 204 bp (indicating the deletion of 66 bases, i.e. 22 amino acids), respectively (Zhang *et al*., 2009). This protocol has been replicated for *B. fusca*, but it was not possible to discriminate between the Bt-susceptible and -resistant strain of *B. fusca* by using PCR. Thus the primers that were designed for *H. armigera* did not work for *B. fusca* (Venter, 2010). It was therefore important to design primers that are specific for *B. fusca* that could amplify the entire *APN1* gene, as well as *APN* isogenes and other Cry receptor genes potentially involved in Bt-resistance.

Limited molecular data is available for *B. fusca*, which complicates any molecular studies on this species. Also no studies have been found on the phylogenetic relationship between *B. fusca* and other related lepidopteran species. *Cytochrome b* gene sequences are available for *B. fusca* and other lepidopteran species and could be used to determine the phylogenetic relationship between *B. fusca* and other lepidopteran species.

2.10. *Cytochrome b*

Through the use of DNA-sequence data from the mitochondrial genome, phylogenetic relationships among species and/or populations can be estimated. These data are not absolute due to the occurrence of ancestral polymorphisms and multiple substitutions at single nucleotide sites (Assefa *et al*., 2006; Simon *et al*., 1994).

The population genetics and phylogeography of African insects have received little attention despite their diversity and economic importance (Kuchta & Meyer 2001). One such example is *B. fusca*, a major pest in the Vaalharts irrigation scheme. According to Sezonlin *et al*. (2006), *B. fusca* displays significant geographic differences due to its ecological preferences. This may correspond to the patterns of molecular genetic differences that were found in phylogeographical analyses done by these authors. They studied the mitochondrial cytochrome *b* gene, due to its informative nature at the intrageneric level in Lepidoptera (Simmons & Weller 2001). Based on genetic differentiation in mitochondrial DNA they concluded that *B. fusca* populations could be divided into three major groups. This included a homogeneous and geographically isolated population from West Africa, and two populations from East and Central Africa (Kenya) with overlapping distributions (Sezonlin *et al*., 2012). No southern African populations were included in this study of Sezonlin *et al*. (2012).

Although sequence data has accumulated rapidly in the past several years, very limited molecular data are available for *B. fusca*. *Cytochrome b* sequences are thus available for phylogenetic analyses of *B. fusca*. There are shortcomings that have to be considered when this gene is used for phylogenetic analyses. This mainly involves the rate of evolution in the conservative and variable domains (Farias *et al*., 2001). However, these sequences were used in various studies to demonstrate genetic relatedness (Farias *et al*., 2001; Sezonlin *et al*., 2012; Simmons & Weller, 2001).

Sezonlin *et al*. (2012) used *cytochrome b* sequences to clarify the genetic relationship between *B. fusca* populations from the Guineo-Congolian rain forest and Afromontane vegetation mosaics in Cameroon. The study of Sezonlin *et al*. (2012) concluded that *B. fusca* populations in Cameroon recently colonized. This conclusion was based on low genetic differentiation observed within these populations according to a comparison of *cytochrome b* mitochondrial DNA gene.

2.11. Vaalharts irrigation scheme

The Vaalharts irrigation scheme is part of a semi-arid area, between 1050 and 1150 m above sea-level (S24º48'693", E27º38'330"), with an average rainfall of 450 mm per year (Bornman, 1988; Herold & Bailey, 1996). The irrigation scheme covers an area of ~80 km from North to South and 30 km from East to West (Kruger *et al*., 2009). It consists of plots of 25 ha each (Fourie, 2006), but many farmers extended their farms by renting or owning more than one plot (Kruger *et al*., 2009). The total scheme extends over about 113 110 ha of which 35 989 ha is under irrigation (Bornman, 1988).

Monsanto, Pannar, Pioneer Hybrid International, Afgri and Syngenta are the main suppliers of seed to farmers in the scheme. Ninety six percent of farmers plant hybrids deploying event MON 810. These plants produce the Bt-toxin Cry1Ab used to control *B. fusca* and other stem borers (Tabashnik *et al*., 2009; Van Rensburg, 2007). Ultra-short growing season maize cultivars allow farmers to plant an additional crop per year. This results in an annual average of 9000 ha of maize being grown (Kruger *et al*., 2009).

Since 1998, Bt-maize has been planted in the Vaalharts irrigation scheme in South Africa (James, 2009b) to target, amongst others, the stem borer *B. fusca* (Kruger *et al*., 2009). *B. fusca* was already identified as one of the most dominant pests with the most likely resistance risk for Bt-maize in 2004 (Fitt *et al*., 2004). One of the first farmer reports of resistance was made in 2004, where 71% of stalks were found to have live over-wintering *B. fusca* larvae (Van den Berg, 2010). In the 2005-2006 growing season, 2.5% of Vaalharts farmers reported damage to Bt-maize from stem borers. By the 2007-2008 growing season damage has increased to 58.8%. This necessitated the application of insecticides to Bt-maize for stem borer control (Tabashnik *et al*., 2009).

According to Kruger *et al*. (2009), only 7.7% of the farmers in the Vaalharts area planted refuges in 1998, which increased to 92.3% in the 2007-2008 growing season. It is suggested that the initial lack of compliance amongst farmers contributed to the resistance development of *B. fusca* to the Cry1Ab toxin (Kruger *et al*., 2011b). This has led to the Vaalharts irrigation scheme and adjacent areas being a hot spot for field evolved resistance of *B. fusca* to Bt-maize.

2.12. Future outlook for GM crops

A DNA-based method of detecting resistant alleles directly would be more efficient than conventional bioassay-based monitoring methods (Xu *et al*., 2005; Zhang *et al*., 2009), especially when resistance is rare and recessive. Bioassays only detect resistant homozygotes, while DNA-based screening can detect resistance alleles in heterozygotes. The latter method will be more efficient, seeing that inheritance of resistance to the Bt-toxins in pests is usually recessive (Morin *et al*., 2003; Yang *et al*., 2007).

Resistance/susceptibility monitoring also plays a key role in resistance management (Wu, 2007; Zhang *et al*., 2009). Establishment of genetic tools for resistance gene identification is challenging, but once resistance is understood it will provide the opportunity to monitor resistance outbreaks at early stages. Only then can effective resistance management strategies be developed that will prevent or delay resistance development in insect populations (Khajuria *et al*., 2011).

The latest commercial strategy in GM crops is toxin stacking, whereby multiple toxins (that do not cause strong cross resistance to each other) are co-expressed in the same crop. This strategy is based on the basis that certain combinations of Cry toxins are unlikely to be resisted simultaneously (Griffitts & Aroian, 2005). The ideal would be to develop resistance biomarkers that can efficiently differentiate susceptible and resistant insects, regardless of the resistance mechanism, Bt-crop or Cry toxin involved (Jurat-Fuentes *et al*., 2011).

Long-term, sustainable transgenic crop use relies on understanding the mode of Cry1A toxin action and mechanisms of resistance (Banks *et al*., 2003). Prior to understanding the mechanisms of resistance, the genes involved in the Bt-toxin interactions should be

identified. Novel Cry toxin binding proteins and proteins that are differentially expressed in resistant larvae have been successfully identified by means of proteomic approaches. This approach is, however, hampered by the low percentage of lepidopteran sequences available in the proteomic databases (Jurat-Fuentes & Adang, 2006). Further research into this field, as well as transcriptomics, is required, but will be facilitated by the availability of sequence data. For species with very little molecular data, such as *B. fusca*, genome sequencing would be more informative. Further genomic, transcriptomic and proteomic studies can then be used to formulate strategies to prevent or delay resistance development in pest populations.

2.13. Summary of literature review

The literature provided an overview of how important GM crops are, but also the risks associated with it. A brief overview of what GM crops are was also provided. The diversity, structure and function of Cry toxins were discussed. This chapter also dealt with the mechanisms that are involved in toxin action. Several theories about actual mechanisms are provided in Section 2.7. A common mechanism of resistance in lepidopteran pests seems to be caused by mutations in Cry receptor genes. Therefore the most important receptors involved in toxin binding were discussed in detail in Section 2.8. Examples of several lepidopteran pests that have developed resistance to Cry toxins were provided.

The preceding literature provides information relevant to the present study. This study was concerned with the detection of mutations in Cry receptor genes from *B. fusca* using PCR amplification. In order to study mutations, sequences of Cry receptor genes are, however, required. After realizing that the molecular data is limited for *B. fusca*, the rest of the study was therefore an attempt to discover Cry receptor genes. The approach of this study was based on the resistance mechanism in *H. armigera* to the Cry1Ac toxin.

In conclusion, the benefits and risks of GM technology will be important for future risk assessments as more knowledge becomes available (Adenle, 2011). It is therefore important to establish the genes involved in Bt-toxin interactions, the mode of action and mechanisms of resistance (Banks *et al*., 2003; Griffitts & Aroian, 2005; Zhang *et al*.,

2009). This task is, however, easier said than done with the low percentage of lepidopteran sequences available in the databases (Jurat-Fuentes & Adang, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1. Sample collection

Bt-susceptible *Helicoverpa armigera* larvae (Lepidoptera: Noctuidae) and Bt-susceptible and -resistant *Busseola fusca* larvae (Lepidoptera: Noctuidae) were collected from the Agricultural Research Council (ARC), Potchefstroom. The Bt-susceptible *B. fusca* larvae were originally sourced from Kenya as egg pockets and raised at the ARC facilities. Bt-resistant *B. fusca* larvae were collected from Bt-maize in the Vaalharts area and further raised at the ARC facilities. The larvae were placed in petri dishes and transported to the laboratory where the larvae were immediately used for DNA isolation. As mentioned in Chapter 2, *H. armigera* and *B. fusca* have developed resistance to Cry1Ac and Cry1Ab respectively, but the binding-patterns and ï sites of Cry1Ac and Cry1Ab are similar. Thus *H. armigera* was used in this study for comparison purposes.

3.2. Sample preparation

The larvae were placed in 95% ethanol, after which five *H. armigera* larvae were pooled and ground to powder in a sterile DNA-free mortar and pestle using liquid nitrogen (AFROX, SA). The powder was then divided into ten tubes, ~30 mg of material per tube. In the case of *B. fusca*, individual larvae were ground using a sterile mortar and pestle. Liquid nitrogen was not used, since such treatment was found to be too harsh for DNA being isolated from *B. fusca* larvae. This change of isolation was necessary, since this treatment caused considerable shearing of the DNA. The larval material was also divided into tubes, ~30 mg of material per tube, prior to DNA isolation. Four tubes could be prepared from the larval material of each *B. fusca* individual.

3.3. DNA isolation

A NucleoSpin® Tissue Kit (Macherey-Nagel, Germany) was used to isolate genomic DNA from *H. armigera* and *B. fusca* larvae according to the protocol provided by the manufacturer. The main steps of the isolation process include the lyses of sample material; the binding of DNA to a silica membrane; a washing step to rid DNA of

contaminants and finally the elution of pure genomic DNA. The isolated DNA was stored at -20 °C until further use.

Prior to storage, DNA was characterized spectrophotometrically and electrophoretically. A NanoDropTM 1000 Spectrophotometer (Thermo Fischer Scientific, US) was used to determine DNA concentrations (ng/µl) and quality ($A_{260\ nm}$:$A_{280\ nm}$ ratios). The integrity of the isolated DNA was also determined by using a 1% (w/v) TAE agarose (WhiteSci, USA) gel containing 0.001 mg/ml ethidium bromide (Bio-Rad, UK).

Electrophoresis was conducted in a wide mini-sub cell GT electrophoresis system (Bio-Rad, UK) for 60 min at 80 V. A 1x TAE solution [40 mM Tris (Sigma Aldrich, US), 20 mM acetic acid, glacial (Merck, US) and 1 mM EDTA (Merck, US), pH 8.0] was used as the electrophoresis buffer. A mixture of 5 µl of the isolated DNA and 5 µl 6x Orange Loading dye (Fermentas Life Science, US) was loaded into each well of the gel. A 1 kb (2 µl) molecular weight marker (O GeneRuler, Fermentas Life Science, US) was also loaded into the gel. The gel images were captured by using a ChemiDocÊ MP Imaging System (Bio-Rad, USA) and Image LabÊ (version 4.0.1) software.

3.4. *Cytochrome b* analysis

A primer set amplifying the gene encoding *cytochrome b* (Sezonlin *et al*., 2006) was used as a positive control. Sequences of this gene are available for *B. fusca* on GenBank. The primer set CP1 (5'-GAT GAT GAA ATT TTG GAT C-3') and TRs (5'-TAT TTC TTT ATT ATG TTT TCA AAA C-3') yielded a fragment of 1000 bp. Each 25 µl reaction contained 11.4 µl PCR-grade water, 1X *Pfu* Buffer (Fermentas Life Science, US), 0.2 mM dNTPs, 0.5 µM of each primer (Applied Biosystems, UK), 3 mM $MgSO_4$, 0.75 U *Pfu* DNA polymerase (recombinant) and 50 ng sample DNA. A no-template control (NTC) was included in the amplification process, of which the reaction contained the same reagents as the other samples, except DNA. The purpose of the NTC is to show that PCR products are not due to amplification of contaminants present in the PCR reagents. The PCR cycling conditions were adjusted to an initial denaturation of 5 min at 94 ºC; followed by 40 cycles of 1 min at 94 ºC, 1 min 30 s at 46 ºC and 2 min at 72 ºC. A final extension step of 10 min at 72 ºC was included. The PCR products were resolved by 1.5% (w/v) TAE agarose gel using electrophoresis as described in Section

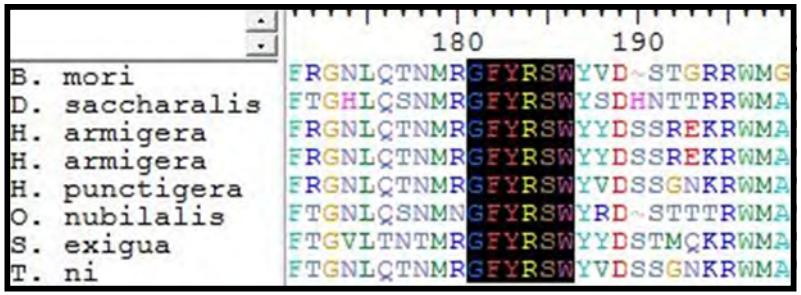
3.3. PCR products were sent to Inqaba Biotech (South Africa) for excision, purification and sequencing. Sequences were analyzed as described in Section 3.7.

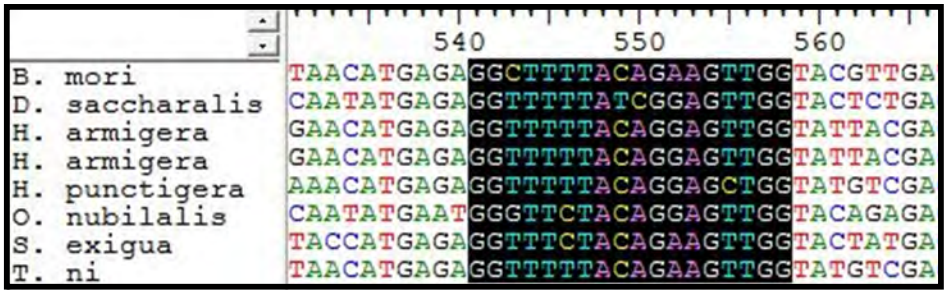
3.5. Design of primers for Cry receptor genes

After many failed attempts to amplify the Cry receptor genes from *B. fusca* using existing primers for other lepidopteran species, it was decided that custom degenerate primers should be designed. For each Cry receptor gene, mRNA sequences (introns excluded) were obtained for several lepidopteran species, since complete gene sequences (introns included) were not available for the genes of interest. mRNA sequences were converted to DNA sequences prior to aligning them using MAFFT online software (http://mafft.cbrc.jp/alignment/server/). Inconsistencies in the alignments were edited manually using BioEdit (version 7.1.3.0) software.

Many conserved regions were observed in the protein sequence alignments of the Cry receptor genes (Appendix A). Conserved regions observed in the protein sequence alignments with the corresponding DNA sequences are shown in Figures 3.1 to 3.8. The full DNA sequence alignment of each Cry receptor gene is shown in Appendix B. Degenerate primers (Table 3.1) were designed manually based on these conserved regions. The positions of the primers are shown in Figure 3.9 (not drawn to scale).

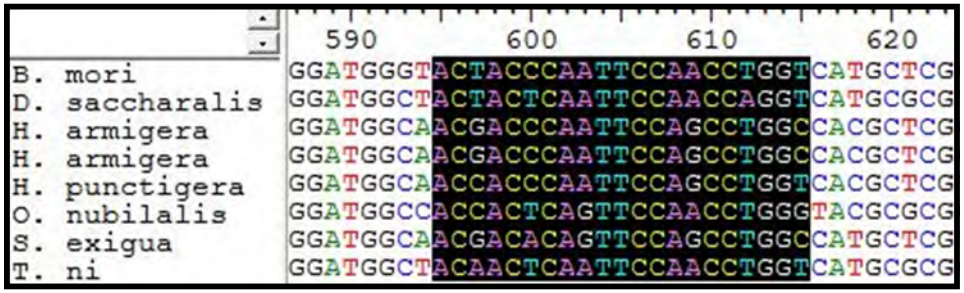
(a)


180
190
B. mori FRGNLQTNMRGFYRSWYVD~STGRRWMG
D. saccharalis FTGHLQSNMRGFYRSWYSDHNTTRRWMA
H. armigera FRGNLQTNMRGFYRSWYYDSSREKRWMA
H. armigera FRGNLQTNMRGFYRSWYYDSSREKRWMA
H. punctigera FRGNLQTNMRGFYRSWYVDSSGNKRWMA
O. nubilalis FTGNLQSNMNGFYRSWYRD~STTTRWMA
S. exigua FTGVLTNTMRGFYRSWYYDSTMQKRWMA
T. ni FTGNLQTNMRGFYRSWYVDSSGNKRWMA

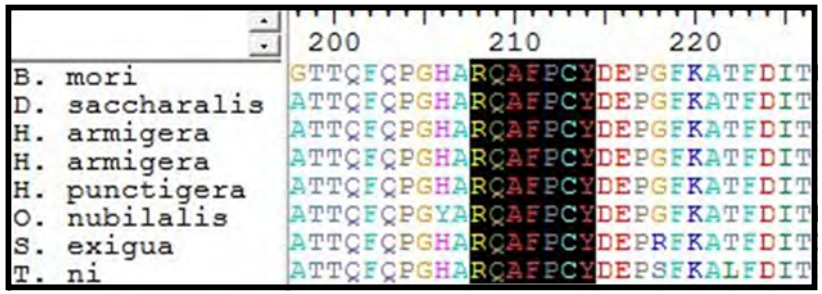

540
550
560
B. mori TAACATGAGAGGCTTTTACAGAAGTTGGTACGTTGA
D. saccharalis CAATATGAGAGGTTTTTATCGGAGTTGGTACTCTGA
H. armigera GAACATGAGAGGTTTTTACAGGAGTTGGTATTACGA
H. armigera GAACATGAGAGGTTTTTACAGGAGTTGGTATTACGA
H. punctigera AAACATGAGAGGTTTTTACAGGAGCTGGTATGTCGA
O. nubilalis CAATATGAATGGGTTCTACAGGAGTTGGTACAGAGA
S. exigua TACCATGAGAGGTTTCTACAGAAGTTGGTACTATGA
T. ni TAACATGAGAGGTTTTTACAGAAGTTGGTATGTCGA

(b)


190
200
210
B. mori VD~STGRRWMGTTQFQPGHARQAFPC
D. saccharalis SDHNTTRRWMATTQFQPGHARQAFPC
H. armigera YDSSREKRWMATTQFQPGHARQAFPC
H. armigera YDSSREKRWMATTQFQPGHARQAFPC
H. punctigera VDSSGNKRWMATTQFQPGHARQAFPC
O. nubilalis RD~STTTRWMATTQFQPGYARQAFPC
S. exigua YDSTMQKRWMATTQFQPGHARQAFPC
T. ni VDSSGNKRWMATTQFQPGHARQAFPC


590
600
610
620
B. mori GGATGGGTACTACCCAATTCCAACCTGGTCATGCTCG
D. saccharalis GGATGGCTACTACTCAATTCCAACCAGGTCATGCGCG
H. armigera GGATGGCAACGACCCAATTCCAGCCTGGCCACGCTCG
H. armigera GGATGGCAACGACCCAATTCCAGCCTGGCCACGCTCG
H. punctigera GGATGGCAACCACCCAATTCCAGCCTGGTCACGCTCG
O. nubilalis GGATGGCCACCACTCAGTTCCAACCTGGGTACGCGCG
S. exigua GGATGGCAACGACACAGTTCCAGCCTGGCCATGCTCG
T. ni GGATGGCTACAACTCAATTCCAACCTGGTCATGCGCG

(c)


200
210
220
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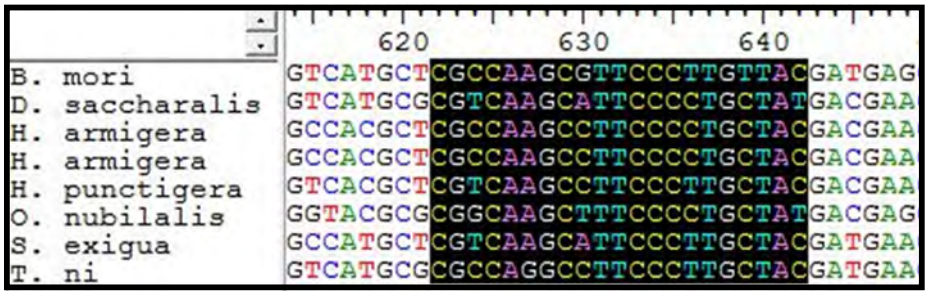
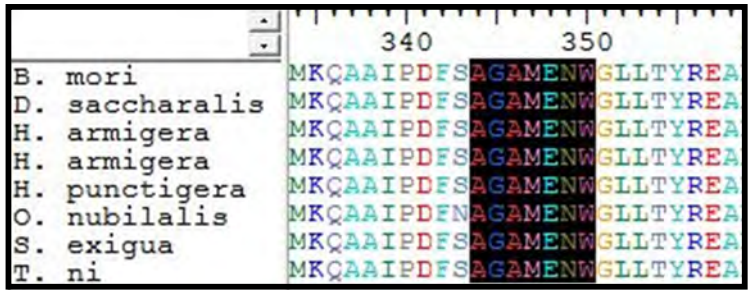
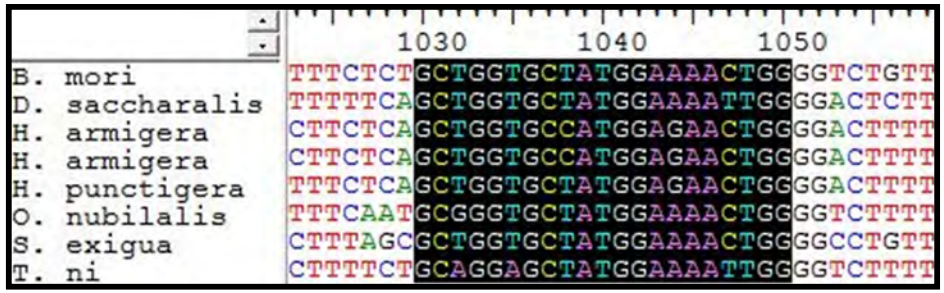
D. saccharalis ATTQFQPGHARQAFPCYDEPGFKATFDIT
H. armigera ATTQFQPGHARQAFPCYDEPGFKATFDIT
H. armigera ATTQFQPGHARQAFPCYDEPGFKATFDIT
H. punctigera ATTQFQPGHARQAFPCYDEPGFKATFDIT
O. nubilalis ATTQFQPGYARQAFPCYDEPGFKATFDIT
S. exigua ATTQFQPGHARQAFPCYDEPRFKATFDIT
T. ni ATTQFQPGHARQAFPCYDEPSFKALFDIT

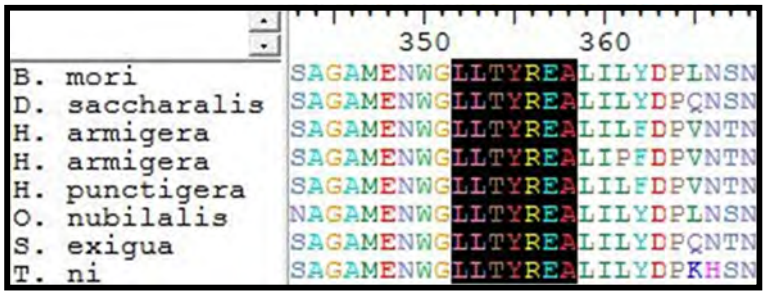

620
630
640
B. mori GTCATGCTCGCCAAGCGTTCCCTTGTTACGATGAG
D. saccharalis GTCATGCGCGTCAAGCATTCCCCTGCTATGACGAA
H. armigera GCCACGCTCGCCAAGCCTTCCCCTGCTACGACGAA
H. armigera GCCACGCTCGCCAAGCCTTCCCCTGCTACGACGAA
H. punctigera GTCACGCTCGTCAAGCCTTCCCTTGCTACGACGAA
O. nubilalis GGTACGCGCGGCAAGCTTTCCCCTGCTATGACGAG
S. exigua GCCATGCTCGTCAAGCATTCCCTTGCTACGATGAA
T. ni GTCATGCGCGCCAGGCCTTCCCTTGCTACGATGAA

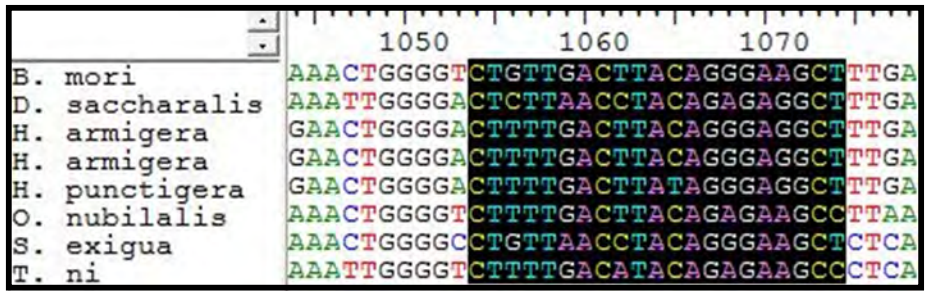
(d)


340
350
B. mori MKQAAIPDFSAGAMENWGLLTYREA
D. saccharalis MKQAAIPDFSAGAMENWGLLTYREA
H. armigera MKQAAIPDFSAGAMENWGLLTYREA
H. armigera MKQAAIPDFSAGAMENWGLLTYREA
H. punctigera MKQAAIPDFSAGAMENWGLLTYREA
O. nubilalis MKQAAIPDFNAGAMENWGLLTYREA
S. exigua MKQAAIPDFSAGAMENWGLLTYREA
T. ni MKQAAIPDFSAGAMENWGLLTYREA

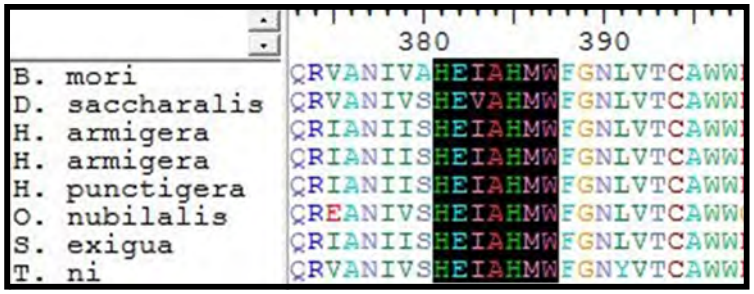

1030
1040
1050
B. mori TTTCTCTGCTGGTGCTATGGAAAACTGGGGTCTGTT
D. saccharalis TTTTTCAGCTGGTGCTATGGAAAATTGGGGACTCTT
H. armigera CTTCTCAGCTGGTGCCATGGAGAACTGGGGACTTTT
H. armigera CTTCTCAGCTGGTGCCATGGAGAACTGGGGACTTTT
H. punctigera TTTCTCAGCTGGTGCTATGGAGAACTGGGGACTTTT
O. nubilalis TTTCAATGCGGGTGCTATGGAAAACTGGGGTCTTTT
S. exigua CTTTAGCGCTGGTGCTATGGAAAACTGGGGCCTGTT
T. ni CTTTTCTGCAGGAGCTATGGAAAATTGGGGTCTTTT

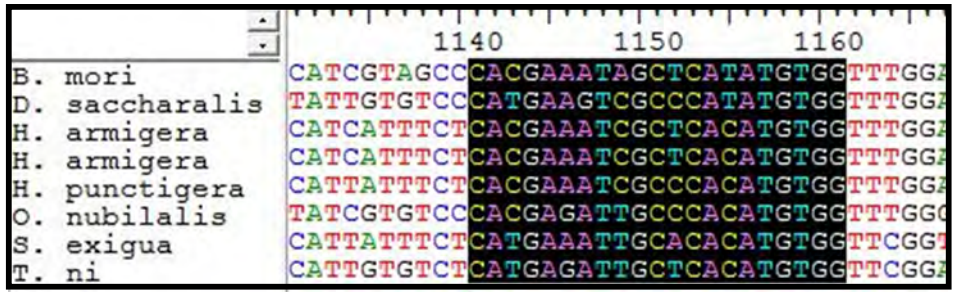
(e)


350
360
B. mori SAGAMENWGLLTYREALILYDPLNSN
D. saccharalis SAGAMENWGLLTYREALILYDPQNSN
H. armigera SAGAMENWGLLTYREALILFDPVNTN
H. armigera SAGAMENWGLLTYREALIPFDPVNTN
H. punctigera SAGAMENWGLLTYREALILFDPVNTN
O. nubilalis NAGAMENWGLLTYREALILYDPLNSN
S. exigua SAGAMENWGLLTYREALILYDPQNTN
T. ni SAGAMENWGLLTYREALILYDPKHSN

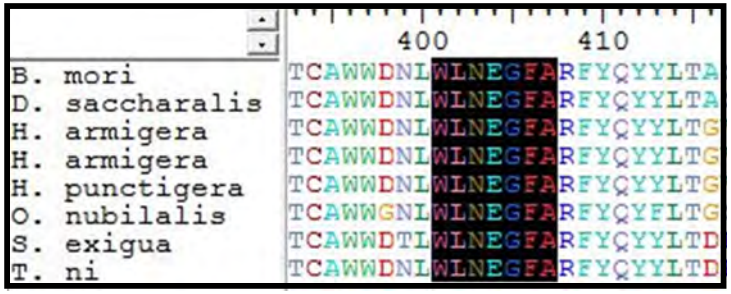

1050
1060
1070
B. mori AAACTGGGGTCTGTTGACTTACAGGGAAGCTTTGA
D. saccharalis AAATTGGGGACTCTTAACCTACAGAGAGGCTTTGA
H. armigera GAACTGGGGACTTTTGACTTACAGGGAGGCTTTGA
H. armigera GAACTGGGGACTTTTGACTTACAGGGAGGCTTTGA
H. punctigera GAACTGGGGACTTTTGACTTATAGGGAGGCTTTGA
O. nubilalis AAACTGGGGTCTTTTGACTTACAGAGAAGCCTTAA
S. exigua AAACTGGGGCCTGTTAACCTACAGGGAAGCTCTCA
T. ni AAATTGGGGTCTTTTGACATACAGAGAAGCCCTCA

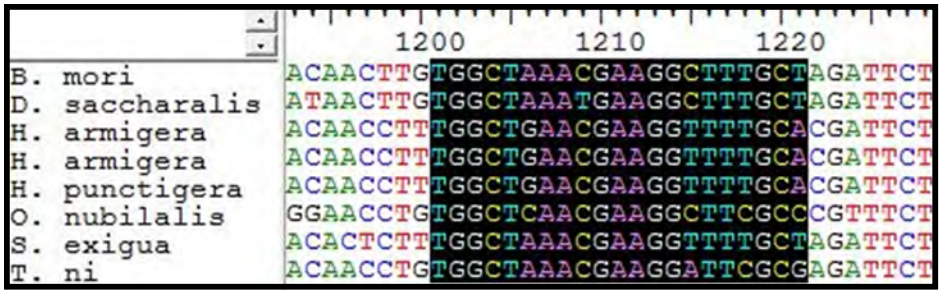
(f)


380
390
B. mori QRVANIVAHEIAHMWFGNLVTCAWW
D. saccharalis QRVANIVSHEVAHMWFGNLVTCAWW
H. armigera QRIANIISHEIAHMWFGNLVTCAWW
H. armigera QRIANIISHEIAHMWFGNLVTCAWW
H. punctigera QRIANIISHEIAHMWFGNLVTCAWW
O. nubilalis QREANIVSHEIAHMWFGNLVTCAWW
S. exigua QRIANIISHEIAHMWFGNLVTCAWW
T. ni QRVANIVSHEIAHMWFGNYVTCAWW

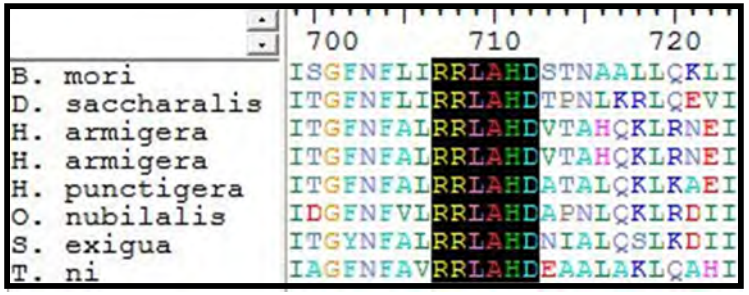

1140
1150
1160
B. mori CATCGTAGCCCACGAAATAGCTCATATGTGGTTTGG
D. saccharalis TATTGTGTCCCATGAAGTCGCCCATATGTGGTTTGG
H. armigera CATCATTTCTCACGAAATCGCTCACATGTGGTTTGG
H. armigera CATCATTTCTCACGAAATCGCTCACATGTGGTTTGG
H. punctigera CATTATTTCTCACGAAATCGCCCACATGTGGTTTGG
O. nubilalis TATCGTGTCCCACGAGATTGCCCACATGTGGTTTGG
S. exigua CATTATTTCTCATGAAATTGCACACATGTGGTTCGG
T. ni CATTGTGTCTCATGAGATTGCTCACATGTGGTTCGG

(g)





(h)



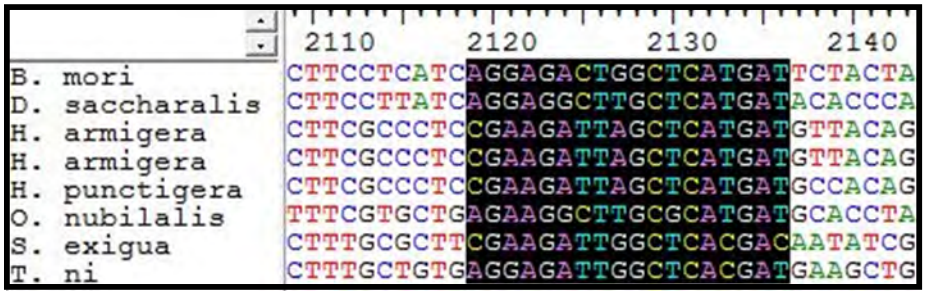
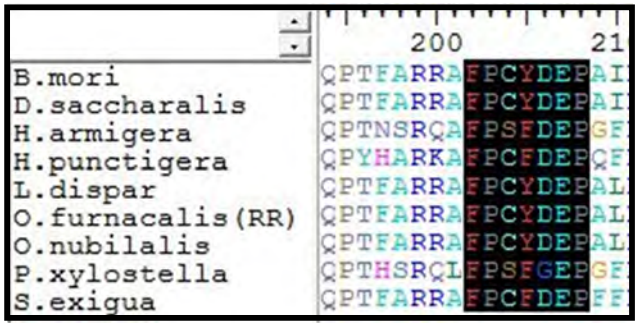
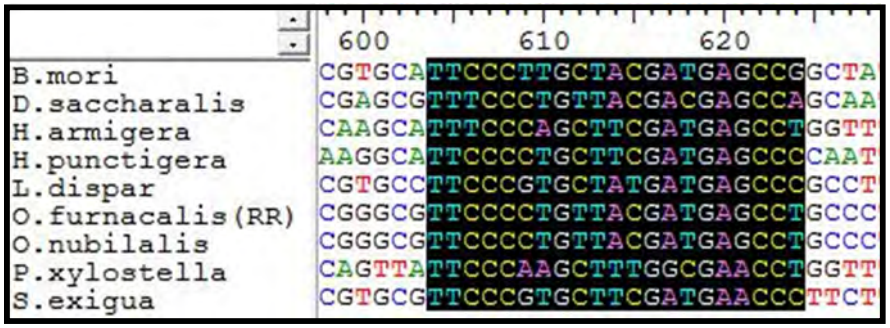


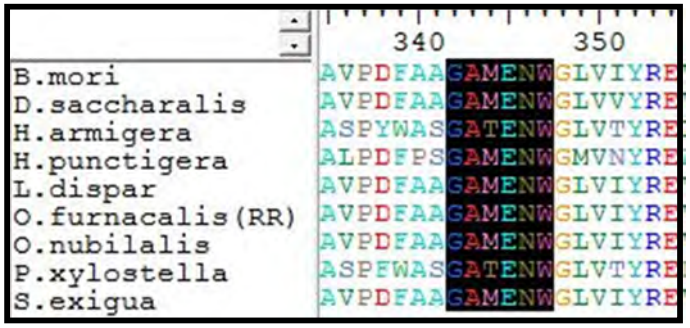
Figure 3.1: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *APN1*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) APN1AF, (b) APN1BF, (c) APN1CR, (d) APN1DR, (e) APN1EF, (f) APN1FF, (g) APN1GR and (h) APN1HR. The complete protein and DNA sequence alignment of APN1 is shown in Figure A.1 (Appendix A) and B.1 (Appendix B), respectively.

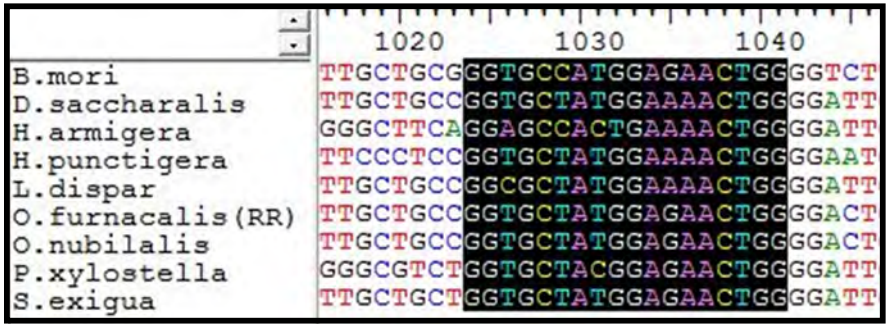
(a)


200
B.mori
D.saccharalis
H.armigera
H.punctigera
L.dispar
O.furnacalis(RR)
O.nubilalis
P.xylostella
S.exigua

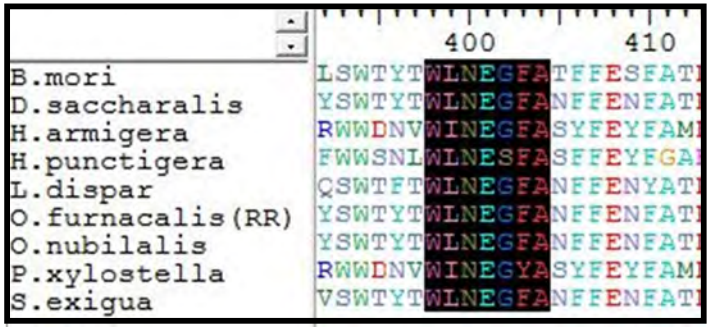

600
610
620
B.mori
D.saccharalis
H.armigera
H.punctigera
L.dispar
O.furnacalis(RR)
O.nubilalis
P.xylostella
S.exigua

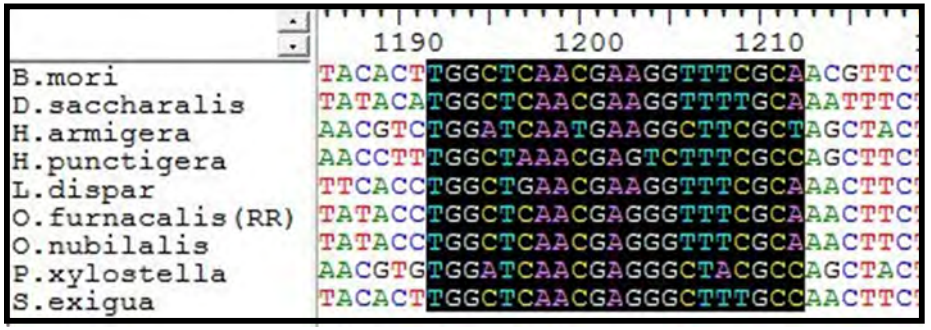
(b)


340
350
B.mori
D.saccharalis
H.armigera
H.punctigera
L.dispar
O.furnacalis(RR)
O.nubilalis
P.xylostella
S.exigua


1020
1030
1040
B.mori
D.saccharalis
H.armigera
H.punctigera
L.dispar
O.furnacalis(RR)
O.nubilalis
P.xylostella
S.exigua

(c)


400
410
B.mori
D.saccharalis
H.armigera
H.punctigera
L.dispar
O.furnacalis(RR)
O.nubilalis
P.xylostella
S.exigua


1190
1200
1210
B.mori
D.saccharalis
H.armigera
H.punctigera
L.dispar
O.furnacalis(RR)
O.nubilalis
P.xylostella
S.exigua

(d)

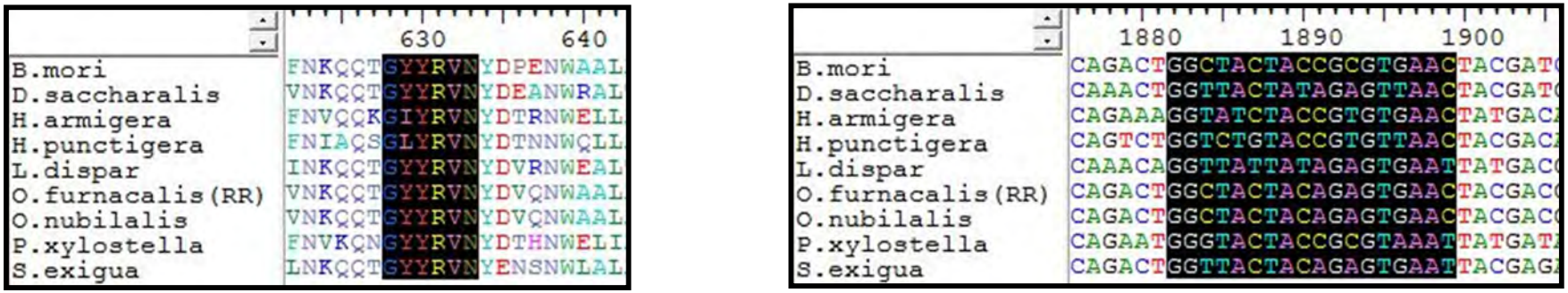
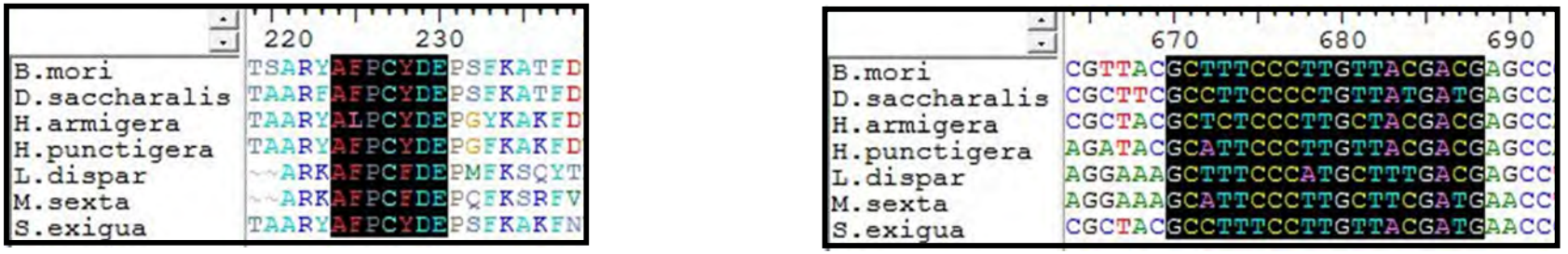
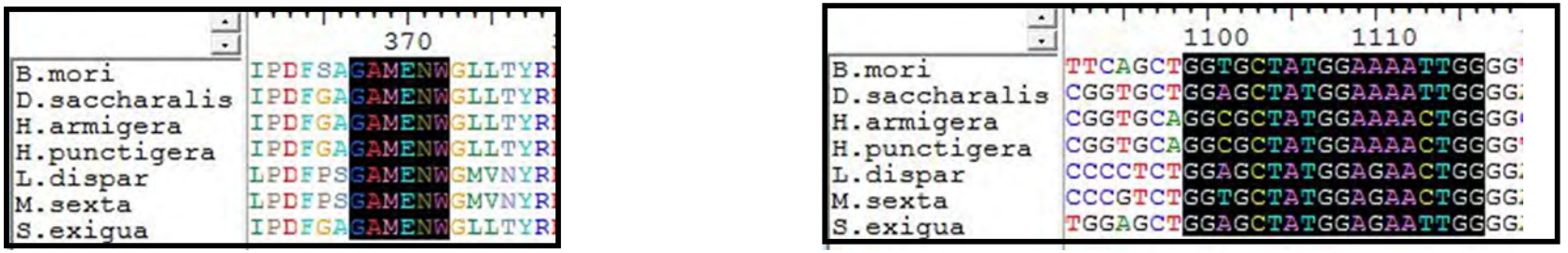


Figure 3.2: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *APN2*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) APN2AF, (b) APN2BF, (c) APN2CR and (d) APN2DR. The complete protein and DNA sequence alignment of APN2 is shown in Figure A.2 (Appendix A) and B.2 (Appendix B), respectively.

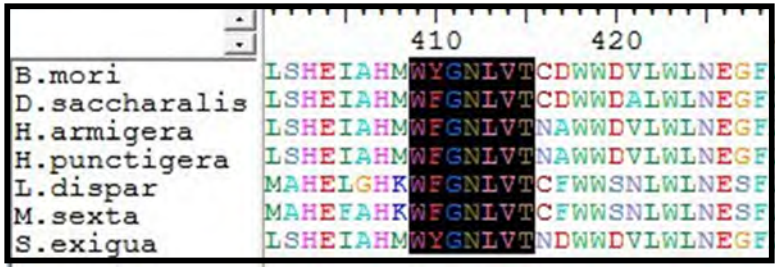
(a)

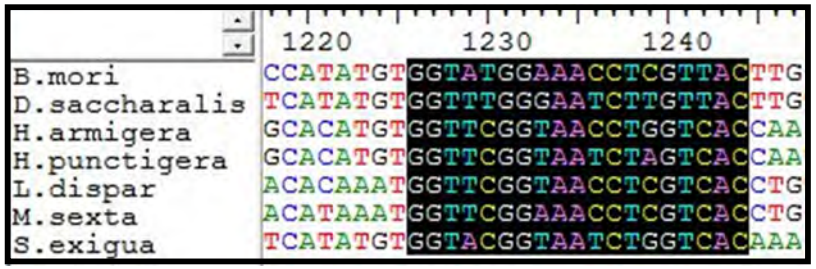


(b)



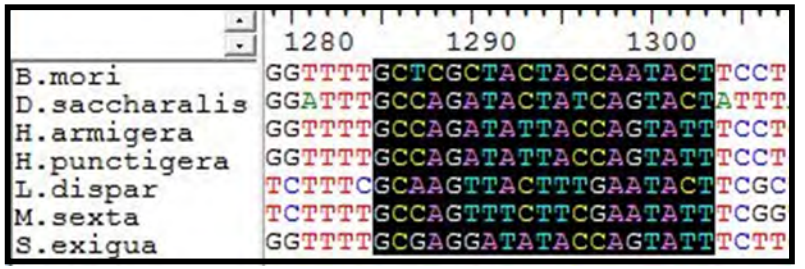
(c)


410
420
B.mori LSHEIAHMWYGNLVTCDWWDVLWLNEGF
D.saccharalis LSHEIAHMWFGNLVTCDWWDALWLNEGF
H.armigera LSHEIAHMWFGNLVTNAWWDVLWLNEGF
H.punctigera LSHEIAHMWFGNLVTNAWWDVLWLNEGF
L.dispar MAHELGHKWFGNLVTCFWWSNLWLNESF
M.sexta MAHEFAHKWFGNLVTCFWWSNLWLNESF
S.exigua LSHEIAHMWYGNLVTNDWWDVLWLNEGF

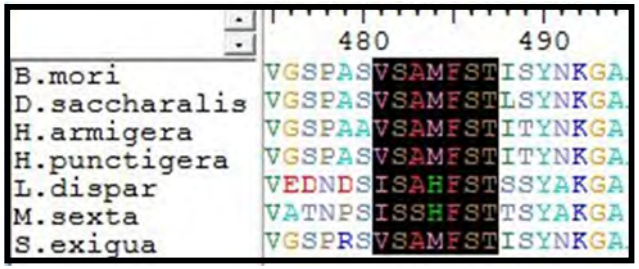

1220
1230
1240
B.mori CCATATGTGGTATGGAAACCTCGTTACTTG
D.saccharalis TCATATGTGGTTTGGGAATCTTGTTACTTG
H.armigera GCACATGTGGTTCGGTAACCTGGTCACCAA
H.punctigera GCACATGTGGTTCGGTAATCTAGTCACCAA
L.dispar ACACAAATGGTTCGGTAACCTCGTCACCTG
M.sexta ACATAAATGGTTCGGAAACCTCGTCACCTG
S.exigua TCATATGTGGTACGGTAATCTGGTCACAAA

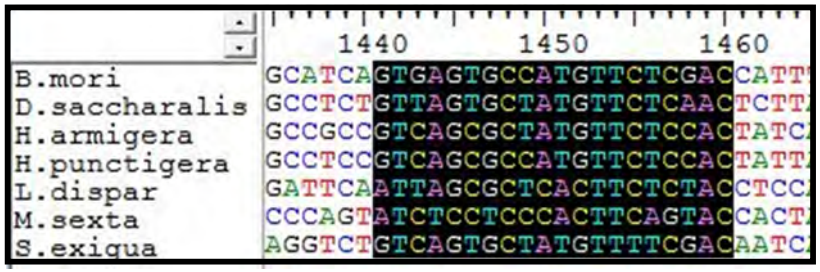
(d)


430
440
B.mori WLNEGFARYYQYFLTDWVE
D.saccharalis WLNEGFARYYQYYLTHWVA
H.armigera WLNEGFARYYQYFLTAWVE
H.punctigera WLNEGFARYYQYFLTAWVE
L.dispar WLNESFASYFEYFAAHWAD
M.sexta WLNESFASFFEYFGAHYAD
S.exigua WLNEGFARIYQYFLTYEVE

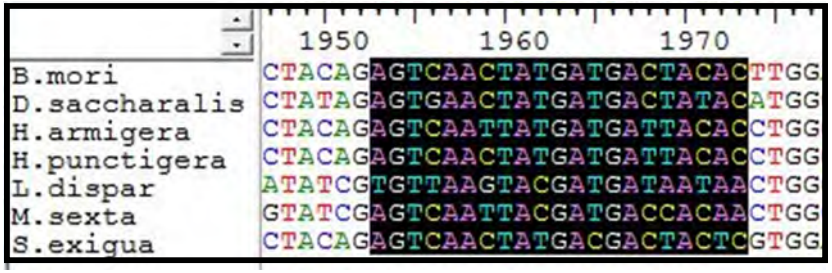

1280
1290
1300
B.mori GGTTTTGCTCGCTACTACCAATACTTCCT
D.saccharalis GGATTTGCCAGATACTATCAGTACTATTT
H.armigera GGTTTTGCCAGATATTACCAGTATTTCCT
H.punctigera GGTTTTGCCAGATATTACCAGTATTTCCT
L.dispar TCTTTCGCAAGTTACTTTGAATACTTCGC
M.sexta TCTTTTGCCAGTTTCTTCGAATATTTCGG
S.exigua GGTTTTGCGAGGATATACCAGTATTTCTT

(e)

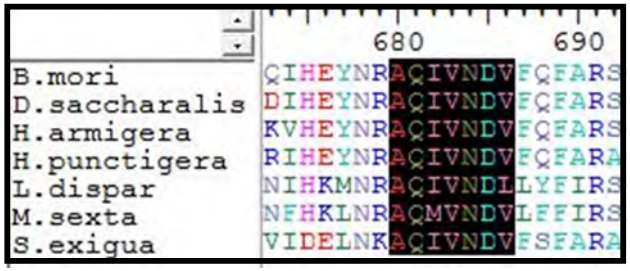

480
490
B.mori VGSPASVSAMFSTISYNKGA
D.saccharalis VGSPASVSAMFSTLSYNKGA
H.armigera VGSPAAVSAMFSTITYNKGA
H.punctigera VGSPASVSAMFSTITYNKGA
L.dispar VEDNDSISAHFSTSSYAKGA
M.sexta VATNPSISSHFSTTSYAKGA
S.exigua VGSPRSVSAMFSTISYNKGA

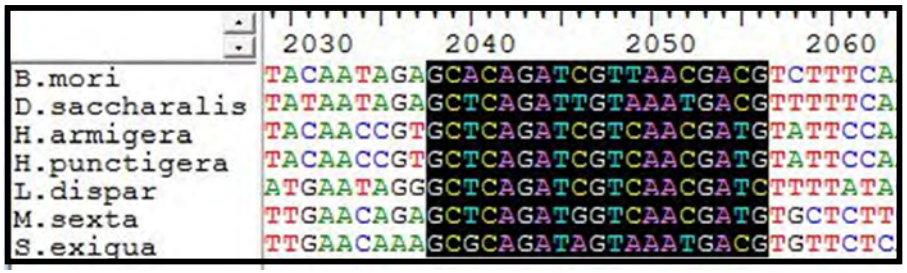

1440
1450
1460
B.mori GCATCAGTGAGTGCCATGTTCTCGACCATT
D.saccharalis GCCTCTGTTAGTGCTATGTTCTCAACTCTT
H.armigera GCCGCCGTCAGCGCTATGTTCTCCACTATC
H.punctigera GCCTCCGTCAGCGCCATGTTCTCCACTATT
L.dispar GATTCAATTAGCGCTCACTTCTCTACCTCC
M.sexta CCCAGTATCTCCTCCCACTTCAGTACCACT
S.exigua AGGTCTGTCAGTGCTATGTTTTCGACAATC

(f)



(g)





(h)

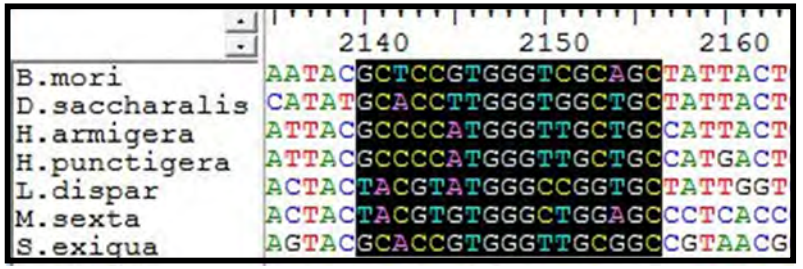
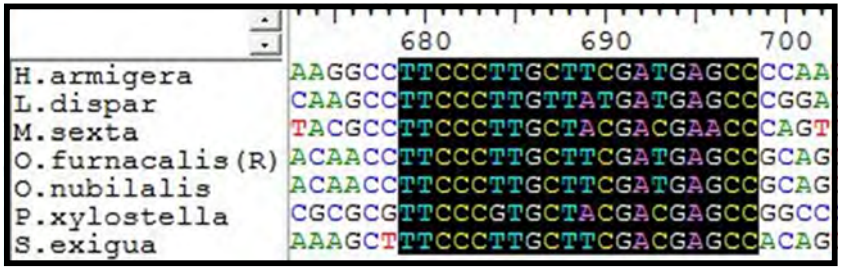


Figure 3.3: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *APN3*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) APN3AF, (b) APN3BF, (c) APN3CR, (d) APN3DR, (e) APN3EF, (f) APN3FF and (g) APN3GR. The complete protein and DNA sequence alignment of APN3 is shown in Figure A.3 (Appendix A) and B.3 (Appendix B), respectively.

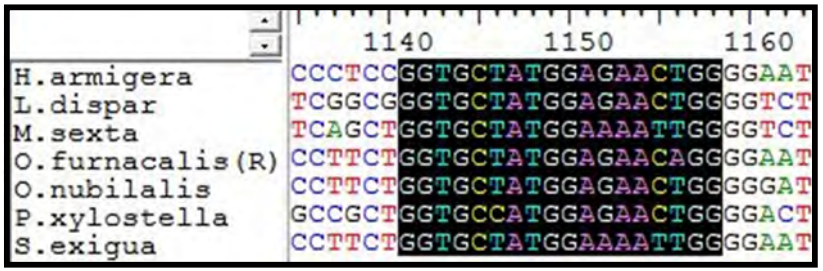
(a)


230
240
H.armigera HVRKAFPCFDEPQFKSRYTI
L.dispar HARQAFPCYDEPGFKAEFDI
M.sexta SARYAFPCYDEPSFKANFDI
O.furnacalis(R) YARTTFPCFDEPQFKSRFVI
O.nubilalis YARTTFPCFDEPQFKSRFVI
P.xylostella FARRAFPCYDEPALKAVFRT
S.exigua HARKAFPCFDEPQFKSRYTI

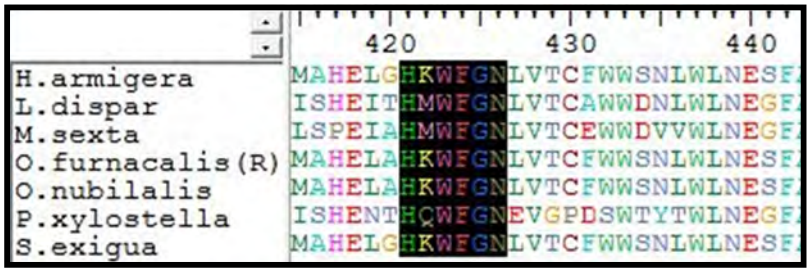

680
690
700
H.armigera AAGGCCTTCCCTTGCTTCGATGAGCCCCAA
L.dispar CAAGCCTTCCCTTGTTATGATGAGCCCGGA
M.sexta TACGCCTTCCCTTGCTACGACGAACCCAGT
O.furnacalis(R) ACAACCTTCCCTTGCTTCGATGAGCCGCAG
O.nubilalis ACAACCTTCCCTTGCTTCGATGAGCCGCAG
P.xylostella CGCGCGTTCCCGTGCTACGACGAGCCGGCC
S.exigua AAAGCTTTCCCTTGCTTCGACGAGCCACAG

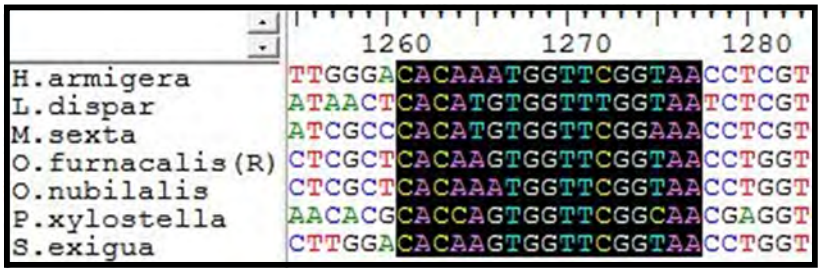
(b)


380
390
H.armigera DFPSGAMENWGMVNYR
L.dispar DFSAGAMENWGLLTYR
M.sexta DFSAGAMENWGLLTYR
O.furnacalis(R) DFPSGAMENRGMVNYR
O.nubilalis DFPSGAMENWGMVNYR
P.xylostella DFAAGAMENWGLVIYR
S.exigua DFPSGAMENWGMVNYR


1140
1150
1160
H.armigera CCCTCCGGTGCTATGGAGAACTGGGGAAT
L.dispar TCGGCGGGTGCTATGGAGAACTGGGGTCT
M.sexta TCAGCTGGTGCTATGGAAAATTGGGGTCT
O.furnacalis(R) CCTTCTGGTGCTATGGAGAACAGGGGAAT
O.nubilalis CCTTCTGGTGCTATGGAGAACTGGGGGAT
P.xylostella GCCGCTGGTGCCATGGAGAACTGGGGACT
S.exigua CCTTCTGGTGCTATGGAAAATTGGGGAAT

(c)


420
430
440
H.armigera MAHELGHKWFGNLVTCFWWSNLWLNESF
L.dispar ISHEITHMWFGNLVTCAWWDNLWLNEGF
M.sexta LSPEIAHMWFGNLVTCEWWDVVWLNEGF
O.furnacalis(R) MAHELAHKWFGNLVTCFWWSNLWLNESF
O.nubilalis MAHELAHKWFGNLVTCFWWSNLWLNESF
P.xylostella ISHENTHQWFGNEVGPDSWTYTWLNEGF
S.exigua MAHELGHKWFGNLVTCFWWSNLWLNESF


1260
1270
1280
H.armigera TTGGGACACAAATGGTTCGGTAACCTCGT
L.dispar ATAACTCACATGTGGTTTGGTAATCTCGT
M.sexta ATCGCCCACATGTGGTTCGGAAACCTCGT
O.furnacalis(R) CTCGCTCACAAGTGGTTCGGTAACCTGGT
O.nubilalis CTCGCTCACAAATGGTTCGGTAACCTGGT
P.xylostella AACACGCACCAGTGGTTCGGCAACGAGGT
S.exigua CTTGGACACAAGTGGTTCGGTAACCTGGT

(d)

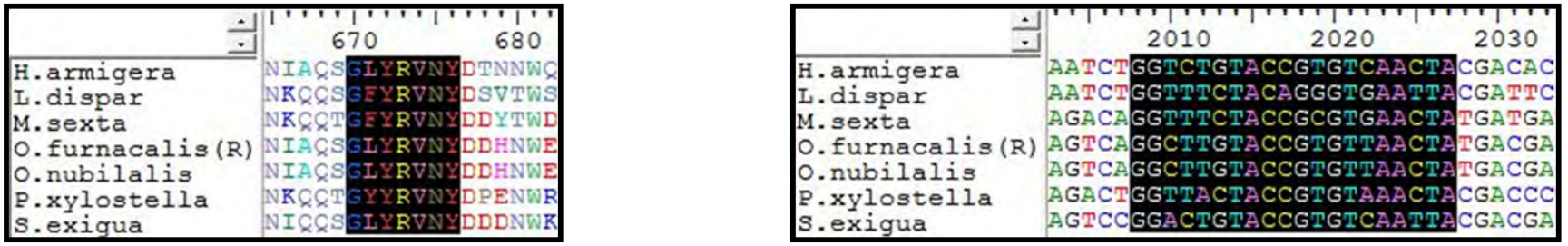
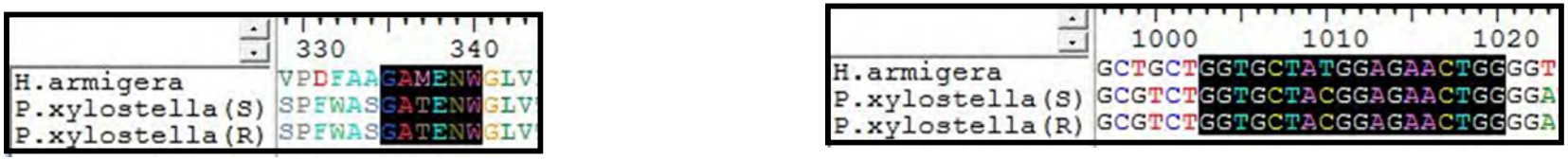


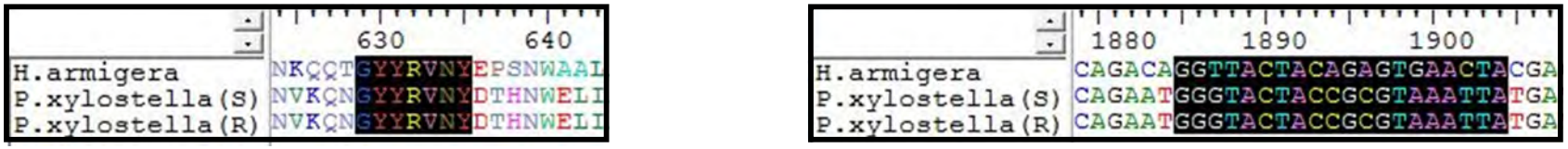
Figure 3.4: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *APN4*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) APN4AF, (b) APN4BF, (c) APN4CR and (d) APN4DR. The complete protein and DNA sequence alignment of APN4 is shown in Figure A.4 (Appendix A) and B.4 (Appendix B), respectively.

(a)



(b)

(c)



(d)

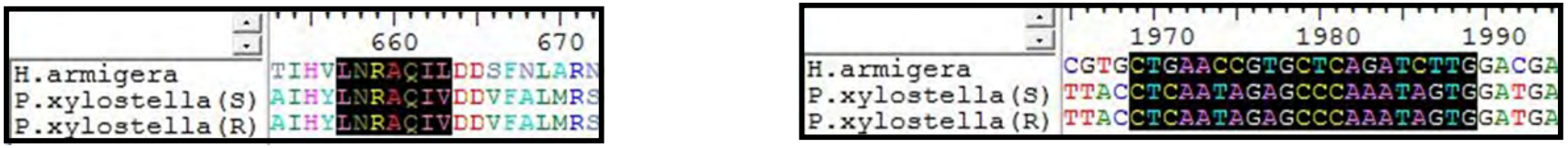


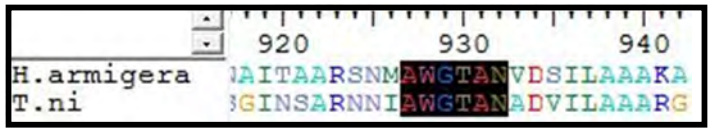
Figure 3.5: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *APN5*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) APN5AF, (b) APN5BF, (c) APN5CR and (d) APN5DR. The complete protein and DNA sequence alignment of APN5 is shown in Figure A.5 (Appendix A) and B.5 (Appendix B), respectively.

(a)

(b)

(c)

(d)



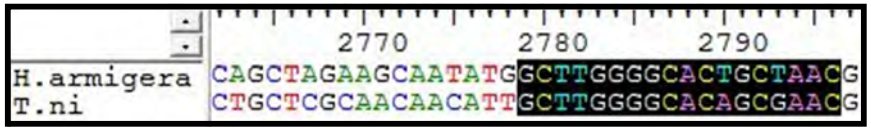
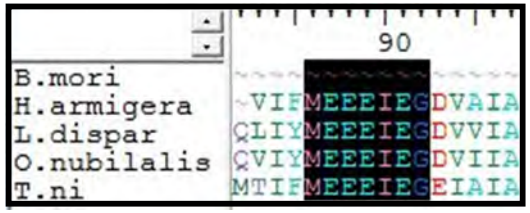
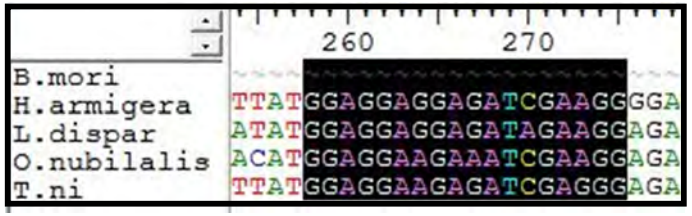


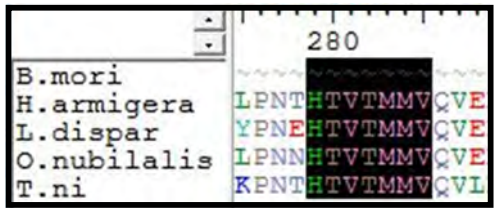
Figure 3.6: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *APN6*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) APN6AF, (b) APN6BF, (c) APN6CR and (d) APN6DR. The complete protein and DNA sequence alignment of APN6 is shown in Figure A.6 (Appendix A) and B.6 (Appendix B), respectively.

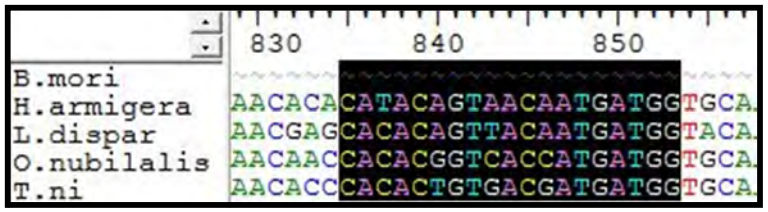
(a)



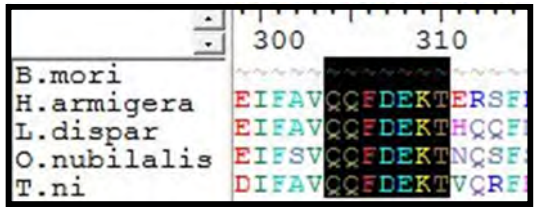


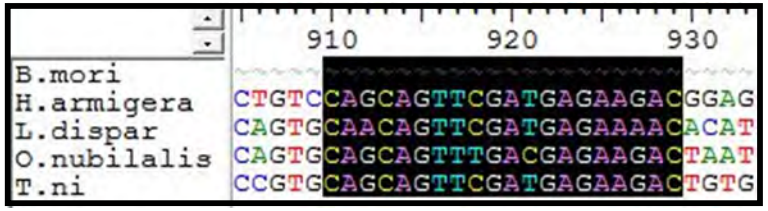
(b)



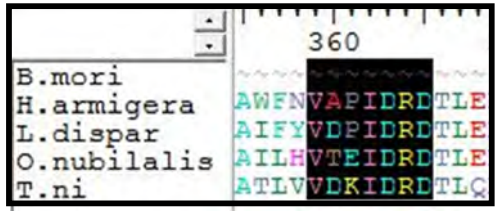


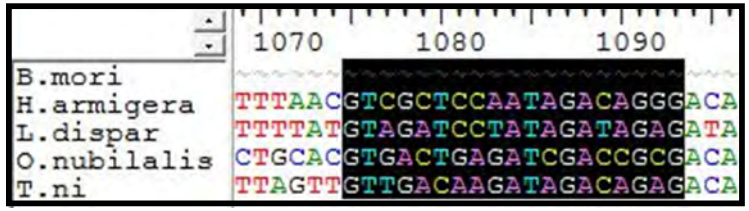
(c)



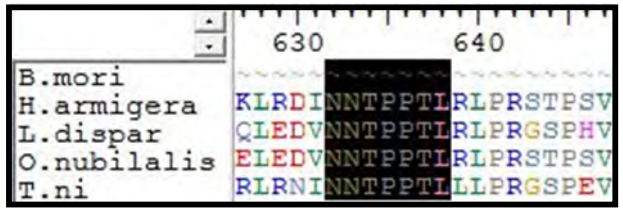


(d)


360
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
AWFNVAPIDRDTLE
AIFYVDPIDRDTLE
AILHVTEIDRDTLE
ATLVVDKIDRDTLQ


1070
1080
1090
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
TTTAACGTCGCTCCAATAGACAGGGACA
TTTTATGTAGATCCTATAGATAGAGATA
CTGCACGTGACTGAGATCGACCGCGACA
TTAGTTGTTGACAAGATAGACAGAGACA

(e)


630
640
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
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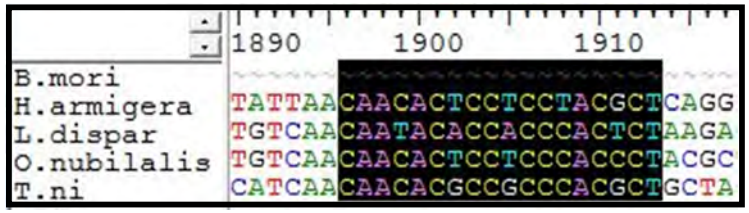
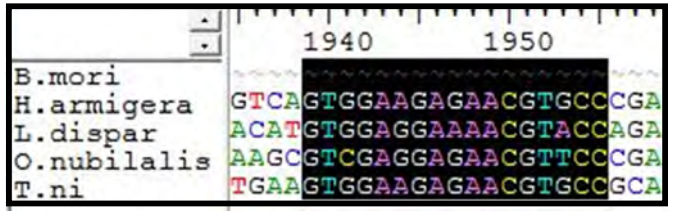
QLEDVNNTPPTLRLPRGSPHV
ELEDVNNTPPTLRLPRSTPSV
RLRNINNTPPTLLLPRGSPEV

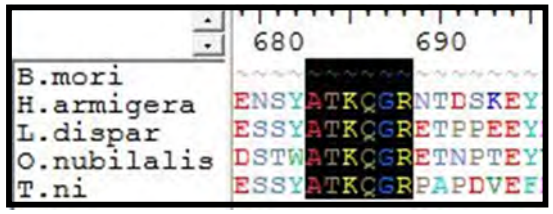

1890
1900
1910
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
TATTAACAACACTCCTCCTACGCTCAGG
TGTCAACAATACACCACCCACTCTAAGA
TGTCAACAACACTCCTCCCACCCTACGC
CATCAACAACACGCCGCCCACGCTGCTA

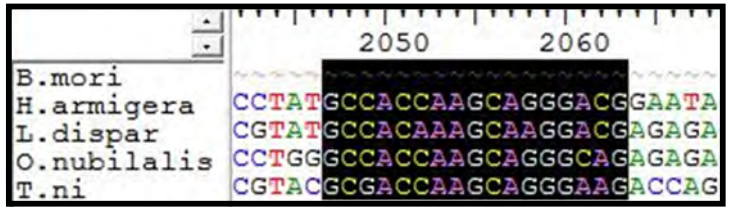
(f)


650
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
STPSVEENVPDGFVIP
GSPHVEENVPEGYLIT
STPSVEENVPEGYEIS
GSPEVEENVPQDFVIP


1940
1950
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
GTCAGTGGAAGAGAACGTGCCCGA
ACATGTGGAGGAAAACGTACCAGA
AAGCGTCGAGGAGAACGTTCCCGA
TGAAGTGGAAGAGAACGTGCCGCA

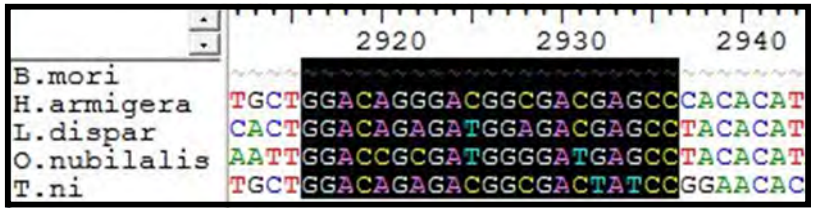
(g)


680
690
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
ENSYATKQGRNTDSKEY
ESSYATKQGRETPPEEY
DSTWATKQGRETNPTEY
ESSYATKQGRPAPDVEF


2050
2060
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
CCTATGCCACCAAGCAGGGACGGAATA
CGTATGCCACAAAGCAAGGACGAGAGA
CCTGGGCCACCAAGCAGGGCAGAGAGA
CGTACGCGACCAAGCAGGGAAGACCAG

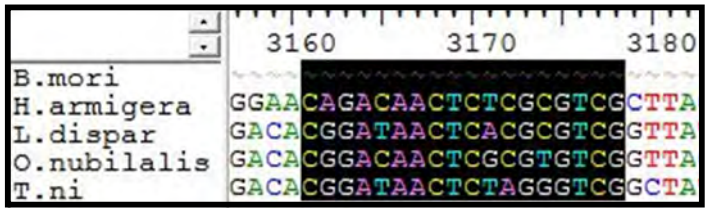
(h)


970
980
B.mori
H.armigera GELLDRDGDEPTHRIF
L.dispar HETLDRDGDEPTHRIF
O.nubilalis DEKLDRDGDEPTHTIF
T.ni DTVLDRDGDYPEHTIF


2920
2930
2940
B.mori
H.armigera TGCTGGACAGGGACGGCGACGAGCCCACACAT
L.dispar CACTGGACAGAGATGGAGACGAGCCTACACAT
O.nubilalis AATTGGACCGCGATGGGGATGAGCCTACACAT
T.ni TGCTGGACAGAGACGGCGACTATCCGGAACAC

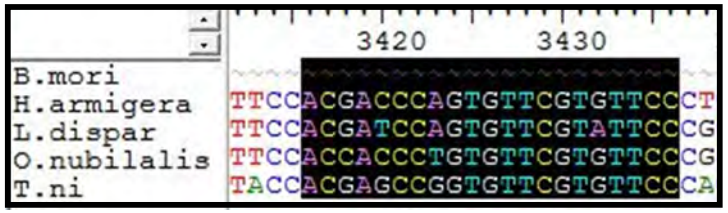
(i)


1050
1060
B.mori
H.armigera DEPGTDNSRVAYAITGL
L.dispar DEPDTDNSRVGYAILGL
O.nubilalis DEPDTDNSRVGYGILDL
T.ni DEPDTDNSRVGYAILGL

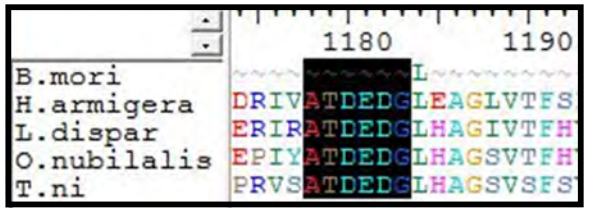

3160
3170
3180
B.mori
H.armigera GGAACAGACAACTCTCGCGTCGCTTA
L.dispar GACACGGATAACTCACGCGTCGGTTA
O.nubilalis GACACGGACAACTCGCGTGTCGGTTA
T.ni GACACGGATAACTCTAGGGTCGGCTA

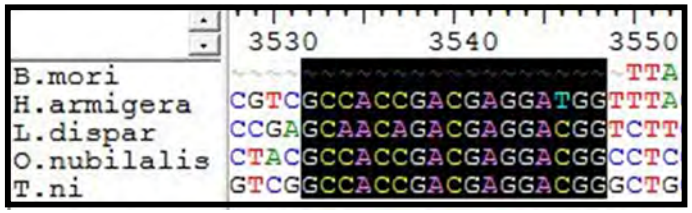
(j)


1140
1150
B.mori
H.armigera YNFHDPVFVFPQPGSTI
L.dispar YNFHDPVFVFPLPGSTI
O.nubilalis YNFHHPVFVFPQPDSVI
T.ni YNYHEPVFVFPQAGNTF

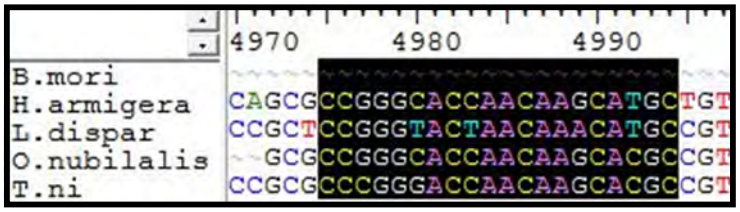

3420
3430
B.mori
H.armigera TTCCACGACCCAGTGTTCGTGTTCCCT
L.dispar TTCCACGATCCAGTGTTCGTATTCCCG
O.nubilalis TTCCACCACCCTGTGTTCGTGTTCCCG
T.ni TACCACGAGCCGGTGTTCGTGTTCCCA

(k)

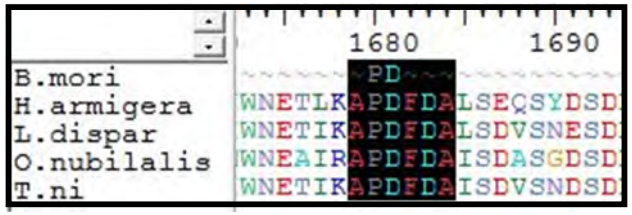

1180
1190
B.mori L
H.armigera DRIVATDEDGLEAGLVTFS
L.dispar ERIRATDEDGLHAGIVTFH
O.nubilalis EPIYATDEDGLHAGSVTFH
T.ni PRVSATDEDGLHAGSVSFS

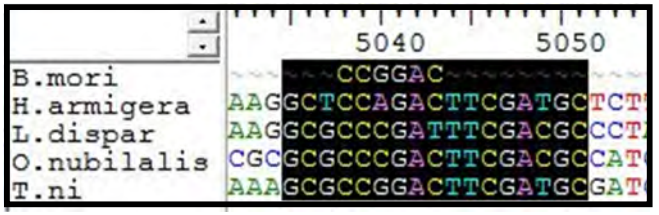

3530
3540
3550
B.mori TTA
H.armigera CGTCGCCACCGACGAGGATGGTTTA
L.dispar CCGAGCAACAGACGAGGACGGTCTT
O.nubilalis CTACGCCACCGACGAGGACGGCCTC
T.ni GTCGGCCACCGACGAGGACGGGCTG

(l)

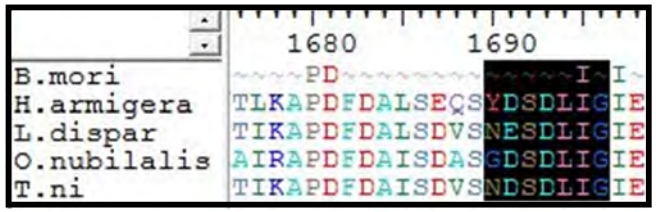


(m)





(n)



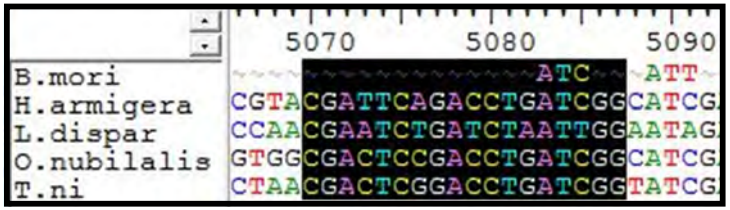
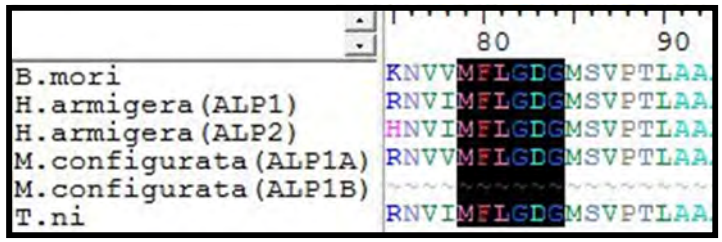
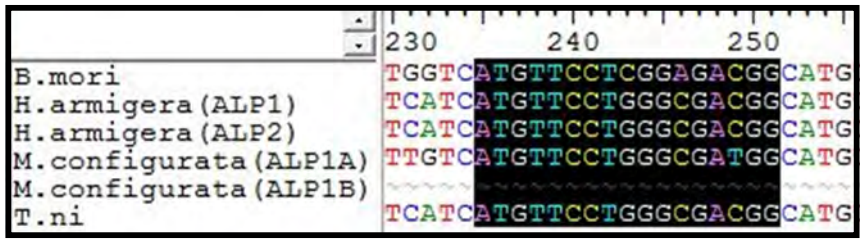


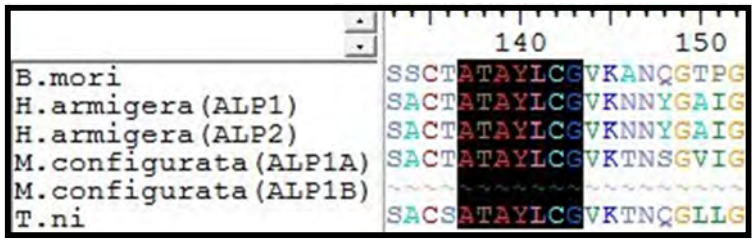
Figure 3.7: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *cadherin*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) CADAF, (b) CADBF, (c) CADCR, (d) CADDF, (e) CADER, (f) CADFF, (g) CADGR, (h) CADHF, (i) CADIR, (j) CADJF, (k) CADKR, (l) CADLF, (m) CADMR, and (n) CADNR. The complete protein and DNA sequence alignment of cadherin is shown in Figure A.7 (Appendix A) and B.7 (Appendix B), respectively.

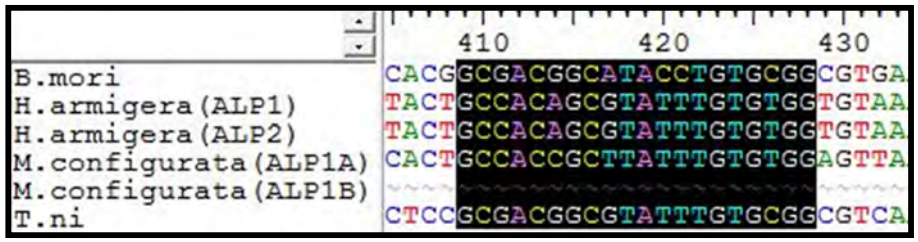
(a)


80
90
B.mori KNVVMFLGDGMSVPTLAA
H.armigera(ALP1) RNVIMFLGDGMSVPTLAA
H.armigera(ALP2) HNVIMFLGDGMSVPTLAA
M.configurata(ALP1A) RNVVMFLGDGMSVPTLAA
M.configurata(ALP1B)
T.ni RNVIMFLGDGMSVPTLAA

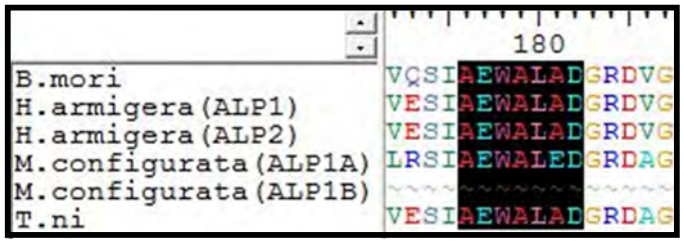

230
240
250
B.mori TGGTCATGTTCCTCGGAGACGGCATG
H.armigera(ALP1) TCATCATGTTCCTGGGCGACGGCATG
H.armigera(ALP2) TCATCATGTTCCTGGGCGACGGCATG
M.configurata(ALP1A) TTGTCATGTTCCTGGGCGATGGCATG
M.configurata(ALP1B)
T.ni TCATCATGTTCCTGGGCGACGGCATG

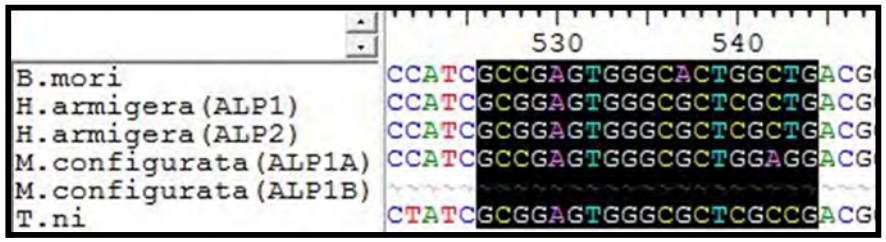
(b)


140
150
B.mori SSCTATAYLCGVKANQGTPG
H.armigera(ALP1) SACTATAYLCGVKNNYGAIG
H.armigera(ALP2) SACTATAYLCGVKNNYGAIG
M.configurata(ALP1A) SACTATAYLCGVKTNSGVIG
M.configurata(ALP1B)
T.ni SACSATAYLCGVKTNQGLLG


410
420
430
B.mori CACGGCGACGGCATACCTGTGCGGCGTGA
H.armigera(ALP1) TACTGCCACAGCGTATTTGTGTGGTGTAA
H.armigera(ALP2) TACTGCCACAGCGTATTTGTGTGGTGTAA
M.configurata(ALP1A) CACTGCCACCGCTTATTTGTGTGGAGTTA
M.configurata(ALP1B)
T.ni CTCCGCGACGGCGTATTTGTGCGGCGTCA

(c)


180
B.mori VQSIAEWALADGRDVG
H.armigera(ALP1) VESIAEWALADGRDVG
H.armigera(ALP2) VESIAEWALADGRDVG
M.configurata(ALP1A) LRSIAEWALEDGRDAG
M.configurata(ALP1B)
T.ni VESIAEWALADGRDAG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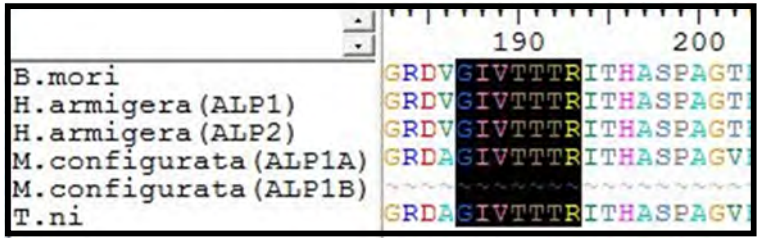
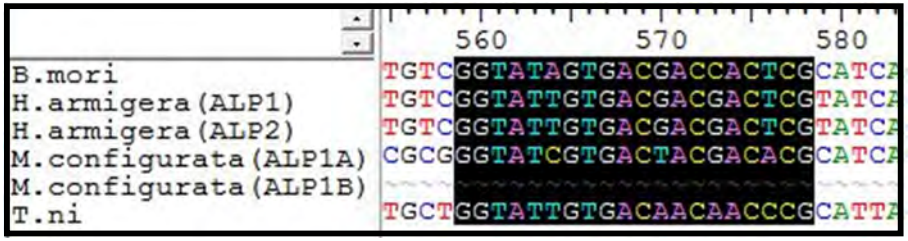
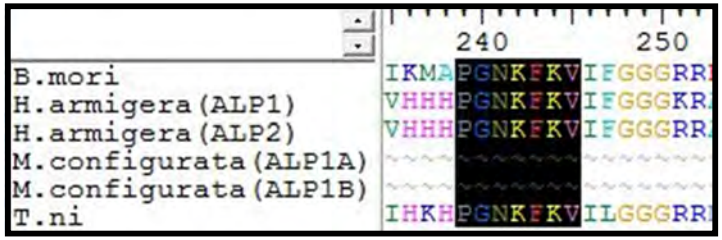
540
B.mori CCATCGCCGAGTGGGCACTGGCTGACG
H.armigera(ALP1) CCATCGCGGAGTGGGCGCTCGCTGACG
H.armigera(ALP2) CCATCGCGGAGTGGGCGCTCGCTGACG
M.configurata(ALP1A) CCATCGCCGAGTGGGCGCTGGAGGACG
M.configurata(ALP1B)
T.ni CTATCGCGGAGTGGGCGCTCGCCGACG

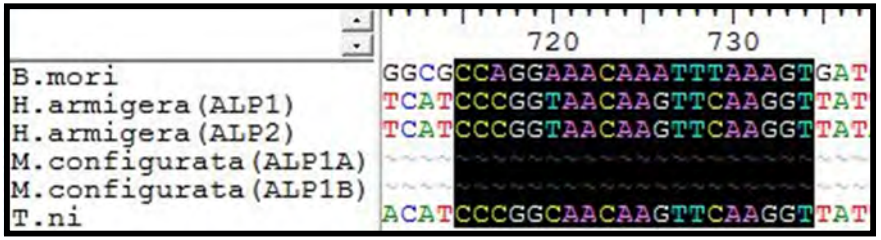
(d)


190
200
B.mori
H.armigera(ALP1)
H.armigera(ALP2)
M.configurata(ALP1A)
M.configurata(ALP1B)
T.ni

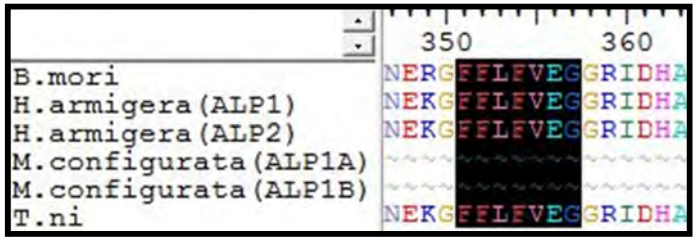

560
570
580
B.mori
H.armigera(ALP1)
H.armigera(ALP2)
M.configurata(ALP1A)
M.configurata(ALP1B)
T.ni

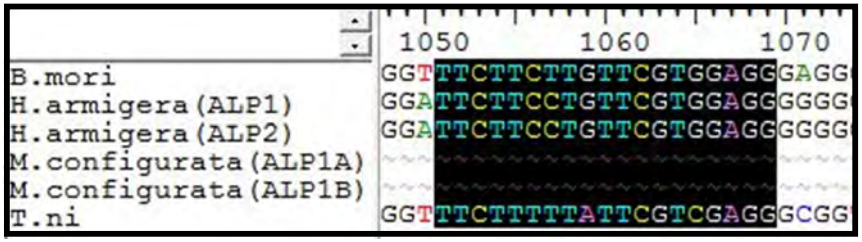
(e)


240
250
B.mori
H.armigera(ALP1)
H.armigera(ALP2)
M.configurata(ALP1A)
M.configurata(ALP1B)
T.ni

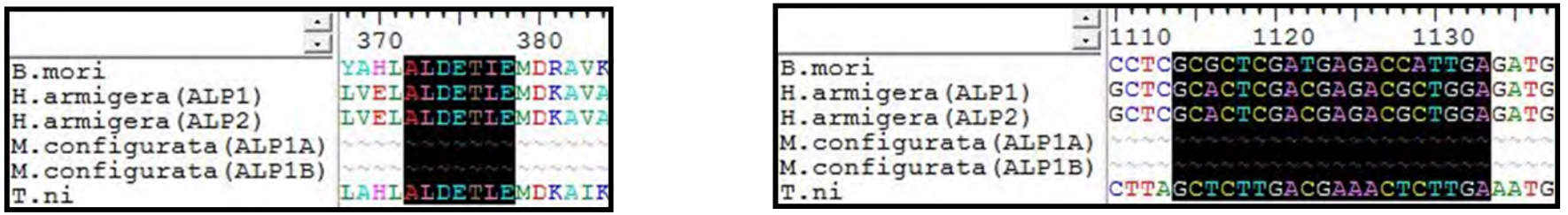

720
730
B.mori
H.armigera(ALP1)
H.armigera(ALP2)
M.configurata(ALP1A)
M.configurata(ALP1B)
T.ni

(f)

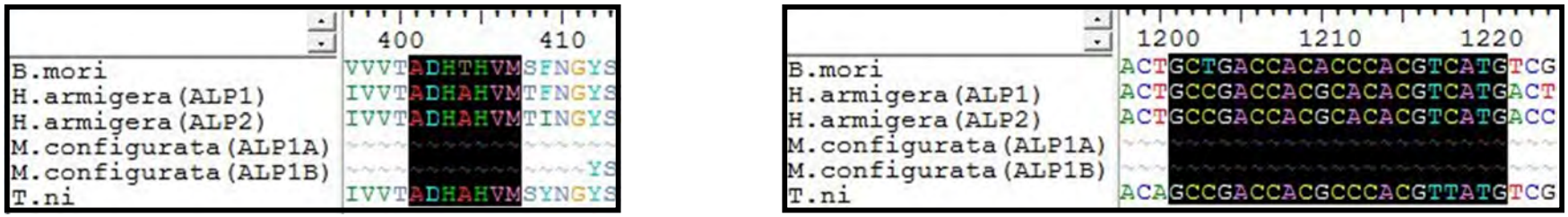

350
360
B.mori
H.armigera(ALP1)
H.armigera(ALP2)
M.configurata(ALP1A)
M.configurata(ALP1B)
T.ni


1050
1060
1070
B.mori
H.armigera(ALP1)
H.armigera(ALP2)
M.configurata(ALP1A)
M.configurata(ALP1B)
T.ni

(g)



(h)



(i)

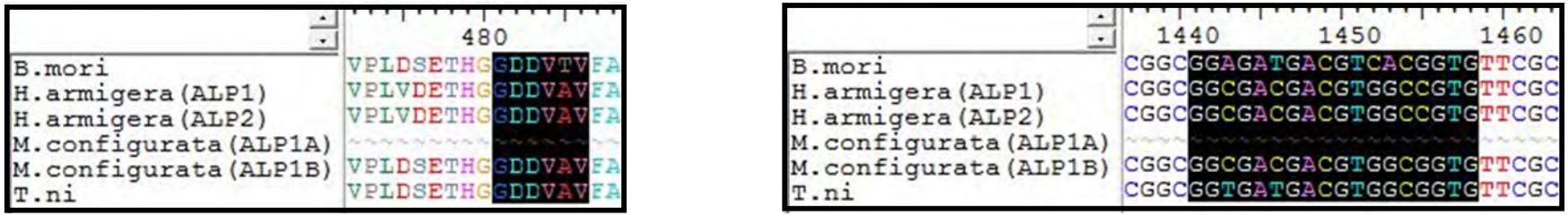


Figure 3.8: Illustrations of conserved regions observed in the protein sequence alignment and the corresponding DNA sequence alignment of *ALP*. Protein and DNA sequence alignments indicate the positions of the following primers: (a) ALPAF, (b) ALPBF, (c) ALPCR, (d) ALPDF, (e) ALPER, (f) ALPFF, (g) ALPGR, (h) ALPHR, and (i) ALPIR. The complete protein and DNA sequence alignment of ALP is shown in Figure A.8 (Appendix A) and B.8 (Appendix B), respectively.

Table 3.1: Degenerate primers designed for several Cry receptor genes of Lepidoptera.

Nr.	Primer	5'→3' Sequence	Region (Protein)	Region (DNA)	Length	G+C%	Tm
1	APN1AF	GGB TTY TAY MGR AGY TGG	183-188	547-564	18	33.33	50.79
2	APN1BF	ACN ACH CAR TTC CAR CCW GG	201-207	601-620	20	45	58.35
3	APN1CR	TAR CAR GGG AAN GCY TGV CG	210-216	628-647	20	45	58.35
4	APN1DR	CCA RTT YTC CAT RGC WCC HGC	346-352	1036-1056	21	47.62	60.61
5	APN1EF	CTB TTR ACH TAY AGR GAR GC	354-360	1060-1079	20	30	52.20
6	APN1FF	CAY GAR RTH GCH CAY ATG TGG	383-389	1147-1167	21	38.10	56.71
7	APN1GR	GCR AAD CCT TCR TTB AGC	403-409	1209-1226	18	38.89	53.07
8	APN1HR	RTC RTG MGC HAR YCT YC	709-714	2126-2142	17	35.29	49.96
9	APN2AF	TTY CCN WGY TWY GRY GAR CC	198-204	592-611	20	35	54.25
10	APN2BF	GGH GCY AYK GAR AAC TGG	337-342	1009-1026	18	44.44	55.34
11	APN2CR	GCR WAR SMY TCR TTK AKC C	393-399	1178-1196	19	31.58	51.53
12	APN2DR	TTH ACD CKR TAV WDV CC	623-628	1867-1883	17	23.53	45.14
13	APN3AF	GCH YTY CCH TGY TWY GAY G	224-230	670-688	19	36.84	53.69
14	APN3BF	GGH GCT ATG GAR AAY TGG	367-372	1099-1116	18	44.44	55.34
15	APN3CR	GTR ACN AGR TTH CCR WAC C	409-415	1226-1244	19	36.84	53.69
16	APN3DR	ART AYA SRW ADW WNG KNG C	429-435	1285-1303	19	21.05	47.21
17	APN3EF	GTB AGY GCY ATG TTY TCV AC	481-487	1441-1460	20	35.00	54.25
18	APN3FF	AGT SAA YTA TGA YGA YTA YWC	651-658	1953-1973	21	23.81	50.85

19	APN3GR	CRT CRT TDA CDA TCT GHG C	680-686	2038-2056	19	36.84	36.84
20	APN3HR	GCH GCV ACC CAH GGD GC	714-719	2140-2156	17	64.71	62.02
21	APN4AF	TTC CCK TGY TWY GAY GAR CC	227-233	679-698	20	40	56.30
22	APN4BF	GGT GCT ATG GAR AAY WGG	381-386	1141-1158	18	44.44	55.34
23	APN4CR	TTR CCR AAC CAY WKG TG	421-426	1261-1277	17	35.29	49.96
24	APN4DR	TAR TTN ACV CKG TAS WRD CC	670-676	2008-2027	20	30	52.20
25	APN5AF	GGT GCT AYG GAG AAC TGG	335-340	1003-1020	18	55.56	59.90
26	APN5BF	GAG MWC GKW CAY MAG TGG	372-377	1114-1131	18	44.44	55.34
27	APN5CR	TAR TTY ACK CKG TAG TAM CC	629-635	1885-1904	20	30	52.20
28	APN5DR	CAM KAT YTG RGC WCK RTT SAG	657-663	1969-1989	21	33.33	54.76
29	APN6AF	TTY CCW TGC TGG GAT GAG C	200-206	598-616	19	52.63	60.16
30	APN6BF	GCT GGW ACT GAA AAC TGG	332-337	994-1011	18	50	57.62
31	APN6CR	TGA TCC AGG TGT TAC TCC	384-390	1151-1168	18	50	57.62
32	APN6DR	GTT MGC WGT GCC CCA AGC	927-932	2779-2796	18	61.11	62.18
33	CADAF	GGA GGA RGA RAT MGA RGG	86-92	258-275	18	44.44	55.34
34	CADBF	CAY ACD GTN ACV ATG ATG G	279-285	835-853	19	36.84	53.69
35	CADCR	GTY TTC TCR TCR AAC TGY TG	304-310	910-929	20	35	54.25
36	CADDF	GTN RMY VMD ATM GAY MGV G	359-365	1075-1093	19	21.05	47.21
37	CADER	AGV GTR GGH GGH GTR TTG	632-638	1896-1913	18	44.44	55.34
38	CADFF	GTS GAR GAR AAC GTD CC	647-652	1939-1955	17	47.06	54.78
39	CADGR	CKK CCY TGC TTK GTS GC	683-688	2047-2063	17	52.94	57.19

40	CADHF	GGA CMG VGA YGG VGA YKA KCC	972-979	2916-2936	21	47.62	60.61
41	CADIR	CGA CVC KHG AGT TRT CYG	1054-1060	3161-3178	18	44.44	55.34
42	CADJF	ACS ABC CDG TGT TCG TRT TCC	1139-1146	3416-3436	21	47.62	47.62
43	CADKR	CCR TCC TCG TCK GTK GC	1178-1183	3532-3548	17	58.82	59.60
44	CADLF	CCS GGB ACY AAC AAR CAY GC	1659-1665	4975-4994	20	50	60.40
45	CADMR	GCR TCG AAR TCB GGM GC	1679-1684	5035-5051	17	52.94	57.19
46	CADNR	CCR ATY AGR TCN GAD TCG	1690-1696	5070-5087	18	38.89	53.07
47	ALPAF	ATG TTC CTS GGM GAY GG	79-84	235-251	17	52.94	57.19
48	ALPBF	GCS ACV GCD TAY YTG TGY GG	137-143	409-428	20	50	60.40
49	ALPCR	CVK CSA GYG CCC ACT CSG C	176-182	526-544	19	68.42	66.64
50	ALPDF	GGT ATH GTG ACD ACV ACH CG	187-193	559-578	20	45	58.35
51	ALPER	ACY TTR AAY TTG TTD CCK GG	239-245	715-734	20	30	52.20
52	ALPFF	TTC TTY YTR TTC GTS GAG G	351-357	1051-1069	19	36.84	53.69
53	ALPGR	TCM AKV GTY TCR TCR AGH GC	372-378	1114-1133	20	35	54.25
54	ALPHR	CAT RAC GTG KGY GTG GTC RGC	401-407	1201-1221	21	52.38	62.57
55	ALPIR	CAC SGY SAC GTC RTC DCC	481-486	1441-1458	18	61.11	62.18

***APN = aminopeptidase N, CAD = cadherin, ALP = alkaline phosphatase, N = A/G/C/T, H = A/C/T, W = A/T, R = A/G, Y = C/T**

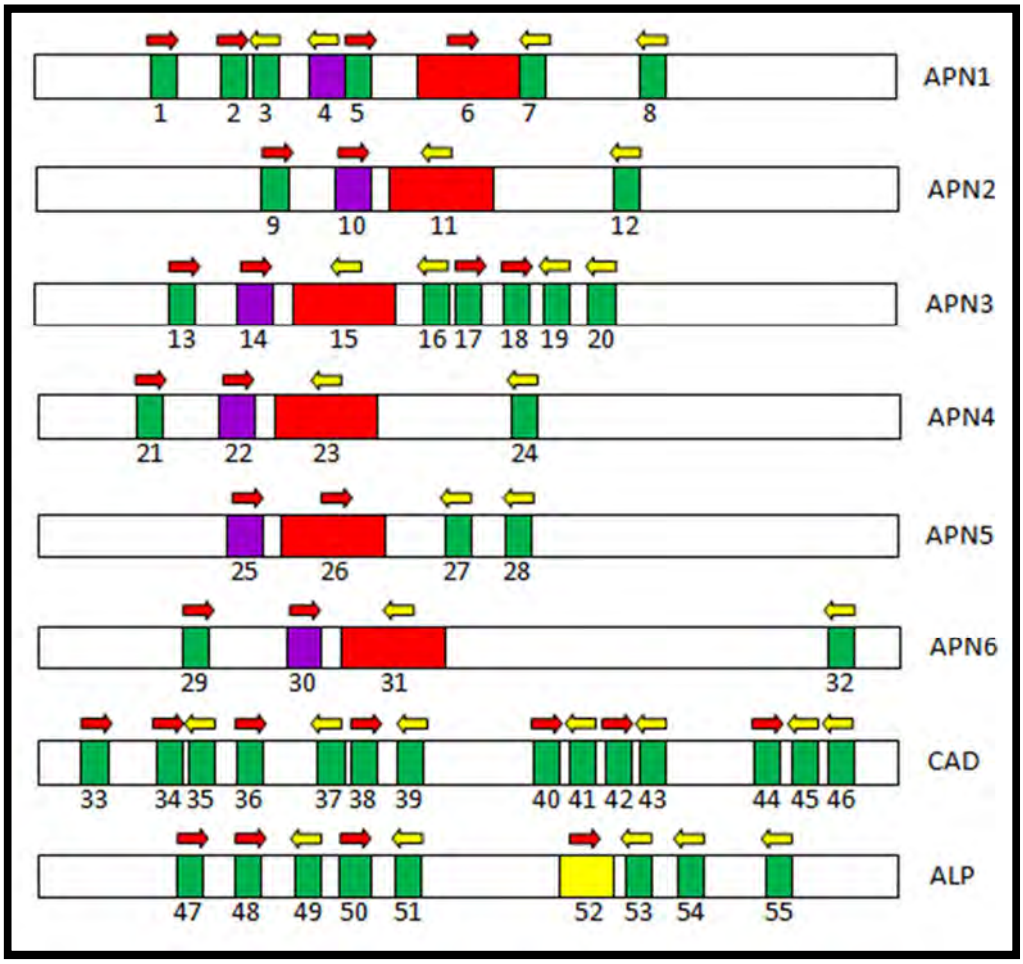


Figure 3.9: Schematic illustration indicating forward (red) and reverse (yellow) primer positions on sequence alignments of Cry receptor genes, *aminopeptidase* (*APN*), *cadherin* (*CAD*) and *alkaline phosphatase* (*ALP*). Purple and red regions indicate conserved motifs, GAMENWG and HEXXH(X)$_{18}$E, respectively, that have been observed in all the APN protein sequences. The yellow region indicates the GFFLFVEGGR conserved motif present in ALP. Green regions indicate other conserved regions that have been observed in the protein sequences of the Cry receptor genes. Numbers indicated beneath the conserved regions correspond to the primer numbers given in Table 3.1. The complete protein and DNA sequence alignments are shown in Appendix A and B, respectively.

3.6. Amplification of Cry receptor genes

DNA isolated from *H. armigera* and *B. fusca* was amplified using custom designed degenerate primers (Table 3.1). The primers were synthesized by Applied Biosystems (UK). Each 25 µl reaction contained 5 µl PCR-grade water, 1X PCR Master Mix (Fermentas Life Science, US), 0.5 µM of each primer (Applied Biosystems, UK) and 50 ng sample DNA. A no-template control (NTC) was included for each primer combination

in the amplification process. A touch-down PCR was performed with an I-Cycler thermal cycler (Bio-Rad, UK). The PCR cycling conditions were an initial denaturation of 5 min at 94 ºC; followed by 20 cycles of 1 min at 94 ºC, 1 min 30 s at 56 ºC (decrease in 0.5 ºC each cycle) and 2 min at 72 ºC. This was followed by another 30 cycles of 1 min at 94 ºC, 1 min 30 s at 46 ºC and 2 min at 72 ºC, and concluded with a final extension step of 10 min at 72 ºC.

DNA amplification was confirmed by performing electrophoresis of the PCR products on a 1.5% (w/v) TAE agarose (WhiteSci, USA) gel containing 0.001 mg/ml ethidium bromide (Bio-Rad, UK). Electrophoresis conditions are described in Section 3.3. A 1 kb (2 µl) and 100 bp (2.5 µl) molecular weight marker (O6GeneRuler, Fermentas Life Science, US) were loaded into the gel to determine the fragment sizes of the Cry receptor gene amplicons.

The amplicons obtained for *H. armigera* and *B. fusca* with the PCR process were excised from the agarose gel and placed in sterile Eppendorf tubes, after which 50 ε l of PCR-grade water was added to each tube. Tubes were centrifuged for 10 min. The PCR products in the eluent were then used as template for re-amplification of the genes with the respective amplification primers under the same cycling conditions. Band excision and re-amplification were repeated until single amplicons were obtained. Re-amplification was confirmed by performing electrophoresis of the PCR products as described in Section 3.3. The approach of re-amplification was decided on when sequencing reactions continuously failed when bands were directly purified and sequenced after excision. Even though the NucleoSpin® Extract II Kit (Macherey-Nagel, Germany) is designed for purification of PCR products from gel fragments, loss of product yield resulted in failed sequencing reactions. The re-amplification approach was consequently pursued.

3.7. Sequence analysis of PCR amplicons

PCR products were purified using a NucleoSpin® Extract II Kit (Macherey-Nagel, Germany) and used as template in DNA sequencing reactions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The DNA sequencing reactions were purified using a ZR (Zymo Research) Sequencing Clean-up KitÊ (The Epigenetics Company, USA) and sequenced in both directions by Karen

Jordaan (North-West University, Potchefstroom Campus, South Africa) using an ABI 3130 Genetic Analyzer (Applied Biosystems, UK). Geospiza Finch TV (version 1.4) software was used to view and edit the DNA sequence chromatograms.

MAFFT online software (http://mafft.cbrc.jp/alignment/server/) was used to align the forward and reverse sequences of each product, after which BioEdit (version 7.1.3.0) software was used to create consensus sequences from the forward and reverse sequences. BLAST (Basic Local Alignment Search Tool) searches were performed to compare the forward, reverse and consensus sequences to the GenBank database of sequences, using the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST) Internet server.

3.8. Phylogenetic analysis

Molecular Evolutionary Genetics Analysis (MEGA) (version 4.0.2) software was used to construct a bootstrap phylogenetic tree using the *cytochrome b* gene sequences of *B. fusca*. Evolutionary relationship was inferred using the neighbor-joining method. Five hundred replicates were used in the bootstrap test. The evolutionary distances were computed using the Jukes-Cantor method. Pairwise deletion option was used to eliminate positions containing alignment gaps and missing data. *Cytochrome b* sequences of several related species in the order Lepidoptera were also included. Outgroups were not included in this analysis.

The same software was used to construct bootstrap neighbor-joining phylogenetic trees using the sequences obtained with *aminopeptidase*, *cadherin* and *alkaline phosphatase* for *B. fusca*. Evolutionary relationships and distances were also computed using the neigbor-joining and Jukes-Cantor method, respectively. The sequences of these respective Cry receptor genes for several related species in the order Lepidoptera were obtained from GenBank and included in the analysis.

CHAPTER 4

RESULTS

4.1. DNA isolation

Genomic DNA was successfully isolated from susceptible *Helicoverpa armigera* larvae and susceptible and resistant *Busseola fusca* larvae by means of a NucleoSpin® Tissue Kit (Section 3.3). DNA was isolated from pooled *H. armigera* larvae. Ten tubes were prepared from the pooled larval material. In contrast, DNA was isolated from individual *B. fusca* larvae. After the initial grinding of the larvae, the material of each larva was divided into four tubes (a-d).

Figure 4.1 shows the electrophoresis results of the DNA isolated from susceptible *H. armigera* larvae. It illustrates the quantity and quality of the isolated DNA. High quantities of DNA can be seen in this figure. Fragmentation is evident in all the samples.

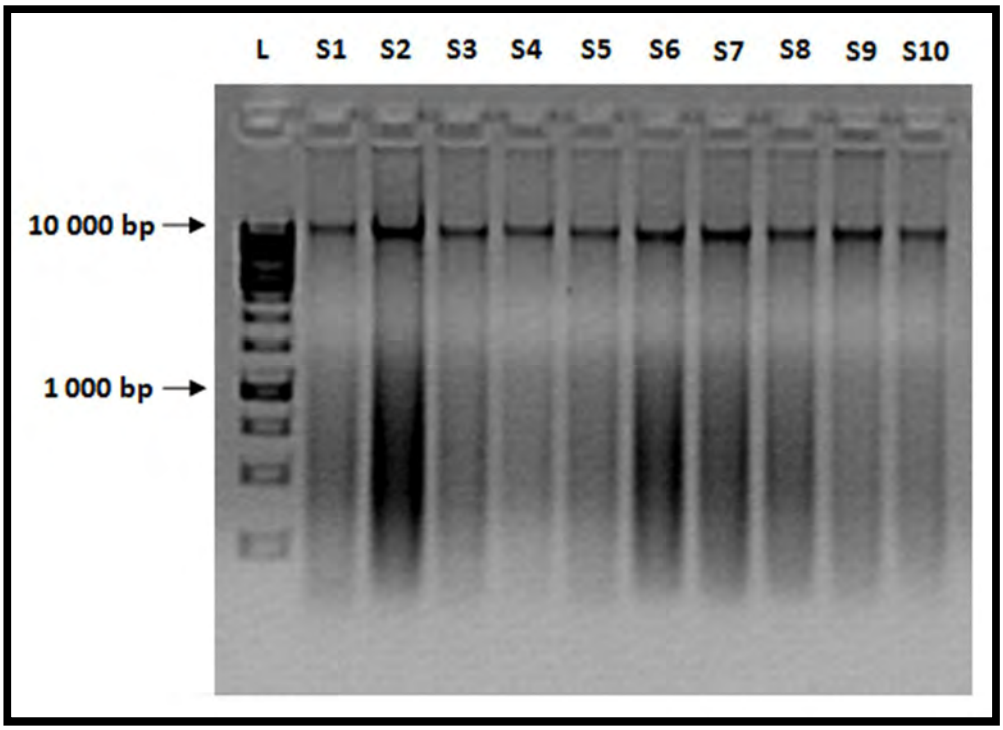


Figure 4.1: A negative image of a 1% (w/v) agarose gel depicting the DNA isolated from pooled susceptible *Helicoverpa armigera* larvae. L = 1 kb molecular weight marker.

These results were confirmed by spectrophotometric measurements (Section 3.3). The average concentration of the isolated DNA was 117.89 ng/εl, with a minimum and maximum concentration of 62.50 ng/εl and 225.52 ng/εl, respectively. Although the concentration varied between the different samples, all samples yielded sufficient concentrations of DNA for amplification. The quality of DNA was determined by the ratio of absorbance at 260 and 280 nm ($A_{260\ nm}/A_{280\ nm}$). A ratio below 1.7 and above 1.9 is indicative of protein and RNA contamination, respectively (Santella, 2006). The lowest and highest 260 nm:280 nm ratio values were 1.55 and 2.11, respectively. The average quality of the isolated DNA was 1.92, which indicates that the samples were only slightly contaminated with RNA. Genomic DNA isolated from *H. armigera* was of sufficient quality and quantity for PCR amplification.

Figures 4.2 and 4.3 show the electrophoresis results of the DNA isolated from susceptible and resistant *B. fusca* larvae, respectively. It illustrates the quantity and quality of the isolated DNA. Diffused fragmentation was observed in the susceptible *B. fusca* samples.

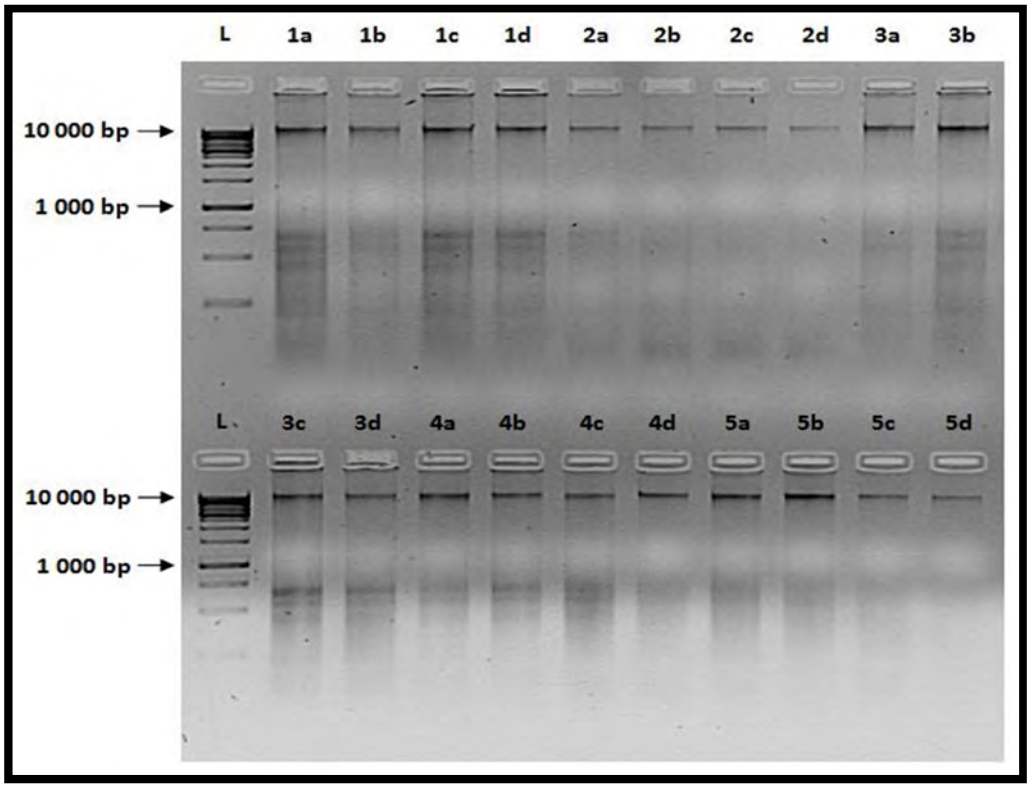


Figure 4.2: A negative image of a 1% (w/v) agarose gel depicting the DNA isolated from five susceptible *Busseola fusca* larvae, with four replicates of each. L = 1 kb molecular weight marker.

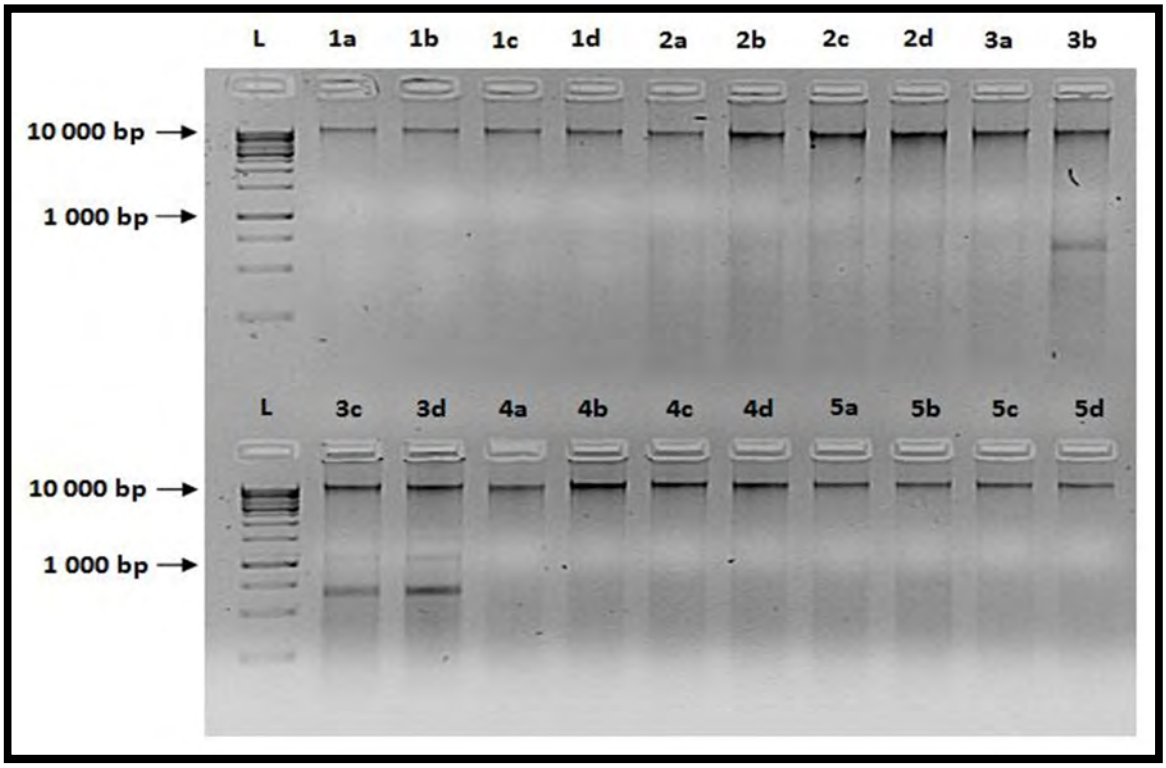


Figure 4.3: A negative image of a 1% (w/v) agarose gel depicting the DNA isolated from five resistant *Busseola fusca* larvae, with four replicates of each. L = 1 kb molecular weight marker.

These results were confirmed by spectrophotometric measurements (Section 3.3). The average concentration of the isolated DNA was 72.32 ng/εl and 43.35 ng/εl for susceptible and resistant *B. fusca* larvae, respectively. Minimum and maximum concentrations of DNA isolated from susceptible *B. fusca* larvae were 8.31 ng/εl and 192.09 ng/εl, respectively. The minimum and maximum concentration of DNA isolated from resistant *B. fusca* larvae were 16.37 ng/εl and 109.68 ng/εl, respectively. Although the concentration varied between the different samples, all samples yielded sufficient concentrations of DNA for amplification. The quality of DNA was determined by the ratio of absorbance at 260 and 280 nm ($A_{260\ nm}/A_{280\ nm}$). The lowest and highest $A_{260\ nm}/A_{280\ nm}$ ratio values were 1.78 and 2.62 for susceptible *B. fusca* larvae, and 1.77 and 2.12 for resistant *B. fusca* larvae, respectively. The average $A_{260\ nm}/A_{280\ nm}$ ratio values of the isolated DNA were 2.02 and 1.96 for susceptible and resistant *B. fusca* larvae, respectively. This indicates that both the samples sets were only slightly contaminated with RNA. Genomic DNA isolated from *B. fusca* larvae was of sufficient quality and quantity for PCR amplification.

4.2. *Cytochrome b* analysis

Genomic DNA isolated from *B. fusca* was used for amplification of *cytochrome b* under the conditions described in Section 3.4. The PCR results are indicated in Figure 4.4. A no-template control (NTC) was included to illustrate that PCR reagents were not contaminated, thus signifying that any fragments obtained with the samples are a result of PCR products that were produced during successful amplification.

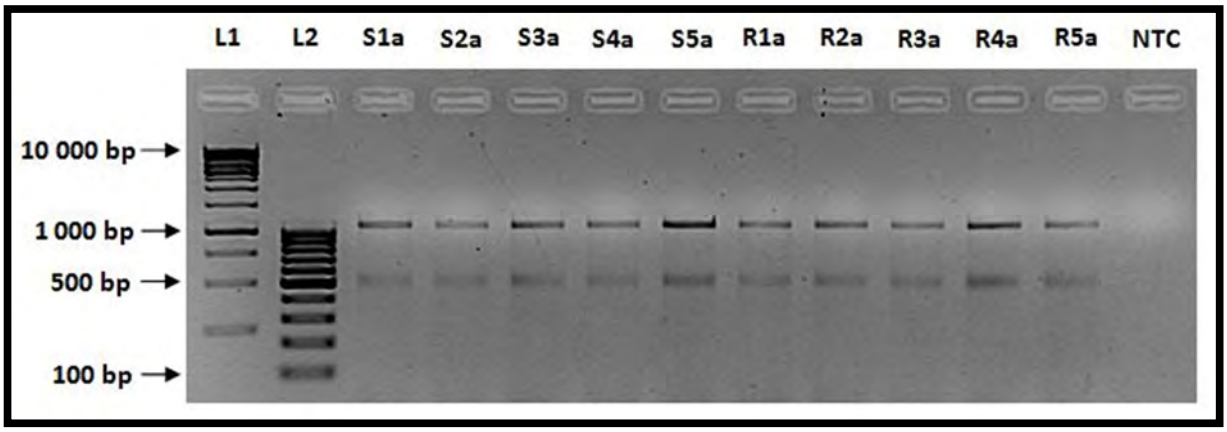


Figure 4.4: A negative image of the PCR results for *cytochrome b* from *B. fusca* on a 1.5% (w/v) agarose gel. L1 = 1 kb molecular weight marker, L2 = 100 bp molecular weight marker, S = susceptible, R = resistant, NTC = no-template control. A 1000 bp fragment, as expected, was obtained for all the samples.

Amplification of *cytochrome b* was successful for all the samples. The expected 1000 bp fragment was obtained for all the samples, although a second non-specific fragment of ~500 bp was also present. Product yield was relatively similar for all the samples. PCR products were sent to Inqaba Biotech (South Africa), where only the fragments of interest (1000 bp) were excised, purified and sequenced. The sequences obtained were used in phylogenetic analysis (Section 3.8).

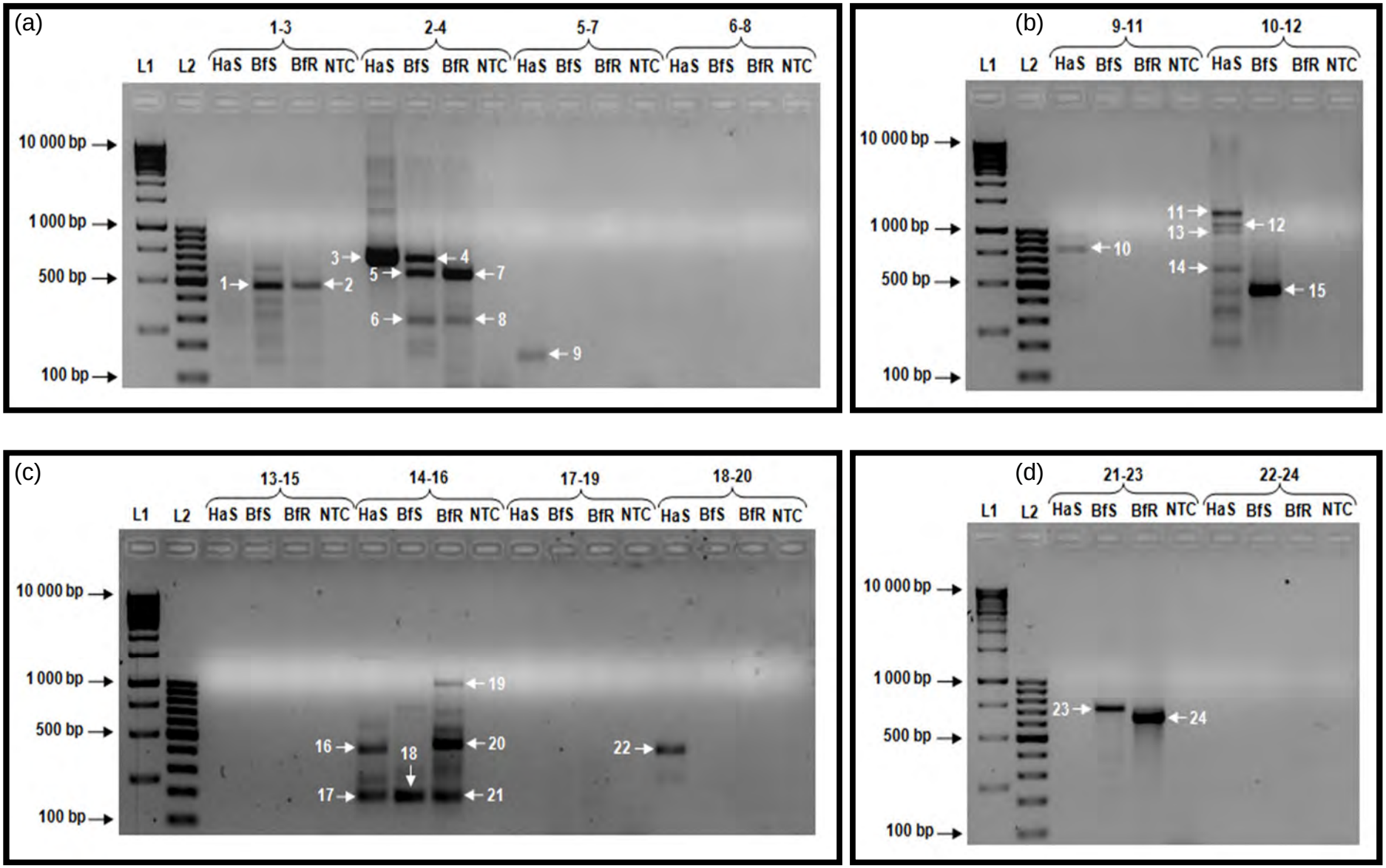
4.3. Amplification of Cry receptor genes

Genomic DNA isolated from *H. armigera* and *B. fusca* was used for amplification of Cry receptor genes (*aminopeptidase N*, *cadherin* and *alkaline phosphatase*) under the conditions described in Section 3.6 using degenerate primers (Table 3.1). PCR results are indicated in Figures 4.5 to 4.8. Diffused smears were observed in the amplification results of the Cry receptor genes. A no-template control (NTC) was included for each primer set in order to illustrate that PCR reagents are not contaminated.

In the events where multiple bands were obtained, all distinct bands (indicated by white arrows) were excised and re-amplified (Section 3.6). All the fragments are numbered to simplify the results obtained with sequence and phylogenetic results. Very faint bands that were produced by these primer sets were not used for further analyses. Re-amplification was repeated until single amplicons, of the same size as the original fragments, were obtained. Re-amplification results for APN1 are illustrated in Figure 4.6. Other re-amplification results are not shown. Multiple bands can be ascribed to the low annealing temperature that was used. A slight increase in annealing temperature, even just 0.5 °C, resulted in unsuccessful amplification. Single amplicon PCR products were purified and sequenced according to the methods described in Section 3.7. Results of sequence analysis are indicated in Section 4.4.

4.3.1 Aminopeptidase N (APN)

Six *aminopeptidase* isogenes were investigated in the present study. For each isogene, two or more primer sets were employed. Figure 4.5a-f illustrates the amplification results for each isogene. Diffused smears are evident in the PCR images for all the samples. A no-template control was included for each primer set to confirm that none of the reagents used for amplification were contaminated.


(a)
1-3
2-4
5-7
6-8
L1
L2
HaS BfS BfR NTC HaS BfS BfR NTC HaS BfS BfR NTC HaS BfS BfR NTC
10 000 bp
1 000 bp
500 bp
100 bp
1
2
3
4
5
6
7
8
9
(b)
9-11
10-12
L1
L2
HaS BfS BfR NTC HaS BfS BfR NTC
10 000 bp
1 000 bp
500 bp
100 bp
10
11
12
13
14
15
(c)
13-15
14-16
17-19
18-20
L1
L2
HaS BfS BfR NTC HaS BfS BfR NTC HaS BfS BfR NTC HaS BfS BfR NTC
10 000 bp
1 000 bp
500 bp
100 bp
16
17
18
19
20
21
22
(d)
21-23
22-24
L1
L2
HaS BfS BfR NTC HaS BfS BfR NTC
10 000 bp
1 000 bp
500 bp
100 bp
23
24

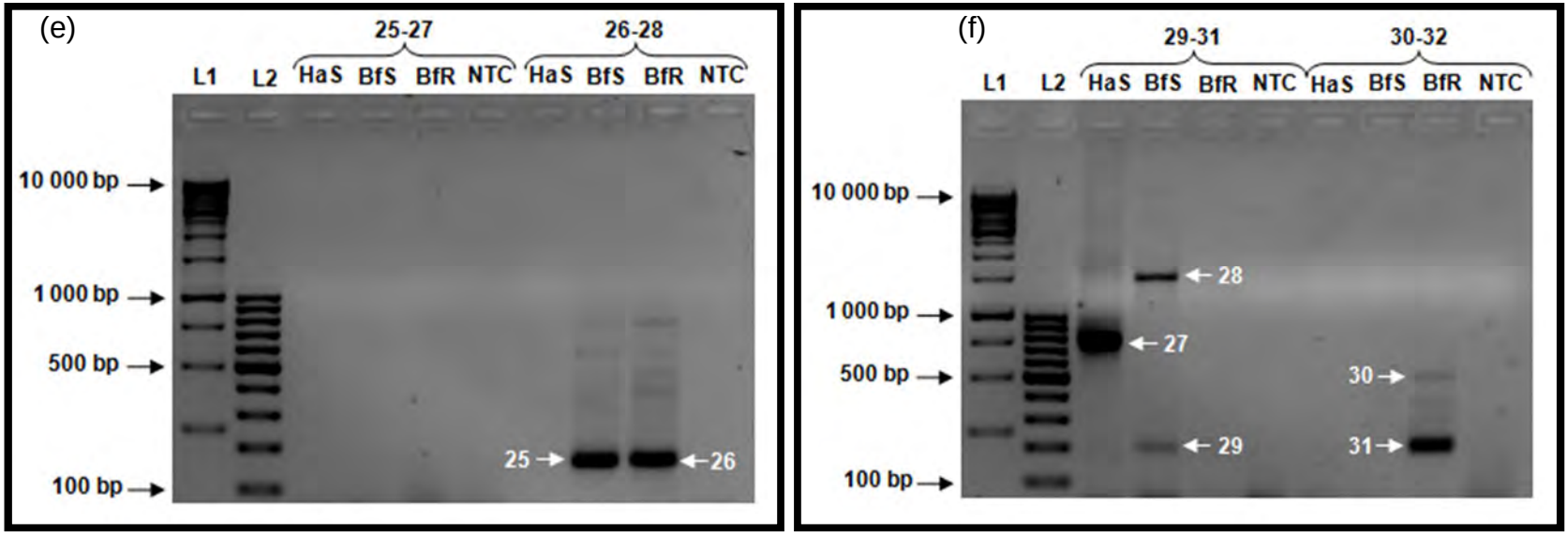


Figure 4.5: Negative images of the amplification results for (a) *APN1,* (b) *APN2*, (c) *APN3*, (d) *APN4*, (e) *APN5* and (f) *APN6* on 1.5% (w/v) agarose gels. The different primer combinations are indicated on top of each gel image (refer to Table 3.1). L1 = 1 kb molecular weight marker, L2 = 100 bp molecular weight marker, Ha = *Helicoverpa armigera*, Bf = *Busseola fusca*, S = susceptible, R = resistant, NTC = no-template control.

Amplification of *APN1* was successful for three primer sets (Figure 4.5a), with amplicons ranging between 160 bp and 680 bp in size. The majority of the bands indicate high product yield. Fragments of similar size were obtained for both the susceptible and resistant *B. fusca* samples using primer sets 1-3 and 2-4, although an additional fragment (~680 bp) was present in the susceptible sample of primer set 2-4. A high intensity fragment of ~660 bp with high intensity was obtained for *H. armigera* with primer set 2-4. A low intensity fragment of ~160 bp was obtained for *H. armigera* with primer set 5-7. Re-amplification was successful for all the excised bands (Figure 4.6).

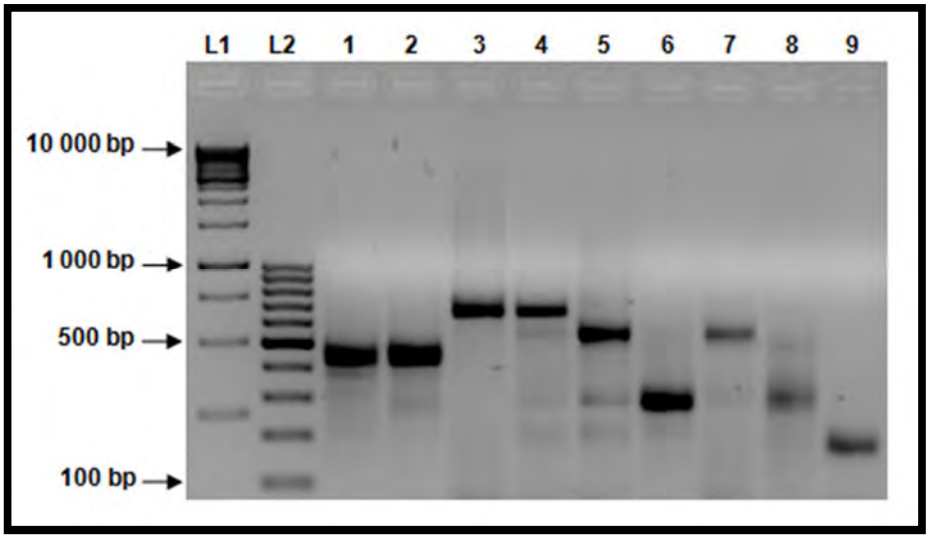


Figure 4.6: A negative image of the re-amplification results for *APN1* on a 1.5% (w/v) agarose gel. L1 = 1 kb molecular weight marker, L2 = 100 bp molecular weight marker. Numbers indicated above the lanes correspond to the numbers of the fragments indicated in Figure 4.5a.

Re-amplification results of *APN1* are illustrated in Figure 4.6. PCR amplicons obtained after several re-amplifications (Figure 4.6) are the same size as the original fragments obtained with the first amplification (Figure 4.5a). Faint bands are still visible for most of the samples. For the fragments of interest yield was, however, sufficient for sequencing at this stage. Sequencing was successful for fragments 1-8, but only fragments 3-5 and 7 showed similarities to *APN* genes from other lepidopteran species. BLAST results for these fragments are indicated in Table 4.2.

Amplification of *APN2* was successful for both primer sets (Figure 4.5b), yielding a fragment of ~800 bp for *H. armigera* using primer set 9-11, whereas primer set 10-12 yielded multiple bands for *H. armigera*. A high intensity band of ~460 bp was obtained

for the susceptible *B.fusca* sample, indicating high quantities of PCR product. Re-amplification was successful for all the excised bands. Sequencing was successful only for fragment 10. The BLAST searches showed similarity to *APN* genes from other lepidopteran species (Table 4.2).

Amplification of *APN3* was successful for two primer sets (Figure 4.5c), yielding multiple fragments (ranging between ~180 bp and ~1000 bp in size) for *H. armigera* and *B. fusca* with primer set 14-16. Primer set 18-20 yielded only one fragment of ~410 bp for *H. armigera*. The majority of the bands were faint and were not used for further analysis. Re-amplification was successful for all the excised bands. Sequencing was, however, successful only for fragment 22. Nonetheless, BLAST searches showed similarity to *APN* genes from other lepidopteran species (Table 4.2).

Amplification of *APN4* was only successful for primer set 21-23 (Figure 4.5d), yielding fragments of ~730 bp and ~650 bp for the susceptible and resistant *B. fusca* sample, respectively. Fragment 24 (~650 bp) was of high intensity, indicating PCR product in high quantities. Re-amplification was not necessary for these two fragments, since single amplicon PCR products were obtained for both samples. Sequencing was successful for both fragments 23 and 24. BLAST results of both fragments showed similarity to *APN* genes from other lepidopteran species (Table 4.2).

Amplification of *APN5* was only successful for primer set 26-28 (Figure 4.5e), yielding a fragment of ~160 bp for both the susceptible and resistant *B. fusca* sample. Even though larger faint bands were visible for each sample, excision and re-amplification was not necessary. The high quantities of PCR products (indicated by the high intensity bands) were sufficient for sequencing reactions (Section 3.7). Sequencing was successful for fragment 26, but did not match any *APN* genes of other lepidopteran species, thus no BLAST results are indicated for *APN5* in Table 4.2.

Amplification of *APN6* was successful for both primer sets (Figure 4.5f). Primer set 29-31 yielded a high intensity band (~770 bp) for the susceptible *H. armigera* sample and two fragments of ~200 bp and ~1530 bp for the susceptible *B. fusca* sample. Primer set 30-32 yielded two fragments of ~210 bp and ~510 bp for the resistant *B. fusca* sample. Band 27 indicates high quantities of PCR product. Re-amplification was successful for all the excised bands. Sequencing was successful for all the fragments. BLAST results

indicated that only fragments 27 and 28 showed similarities with *APN* genes from other lepidopteran species. These results are indicated in Table 4.2.

4.3.2 Cadherin

Many primer sets were employed to amplify the *cadherin* receptor gene. This is due to the many conserved regions that were observed in this gene. Figure 4.7a and b illustrate the amplification results for *cadherin*. Diffused smears are evident in the PCR images. A no-template control was included for each primer set to confirm that none of the reagents used for amplification were contaminated.

Amplification of *cadherin* was successful for all the primer sets, yielding multiple bands (Figure 4.7). Some bands were faint but distinct, whereas others were high in intensity but smeared. Product yield varied for each primer set, with the highest product yield observed for primer set 44-46. Fragment sizes ranged between ~140 bp and ~1820 bp. Re-amplification of the fragments for this gene was particularly challenging. Re-amplification was repeated several times, yet some fragments still failed to re-amplify. Re-amplification was only successful for fragments 39, 46-49, 52, 55 and 58-74. Sequencing was successful for fragments 39, 46, 49, 52, 55, 58, 59, 62-64 and 70. There were, however, no sequence similarities to *cadherin* from other lepidopteran species for any of these sequenced fragments. Therefore these results were not included. Fragments 39, 46 and 62, however, showed similarities to *B. fusca* microsatellite sequences (Table 4.2).

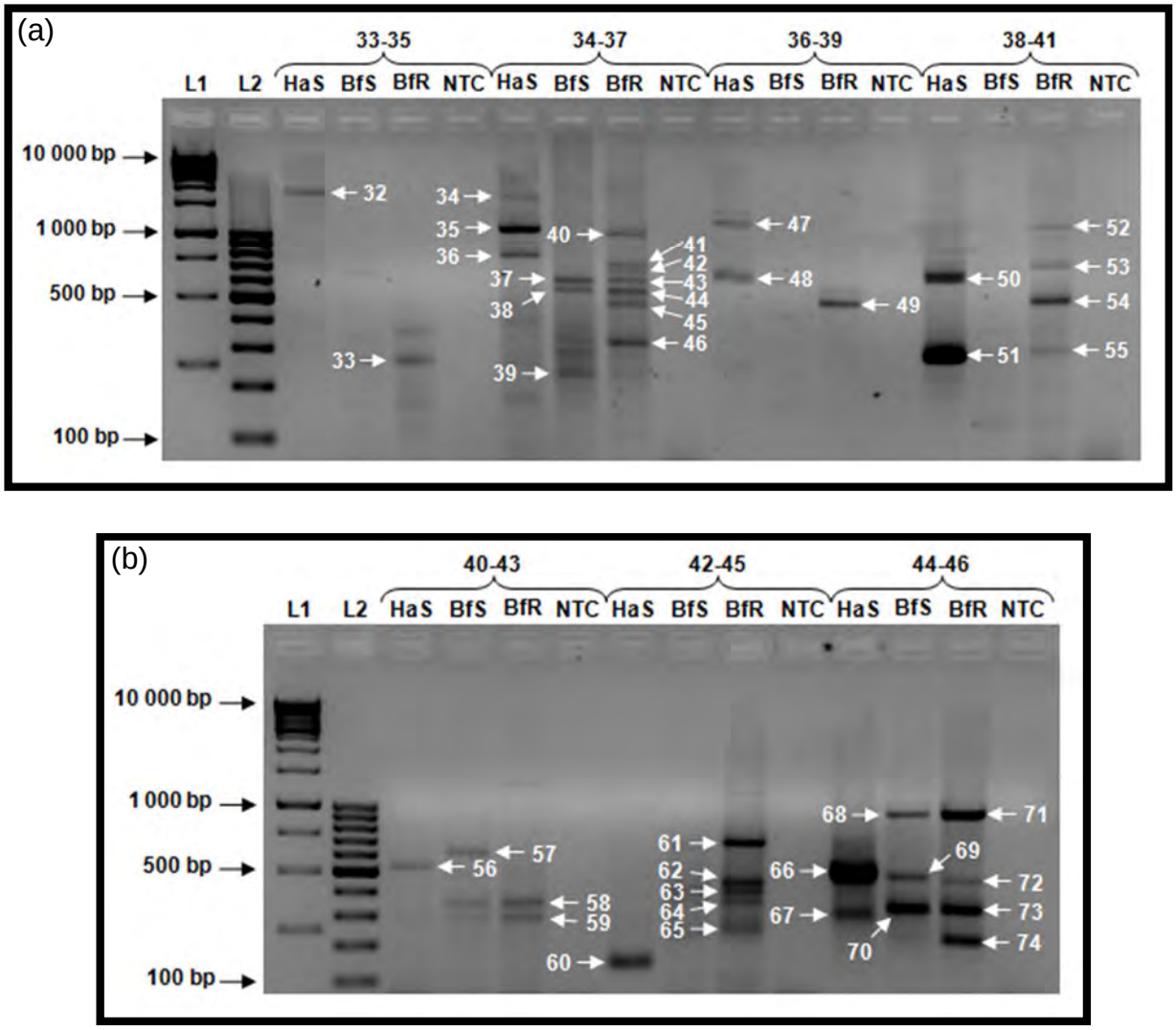


Figure 4.7: Negative images of the amplification results for *cadherin* on 1.5% (w/v) agarose gels. The different primer combinations are indicated on top of the gel images (refer to Table 3.1). L1 = 1 kb molecular weight marker, L2 = 100 bp molecular weight marker, Ha = *Helicoverpa armigera*, Bf = *Busseola fusca*, S = susceptible, R = resistant, NTC = no-template control.

4.3.3 Alkaline phosphatase (ALP)

Five primer sets were employed for the amplification of *alkaline phosphatase*. Figure 4.8a and b illustrate the amplification results for *ALP*. Diffused smears are evident in the PCR images. A no-template control was included for each primer set to confirm that none of the reagents used for amplification were contaminated.

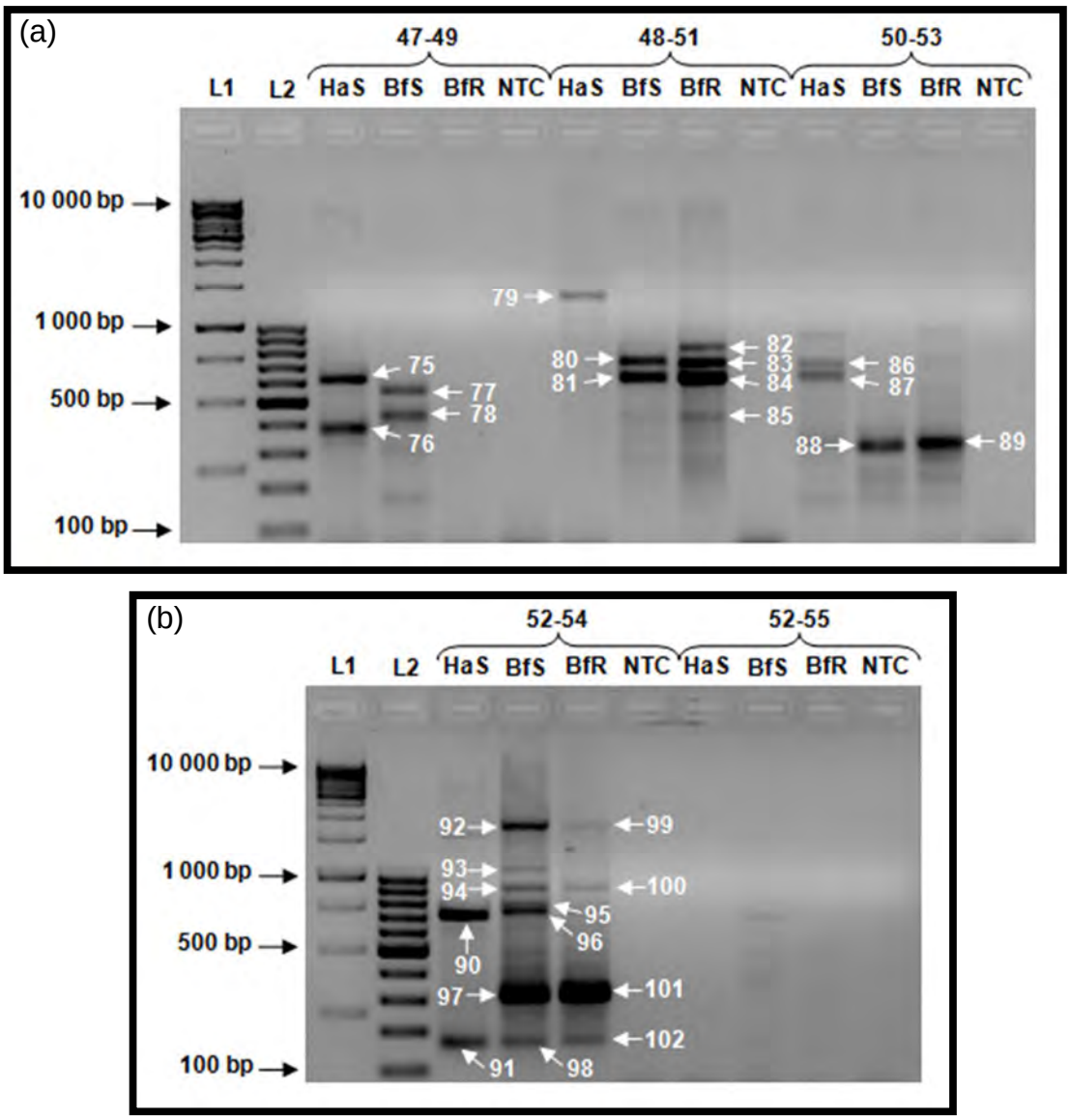


Figure 4.8: Negative images of the amplification results for *ALP* on 1.5% (w/v) agarose gels. The different primer combinations are indicated on top of the gel images (refer to Table 3.1). L1 = 1 kb molecular weight marker, L2 = 100 bp molecular weight marker, Ha = *Helicoverpa armigera*, Bf = *Busseola fusca*, S = susceptible, R = resistant, NTC = no template control.

Amplification of *ALP* was successful for all the primer sets. The quantity of the fragments obtained with primer set 52-55 were, however, not sufficient for further analysis. Four primer sets (47-49, 48-51, 50-53 and 52-54) yielded multiple distinct bands (Figure 4.8). Fragment sizes ranged between ~160 bp and ~1830 bp. The majority of the bands were of high intensity, indicating high product yield. Re-amplification was successful for the majority excised bands, but was unsuccessful for fragments 82, 87, 92, 93, 95 and 98-102. Sequences of fragments 75, 79, 80, 81, 83, 84

and 100 showed similarities to *ALP* from other lepidopteran species and are indicated in Table 4.2. Fragment 87, however, showed similarities to *cadherin* from *H. armigera*.

4.4 Sequence analysis

4.4.1 *Cytochrome b*

The 1000 bp amplicons obtained with the *cytochrome b* primer set (Figure 4.4) were sequenced by Inqaba Biotech (South Africa). BLAST searches were performed on the obtained sequences, wherein the query sequences were compared to sequences in GenBank for similarities. BLAST results for *cytochrome b* are given in Table 4.1.

Table 4.1: BLAST results for *Busseola fusca cytochrome b* sequences, with the % similarity and E values indicated.

Query sequence	Query length	GenBank ID		% Similarity	E value
		Accession number	Description		
S1a	881	AY769586.1	*Busseola fusca* haplotype hapl51-k2 cytochrome b gene	100	0.0
S2a	879			99	0.0
S3a	884			100	0.0
S4a	881			99	0.0
S5a	881			99	0.0
R1a	879	AY769605.1	*Busseola fusca* haplotype hapl70-k2 cytochrome b gene	100	0.0
R2a	926			99	0.0
R3a	873			99	0.0
R4a	882			100	0.0
R5a	880			100	0.0

These results confirmed that the obtained sequences were indeed partial regions of the *cytochrome b* gene of *B. fusca*. Query length varied between 873 and 926 nucleotides for all the sequences. Background noise was insignificantly low for all the *cytochrome b* sequences. For all the *cytochrome b* samples sequence similarity of 99-100% and Expected (E) values of 0.0 were obtained. E values of zero or less indicate that there were high similarities between the query sequences and the matched sequences. For all the query sequences query coverage of 100% was indicated. The query sequences of the susceptible and resistant samples showed high similarity to the *cytochrome b*

gene of *B. fusca* haplotype hapl51-k2 (Accession number AY769586.1) and haplotype hapl70-k2 (Accession number AY769605.1), respectively. These haplotypes were used in a phylogenetic study by Sezonlin *et al*. (2006). In this study, *B. fusca* populations separated into three mitochondrial clades: W (West Africa), KI (East Africa) and KII (Central to East Africa).

Both haplotypes indicated in Table 4.1 clustered together with the KII clade (Central to East Africa) according to the results of Sezonlin *et al*. (2006). These results could be explained for the susceptible samples, since they originated from Kenya. However, the resistant samples are from southern Africa. Sezonlin *et al*. (2006) did not use *B. fusca* larvae from southern Africa in their phylogenetic analysis of the *cytochrome b* gene. This may explain why the resistant samples (from the Vaalharts area) clustered together with the susceptible samples in the KII clade.

The sequences indicated in Table 4.1 were used in phylogenetic analysis (Section 4.5.1). This analysis intended to illustrate the evolutionary relationship of the *cytochrome b* gene among the obtained sequences and various lepidopteran species in the family Noctuidae.

4.4.2 Cry receptor genes

Multiple bands were obtained with the amplification of Cry receptor genes (Figures 4.5 to 4.8). Bands were excised and re-amplified as described in Section 3.6. Single amplicon PCR products were then purified and sequenced. BLAST searches were performed on the obtained sequences, wherein the query sequences were compared to sequences in GenBank for similarities. BLAST results for Cry receptor genes are given in Table 4.2.

Table 4.2: BLAST results for Cry receptor gene sequences.

Gene	Fragment	Sample	Query length	Accession number	Description	% Query coverage	% Similarity	E value
APN1	3F	HaS	215	EU568874.1	*Helicoverpa armigera* Cry1Ac (R) *APN1*	11	100	0.084
				EU568875.1	*Helicoverpa armigera* Cry1Ac (S) *APN1*	11	100	0.084
	3R		224	EU568874.1	*Helicoverpa armigera* Cry1Ac (R) *APN1*	84	99	4.00E-88
				EU568875.1	*Helicoverpa armigera* Cry1Ac (S) *APN1*	84	99	4.00E-88
	4R	BfS	183	EU568874.1	*Helicoverpa armigera* Cry1Ac (R) *APN1*	81	99	2.00E-66
				EU568875.1	*Helicoverpa armigera* Cry1Ac (S) *APN1*	81	99	2.00E-66
	5F	BfS	389	EU568874.1	*Helicoverpa armigera* Cry1Ac (R) *APN1*	60	74	2.00E-31
				EU568875.1	*Helicoverpa armigera* Cry1Ac (S) *APN1*	60	74	2.00E-31
	5R		299	EU568874.1	*Helicoverpa armigera* Cry1Ac (R) *APN1*	34	74	1.00E-07
				EU568875.1	*Helicoverpa armigera* Cry1Ac (S) *APN1*	34	74	1.00E-07
	7F	BfR	214	AY218842.1	*Spodoptera exigua APN1*	28	79	0.002
APN2	10F	HaS	258	AY181026.1	*Helicoverpa armigera APN4*	57	87	7.00E-42
	10R	HaS	295	AY181026.1	*Helicoverpa armigera APN4*	70	99	1.00E-96
APN3	22F	HaS	297	AY279537.1	*Helicoverpa armigera APN3*	18	91	2.00E-11
	22R	HaS	318	AY279537.1	*Helicoverpa armigera APN3*	30	96	5.00E-13
APN4	23R	BfS	224	EU568874.1	*Helicoverpa armigera* Cry1Ac (R) *APN1*	56	70	0.002
				EU568875.1	*Helicoverpa armigera* Cry1Ac (S) *APN1*	56	70	0.002
	24F	BfR	452	HM357836.1	*Mamestra configurata APN8*	6	93	0.68
	24R		536	JQ061146.1	*Bombyx mori APN4*	14	81	1.00E-10
APN6	27F	HaS	210	EU328183.1	*Helicoverpa armigera APN6*	64	98	7.00E-22
	27R		261	EU328183.1	*Helicoverpa armigera APN6*	66	96	1.00E-32
	28F	BfS	424	EU328183.1	*Helicoverpa armigera APN6*	44	79	9.00E-37
	28R	BfS	397	EU328183.1	*Helicoverpa armigera APN6*	21	82	7.00E-13

***APN = Aminopeptidase, F = forward sequence, R = reverse sequence, Ha = *Helicoverpa armigera*, Bf = *Busseola fusca*, S = susceptible, R = resistant**

Gene	Fragment	Sample	Query length	Accession number	Description	% Query coverage	% Similarity	E value
CAD	39R	BfS	136	DQ393654.1	*Busseola fusca* clone BFU067 microsatellite sequence	21	97	0.014
	46R	BfR	220	DQ393654.1	*Busseola fusca* clone BFU067 microsatellite sequence	10	100	1.1
	62R	BfR	306	AY884601.1	*Busseola fusca* clone B3.21 microsatellite sequence	9	97	0.036
ALP	75F	HaS	303	EU729322.1	*Helicoverpa armigera ALP1*	22	93	8.00E-17
				EU729323.1	*Helicoverpa armigera ALP2*	22	93	8.00E-17
	79F	HaS	311	EU729322.1	*Helicoverpa armigera ALP1*	19	98	2.00E-19
				EU729323.1	*Helicoverpa armigera ALP2*	19	98	2.00E-19
	80F	BfS	514	AB379676.1	*Bombyx mori ALP*	12	77	0.018
	80R	BfS	507	AB379676.1	*Bombyx mori ALP*	15	75	1.00E-04
	81F	BfS	350	HM357865.1	*Mamestra configurata ALP1A*	15	91	3.00E-10
				AB379676.1	*Bombyx mori ALP*	16	81	1.00E-03
	81R	BfS	460	EU729322.1	*Helicoverpa armigera ALP1*	12	78	0.057
				EU729323.1	*Helicoverpa armigera ALP2*	12	78	0.057
	83F	BfR	524	AB379676.1	*Bombyx mori ALP*	18	82	0.019
	83R		542	AB379676.1	*Bombyx mori ALP*	14	75	1.00E-04
	84R	BfR	160	HM357865.1	*Mamestra configurata ALP1A*	25	90	4.00E-04
	87F	HaS	273	AY714876.1	*Helicoverpa armigera cadherin*	30	75	9.00E-03
	87R	HaS	327	AY714876.1	*Helicoverpa armigera cadherin*	25	74	0.003
	91F	HaS	105	EU729322.1	*Helicoverpa armigera ALP1*	100	78	6.00E-12
				EU729323.1	*Helicoverpa armigera ALP2*	100	78	6.00E-12
	91R	HaS	100	EU729322.1	*Helicoverpa armigera ALP1*	89	84	8.00E-16
				EU729323.1	*Helicoverpa armigera ALP2*	89	84	8.00E-16

***CAD = Cadherin, ALP = Alkaline phosphatase, F = forward sequence, R = reverse sequence, Ha = *Helicoverpa armigera*, Bf = *Busseola fusca*, S = susceptible, R = resistant**

These results confirmed that the obtained sequences were indeed partial regions of the respective Cry receptor genes from lepidopteran species. Query length varied between 100 and 542 nucleotides for all the sequences. BLAST results may have been affected by the background noise present in some of the Cry receptor gene sequences. For some fragments, sequencing was repeated several times. Failure to sequence some of these fragments, despite being single PCR amplicons in high quantities, may be due to several factors. These include: inefficient primer binding, multiple priming sites, secondary structures, contaminants present in the sample or enzyme slippage during sequencing due to repetitive regions (McGrath, 2011). The exact causes of sequencing failure were not further explored.

For all the Cry receptor genes there was a sequence similarity of 70-100% and Expected (E) values of <0-1.1. Low E values indicate that there was high similarity between the query sequences and the matched sequences (for E values below 0). Receptor protein gene sequences were used in phylogenetic analysis (Section 4.5.2). Phylogenetic analysis was performed to illustrate the evolutionary relationship of the respective Cry receptor genes among the obtained sequences and various lepidopteran species in the family Noctuidae.

4.5 Phylogenetic analysis

Sequences of the 1000 bp *cytochrome b* PCR amplicons were determined by Inqaba Biotech (Section 3.4). These sequences were subjected to BLAST searches (Section 4.4.1). Sequences were also used to construct a bootstrap neighbor-joining phylogenetic tree using MEGA (version 4.0.2) software (Section 3.8). Evolutionary distances were computed using the Jukes-Cantor method. Figure 4.9 illustrates the bootstrap neighbor-joining phylogenetic tree of *cytochrome b* gene sequences from 56 lepidopteran species and 10 susceptible and resistant (five of each) *B. fusca* larvae.

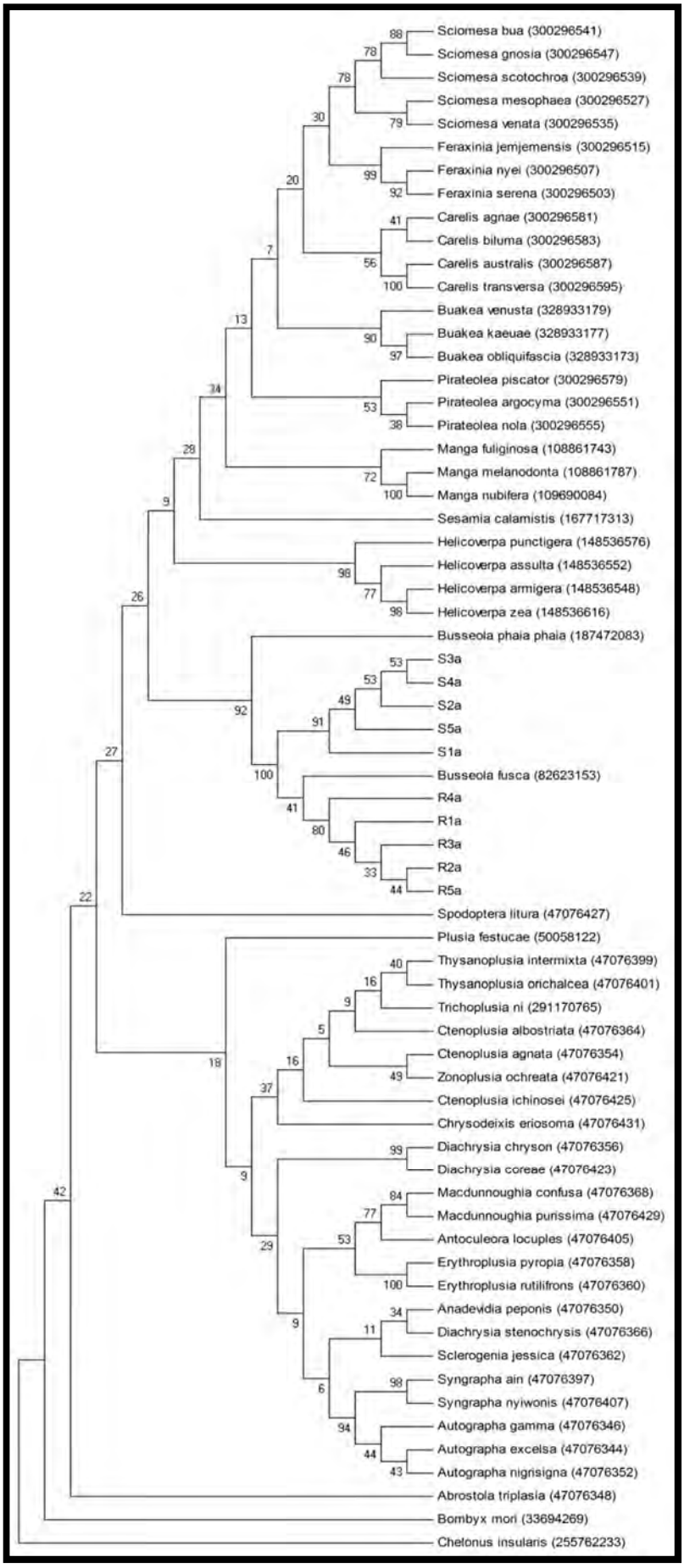
Sequences of the fragments obtained with amplification of Cry receptor genes were also used to construct bootstrap neighbor-joining phylogenetic trees using the same software (Section 3.8). The Jukes-Cantor method was used to compute evolutionary distances. Figures 4.11 to 4.13 illustrate the bootstrap neighbor-joining phylogenetic trees of Cry receptor gene sequences from several lepidopteran species and the obtained sequences indicated in Table 4.2.

4.5.1 *Cytochrome b*

The phylogenetic tree in Figure 4.9 illustrates the evolutionary relationships of the *cytochrome b* gene among 56 lepidopteran species in the family Noctuidae and the sequences obtained with *B. fusca* (Table 4.1). Species of the same genus is clustered together. From this figure it is evident that *Bombyx mori* and *Chelonus insularis* were outgroups in this case. However, for this phylogenetic analysis outgroups were not specified.

Susceptible and resistant *B. fusca* samples formed separate sub-clusters with bootstrap values between 49% and 91%. The *cytochrome b* sequence from *B. fusca* (gi: 82623153) clustered together with the sub-cluster of resistant samples with 41% bootstrap support. This sequence was randomly selected for this phylogenetic analysis. According to the results of Sezonlin *et al*. (2006), this sequence (gi: 82623153) also clustered with the KII clade (Central to East Africa). These results are consistent with the BLAST results (Table 4.1). The phylogenetic relationship between the susceptible and resistant sub-clusters and *B. fusca* (gi: 82623153) is supported by a 100% bootstrap value.

Figure 4.9: A neighbor-joining phylogenetic tree based on an alignment of *cytochrome b* gene sequences obtained after amplification (Table 4.1) and 56 other species in the order Lepidoptera, family Noctuidae. Species and GenBank Gen-Info Identifier (GI) numbers are shown. S = susceptible, R = resistant.



According to the dendogramôs topology, it is evident that *B. fusca* is more closely related to species of the genus *Helicoverpa* (*H. armigera*, *H. assulta*, *H. punctigera* and *H. zea*). A few sequences were selected from the phylogenetic tree in Figure 4.9 to construct a condensed phylogenetic tree (Figure 4.10).

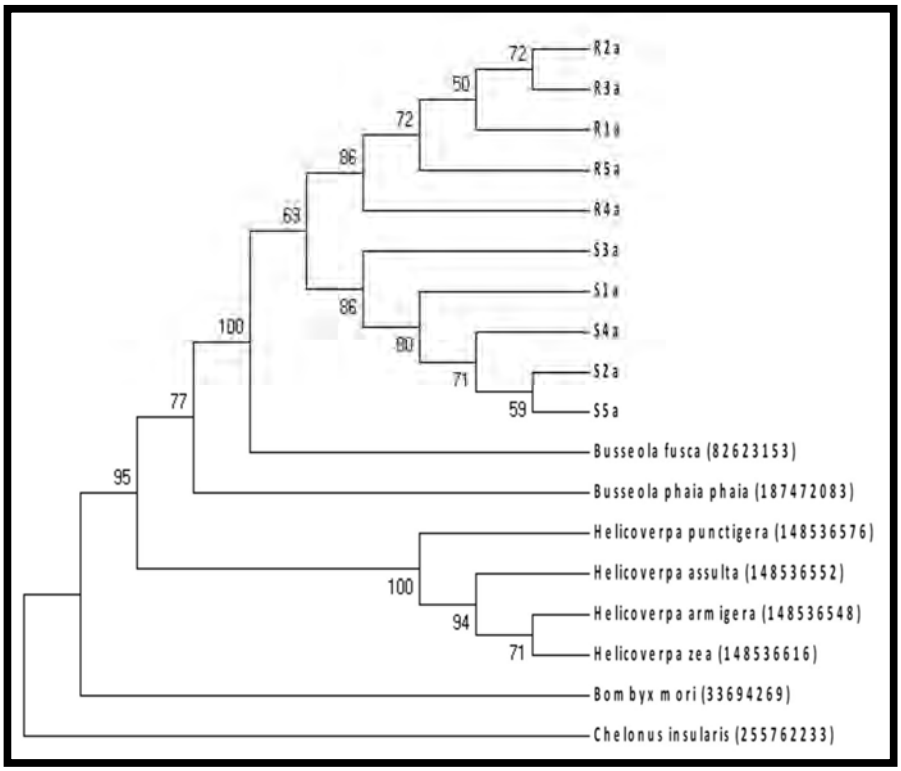


Figure 4.10: A condensed neighbor-joining phylogenetic tree based on an alignment of *cytochrome b* gene sequences obtained after amplification (Table 4.1) and other species in the order Lepidoptera, family Noctuidae. Species and Genbank GenInfo Identifier (GI) numbers are shown. S = susceptible, R = resistant.

Figure 4.10 illustrates a condensed bootstrap neigbor-joining phylogenetic tree, constructed with a few selected sequences from Figure 4.9. According to this figure the phylogenetic tree is divided into four major clusters: (1) *B. fusca* samples (susceptible and resistant), (2) *Busseola* spp., (3) *Helicoverpa* spp. and (4) *Bombyx* and *Chelonus* spp. Genera *Bombyx* and *Chelonus* did not group with any of the other clusters, although these species also belong to the family Noctuidae.

The susceptible and resistant *B. fusca* samples produced two separate sub-clusters, which is an indication of genetic differentiation in two geographically isolated populations of *B. fusca* (Sezonlin *et al*., 2006). Thus the phylogenetic analysis may confirm the separation of *B. fusca* into two groups corresponding to their geographical

positions (susceptible Kenyan samples versus resistant South-African samples) (Section 3.1). In this condensed phylogenetic tree *B. fusca* (gi: 82623153) did not only cluster with the sub-cluster of resistant samples as illustrated in Figure 4.9. In this case it clustered together with both the susceptible and resistant sub-clusters, also supported by a bootstrap value of 100%.

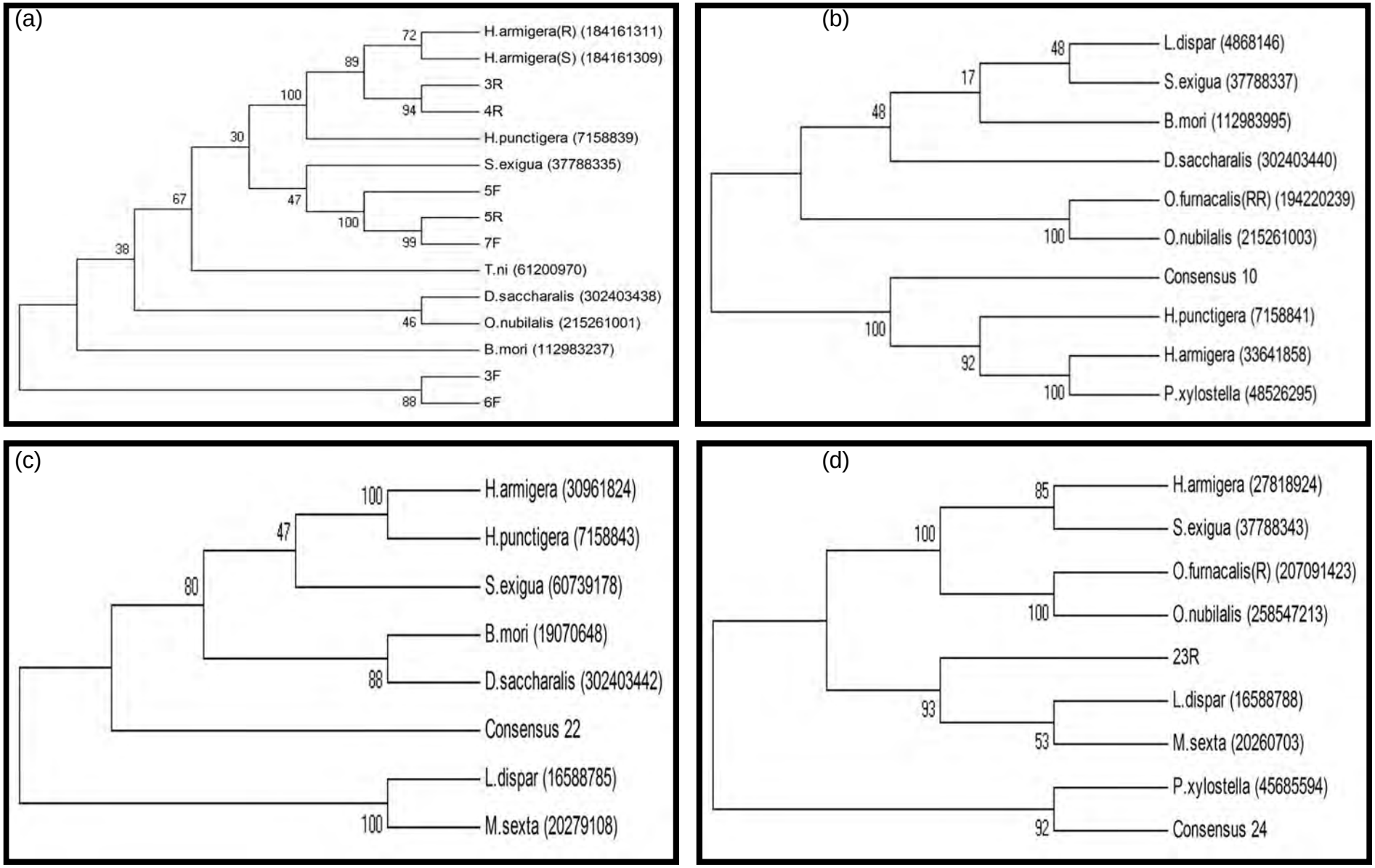
From this condensed phylogenetic tree it is also evident that *B. fusca* is more closely related to species of the genus *Helicoverpa* (*H. armigera*, *H. assulta*, *H. punctigera* and *H. zea*). This relationship is, however, supported with a bootstrap value of 95% in the condensed phylogenetic tree compared to 26% in the extended phylogenetic tree. These results support the approach that was chosen for this present study, which was based on the mechanism of *H. armigera* resistance to Cry1Ac.

4.5.2 Cry receptor genes

Phylogenetic trees in Figures 4.11a-f illustrate the evolutionary relationships of the Cry receptor genes *APN* (isogenes 1 to 6) and *cadherin* among lepidopteran species in the family Noctuidae and the sequences obtained with *B. fusca* (Table 4.2). The lepidopteran receptor gene sequences used in this phylogenetic analysis are the same gene sequences that were used for primer design (Appendix B). No outgroups were specified for any of these phylogenetic trees.

For *APN5*, no sequence data could be generated due to failure of sequencing (fragment 25) and no similarities (fragment 26). Thus, phylogenetic analysis could also not be performed on this gene. It was also not possible to construct a bootstrap neighbor-joining phylogenetic tree with *ALP* gene sequences, due to no common nucleotide sites.

Consensus sequences were prepared for fragments from which both the forward and reverse sequence were determined. However, for *APN1* (Figure 4.11a) separate sequences were used, due to inaccurate clustering observed with the consensus sequences (results not shown). Separate sequences were also used for *APN6* (Figure 4.11e), due to a lack in nucleotide sites for consensus sequences.


(a)
H.armigera(R) (184161311)
H.armigera(S) (184161309)
3R
4R
H.punctigera (7158839)
S.exigua (37788335)
5F
5R
7F
T.ni (61200970)
D.saccharalis (302403438)
O.nubilalis (215261001)
B.mori (112983237)
3F
6F
72
89
100
94
30
67
47
100
99
38
46
88
(b)
L.dispar (4868146)
S.exigua (37788337)
B.mori (112983995)
D.saccharalis (302403440)
O.furnacalis(RR) (194220239)
O.nubilalis (215261003)
Consensus 10
H.punctigera (7158841)
H.armigera (33641858)
P.xylostella (48526295)
48
17
48
100
100
92
100
(c)
H.armigera (30961824)
H.punctigera (7158843)
S.exigua (60739178)
B.mori (19070648)
D.saccharalis (302403442)
Consensus 22
L.dispar (16588785)
M.sexta (20279108)
100
47
80
88
100
(d)
H.armigera (27818924)
S.exigua (37788343)
O.furnacalis(R) (207091423)
O.nubilalis (258547213)
23R
L.dispar (16588788)
M.sexta (20260703)
P.xylostella (45685594)
Consensus 24
85
100
100
93
53
92

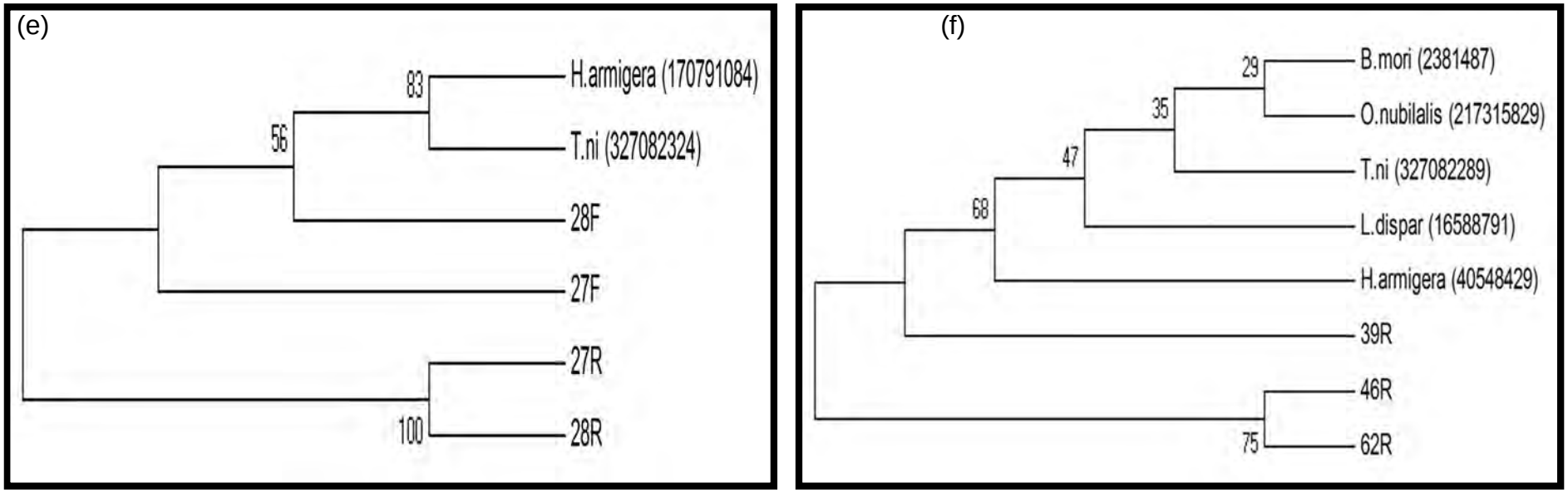


Figure 4.11: Neighbor-joining phylogenetic trees based on an alignment of (a) *APN1*, (b) *APN2*, (c) *APN3*, (d) *APN4* (e) *APN6* and (f) *cadherin* gene sequences obtained after amplification (Table 4.2) and other species in the order Lepidoptera, family Noctuidae. Species and GenBank Gen-Info Identifier (GI) numbers are shown. (R) = resistant, (S) = susceptible. Numbers indicated for fragments in these figures correspond to fragment numbers indicated in Table 4.2.

Figure 4.11a illustrates a neigbor-joining phylogenetic tree for *APN1*. The reverse sequence of fragment 3 (3R) clustered with *H. armigera* (R and S) with 89% bootstrap support. This was expected, because fragment 3 resulted from amplification of *APN1* from *H. armigera* (Figure 4.5a). However, the forward sequence of this fragment (3F) as well as fragment 6 (6F) did not group with any of the lepidopteran species. Fragment 6 resulted from amplification of *APN1* from *B. fusca* (S) (Figure 4.5a). Sequences of fragment 5 (5F and 5R) and fragment 7 (7F) clustered with *S. exigua* with 47% bootstrap support. These results are the first to indicate a phylogenetic relationship between *B. fusca* and other lepidopterans for the *APN1* receptor gene. The reverse sequence of fragment 4 (4R) also clustered with *H. armigera*, even though this fragment resulted from amplification of *B. fusca* DNA. This is supported with an 89% bootstrap value.

Figure 4.11b illustrates a neigbor-joining phylogenetic tree for *APN2*. The consensus sequence of fragment 10 clustered with *Helicoverpa* spp. with 100% bootstrap support. This pattern of clustering was expected, because this fragment resulted from amplification of *APN2* from *H. armigera* (Figure 4.5b).

Figure 4.11c illustrates a neigbor-joining phylogenetic tree for *APN3*. The consensus sequence of fragment 22 did not cluster with any of the lepidopteran species. This fragment resulted from amplification of *APN3* from *H. armigera* (Figure 4.5c). It was thus expected that this consensus sequence would cluster with *Helicoverpa* spp. similar to the clustering observed in Figure 4.5b. No bootstrap support is indicated for clustering of Consensus 22. The same results were obtained when the separate forward and reverse sequences were used for this analysis (results not shown).

Figure 4.11d illustrates a neigbor-joining phylogenetic tree for *APN4*. The reverse sequence of fragment 23 (23R) clustered with *Lymantria dispar* and *Manduca sexta* with bootstrap support of 93%. The consensus sequence of fragment 24 clustered with *P. xylostella* with bootstrap support of 92%. Fragments 23 and 24 resulted from amplification of *APN4* from susceptible and resistant *B. fusca*, respectively. These results thus indicate a

phylogenetic relationship between *B. fusca* and other lepidopterans for the *APN4* receptor gene.

Figure 4.11e illustrates a neigbor-joining phylogenetic tree for *APN6*. Due to no common nucleotide sites between consensus sequences, separate forward and reverse sequences were used in the construction of this phylogenetic tree. Only the forward sequence of fragment 28 (28F) clustered with the two lepidopteran sequences used in this analysis. This was supported by a bootstrap value of 56%. This fragment resulted from amplification of *APN6* from *B. fusca* (S). The reverse sequence of this same fragment, however, formed an outgroup along with the forward and reverse sequences of fragment 27. Fragment 27 resulted from amplification of *APN6* from *H. armigera*. Thus it was expected that the sequences from this fragment would rather cluster with *H. armigera*. This was not, however, the case. These results indicate a phylogenetic relationship between *B. fusca* and other lepidopterans for the *APN6* receptor gene.

Figure 4.11f illustrates a neigbor-joining phylogenetic tree for *cadherin*. The reverse sequences of fragments 39, 46 and 62 resulted from amplification of *cadherin* from *B. fusca* (S and R). These three sequences formed an outgroup in this phylogenetic tree with no bootstrap support indicated. BLAST results indicated that all three these sequences showed sequence homology with *B. fusca* microsatellite sequences (Table 4.2). Thus this pattern of clustering was expected.

4.6 Summary of results

In the present study, genomic DNA was successfully isolated from *H. armigera* (susceptible) and *B. fusca* (susceptible and resistant) larvae. Isolated DNA was used for amplification of the *cytochrome b* and Cry receptor genes.

Cytochrome b gene amplification served the purpose of a control, which illustrated that the isolated DNA was amplifiable. The *cytochrome b* primer set from Sezonlin *et al*. (2006) was employed. The expected fragment length of 1000 bp was obtained for all the susceptible and resistant *B. fusca* samples (five of each). These fragments were sequenced, after

which BLAST searches (Section 4.4.1) and phylogenetic analysis (Section 4.5.1) were performed. The present study was based on the mechanism of *H. armigera* resistance to Cry1Ac. This approach was supported by the phylogenetic analysis, which indicated that *B. fusca* is more closely related to *Helicoverpa* spp.

The Cry receptor genes that were amplified consisted of *aminopeptidase N* (isogenes 1 to 6), *cadherin* and *alkaline phosphatase*. Previous observations concluded that existing primer sets used for the amplification of Cry receptor genes from other lepidopteran species fail to amplify these genes from *B. fusca* (Venter, 2010). Consequently, degenerate primers were designed to amplify these genes from *B. fusca*. The process of primer design is described in detail in Section 3.5.

Amplification was successful for the majority of the primer sets, where multiple bands were obtained in most cases. Bands were excised and re-amplified according to the methods described in Section 3.6 in order to obtain single PCR amplicons to be used for sequencing. Single PCR amplicons were purified and sequenced according to the methods described in Section 3.7.

BLAST searches were performed, wherein the sequences of the PCR amplicons were compared to the GenBank database of sequences. Amplicon sequences that matched Cry receptor gene sequences on GenBank were used in phylogenetic analysis (Section 4.5.2).

The significance of these results will be discussed in Chapter 5.

CHAPTER 5

DISCUSSION

5.1. Introduction

This study was concerned with Cry receptor genes from *Busseola fusca* and mutations potentially involved in Bt-resistance. The aim was to investigate mutations using a PCR detection method. However, in order to study mutations, sequences of Cry receptor genes are essential. However, molecular data for *B. fusca* is limited. Most of this study was thus an attempt to generate sequence data for these genes. *Helicoverpa armigera* and *B. fusca* larvae were collected from the Agricultural Research Council (ARC) in Potchefstroom. Bt-susceptible *B. fusca* larvae were originally sourced from Kenya as egg pockets and raised at the ARC facilities. Bt-resistant larvae were collected from Bt-maize in the Vaalharts area and further raised at the ARC facilities. Genomic DNA was successfully isolated from these larvae and subsequently used for PCR amplification. Sequences of single amplicons were determined and subjected to BLAST searches and phylogenetic analysis.

5.2. DNA isolation

DNA was successfully isolated from *H. armigera* and *B. fusca* larvae. Sufficient quantities of DNA that was of suitable quality for PCR analysis were obtained using the NucleoSpin® Tissue Kit (Macherey-Nagel). This kit was successfully used in previous studies.

In a study by Tomaso *et al*. (2010), several commercial DNA isolation kits were used for isolation of DNA from insects. These authors (Tomaso *et al*., 2010) concluded that the NucleoSpin® Tissue Kit (Macherey-Nagel) yielded intermediate quantities of DNA, whereas QIAGEN and Roche kits generated the highest DNA yield. Although commercial kits are very convenient, these can be very expensive as well.

There was fragmentation of DNA isolated from the *H. armigera* samples (Figure 4.1) as well as the susceptible *B. fusca* samples (Figure 4.2). Fragmentation of the DNA from *H.*

armigera may be due to liquid nitrogen that was used during the DNA isolation process. The use of liquid nitrogen during the isolation of DNA from *B. fusca* was previously found to be too harsh (results not shown).

Fragmentation of DNA resulting from the use of liquid nitrogen observed in this study is consistent with results obtained by Singh *et al*. (2011). These authors observed that when only liquid nitrogen was used prior to DNA isolation from the silkworm, DNA yield was low. However, yield was improved when larval tissue was first fixed in absolute alcohol and then ground in liquid nitrogen. These authors concluded that liquid nitrogen is too harsh when DNA is isolated from insects.

It can thus be concluded that liquid nitrogen is too harsh for DNA isolation from *H. armigera* as well as *B. fusca*. Even without using liquid nitrogen, moderate fragmentation of DNA was observed (susceptible *B. fusca* larvae; Figure 4.2). According to Singh *et al*. (2011), absolute alcohol preserves the DNA, thus preventing mechanical shearing and fragmentation of DNA during isolation. However, larvae were placed in absolute (95%) ethanol prior to DNA isolation (Section 3.2). This implies that one of the steps in the isolation process causes mechanical shearing of the DNA. Shorter incubation time (less than one hour) in the lyses step should be considered. Isolated DNA in this present study was, however, of sufficient quantity and quality for successful amplification.

5.3. *Cytochrome b* analysis

The *cytochrome b* primer set (Section 3.4.) yielded the expected DNA fragment of 1000 bp for all the samples, although a second DNA fragment of ~500 bp was also visible in all the samples. The cycling conditions of Sezonlin *et al*. (2006) were only slightly adapted with an extension step at 72 °C for 2 min instead of 1 min 30 s. Further optimization of the cycling conditions may eliminate the non-specific PCR product (~500 bp).

The PCR products were sequenced by Inqaba Biotech (South Africa). The company first excised the expected fragments of 1000 bp and then purified it. BLAST searches with both forward and reverse sequences indicated that these were *cytochrome b* from *B. fusca*.

Consensus sequences were used for further analysis. The sequences were consistent with those of Sezonlin *et al*. (2006).

Cytochrome b is used for various phylogenetic analyses applications in insects (Sezonlin *et al*., 2012; Simmons & Weller, 2001), vertebrates (Farias *et al*., 2001; Parson *et al*., 2000), plants (Nanasato *et al*. 2005), bacteria (Baymann *et al*., 2012) and fungi (Yin *et al.*, 2012). Applications of the *cytochrome b* gene include: (i) analysis of molecular diversity of *B. fusca* populations in different geographical areas (Sezonlin *et al*., 2012); (ii) resolving phylogenetic relationships at different taxonomic levels (Farias *et al*., 2001; Simmons & Weller, 2001); (iii) identification of vertebrate speciesô biological origin within different vertebrate groups (Parson *et al*., 2000) and (iv) assessment of the evolutionary dynamics of *cytochrome b* gene introns in fungi (Yin *et al*., 2012).

5.4. Amplification of Cry receptor genes

In the present study multiple bands were obtained when degenerate primers were used. The low annealing temperature and other conditions were possibly responsible for the non-specific products (Degen *et al*., 2006; Riedel *et al*., 2012). Changes in the PCR conditions did not provide any improved solutions and it was decided that bands would be excised from the agarose gels and re-amplified. Hereafter all the fragments would then be sequenced.

Ziňtkiewicz *et al*. (1992) proposed that multiple bands are a result of simultaneous amplification from many genomic loci, which may also be the case in this present study. It was, however, necessary to design degenerate primers for this study in order to take into consideration the variable regions in the Cry receptor genes. Another reason for multiple bands may be the use of genomic DNA for PCR. Only mRNA sequences (introns excluded) of the respective receptor genes were available on GenBank and these were used in sequence alignments. Primers were designed based on conserved regions observed in protein sequence alignments of these genes. Thus introns were excluded from these alignments. It is then possible that these degenerate primers amplified regions containing introns. Allen and Mertens (2008) also observed multiple bands with

amplification of genomic DNA. These authors (Allen & Mertens, 2008) suggest that multiple isoforms or pseudogenes within the genome may also result in multiple bands. Multiple bands are therefore not uncommon when genomic DNA is used as template for amplification.

In the case where multiple bands were obtained, defined bands were excised and re-amplified according to the methods described in Section 3.6. Re-amplification was performed under the exact same cycling conditions using the respective amplification primers. In the events where single PCR amplicons were obtained after the first round of amplification, purification and sequencing of the PCR products could be performed immediately (Section 3.7). Re-amplification results are shown for *APN1* only (Figure 4.6).

Several studies have used PCR-based detection methods to discriminate between susceptible and resistant species (Wang *et al*., 2005; Zhang *et al*., 2009). Mutations in Cry receptor genes seem to occur frequently in lepidopteran species, but this is not the only mechanism of resistance. In *H. armigera* a mutation of the *APN1* isogene was associated with Cry1Ac resistance. Zhang *et al*. (2009) developed an allele-specific PCR protocol that could detect this mutation and differentiate between susceptible and resistant *H. armigera* strains. The primer set employed in this protocol of Zhang *et al*. (2009) yielded a single fragment of 270 bp for the susceptible strain and 204 bp for the resistant strain. This indicated a deletion of 66 bases (22 amino acids) which were responsible for the Cry1Ac resistance in *H. armigera*.

The only mutation in the *APN2* isogene that have been reported to date is a deletion outside the Cry1Ac binding site (Zhang *et al*., 2009). It was suggested that resistance is not conferred by this mutation. Six mutations have been identified in the *APN3* isogene from resistant *H. armigera* that could be associated with Cry1Ac resistance (Wang *et al*., 2005). The authors also concluded that, among the *APN3* isogenes, polymorphisms unrelated to resistance are also possible. No further studies could be found where mutations in the *APN3* isogene are implicated as a mechanism of resistance in lepidopteran pests. No studies could be found where mutations in the *APN4* isogene have been detected.

According to Baxter *et al*. (2011), the *APN5* isogene is not linked to the Cry1Ac resistance in *Bombyx mori* or *Plutella xylostella*. These authors also stated that this is most probably also the case in other lepidopteran species. According to Tiewsiri and Wang (2011), Cry1Ac resistance in *Trichoplusia ni* was caused by reduced expression of the *APN1* isogene as well as increased expression of the *APN6* isogene. In this case resistance was not due to mutations in receptor genes. These authors suggested that resistance was rather due to transcription regulation by means of an unidentified *trans*-regulatory mechanism. No studies could be found that demonstrated mutations occurring in the *APN6* isogene.

According to Gahan *et al*. (2001) Cry1Ac resistance in *Heliothis virescens* is caused by a single mutation in *cadherin*. This mutation is generated by the insertion of a transposable element. According to Morin *et al*. (2004) Cry1Ac resistance in *Pectinophora gossypiella* is linked with three alleles (r1, r2 and r3), each carrying a different mutation which codes for an incomplete cadherin protein. In that study, a PCR-based method was developed to distinguish between susceptible and resistant alleles. A goal of the present study was to determine sequences for *cadherin* for *B. fusca*. Some sequences specific for *B. fusca* were generated in the present study.

According to Jurat-Fuentes and Adang (2004) resistance in *H. virescens* also occurred when *alkaline phosphatase* was up- or down-regulated due to mutations. Reduced ALP activity correlated with resistance, although there was no reduced Cry1Ac binding (Jurat-Fuentes & Adang, 2006). According to Jurat-Fuentes *et al*. (2011), reduced ALP levels are common to lepidopteran species that are resistant to Cry toxins. Several studies have confirmed that reduced ALP activity correlated with Cry toxin resistance in pests (Fernandez *et al*., 2006; Hua *et al*., 2009; Jurat-Fuentes & Adang, 2007). There is, however, not a single study that attributes this resistance to mutations in the *alkaline phosphatase* receptor gene.

With all the multiple bands that were obtained with amplification of the respective Cry receptor genes, differences in fragment sizes were observed between the susceptible and

resistant *B. fusca* samples (fragments 4 and 7, 18 and 19/20, 23 and 24, etc.). It is, however, not possible to assign any of these differences to mutational events that resulted in resistance to Cry toxins.

Several additional studies concluded that an alteration in one or more Bt toxin-binding receptors confer Cry toxin resistance to species (Ferré *et al*., 1991; Forcada *et al*., 1999; Lee *et al*., 1995; Wright *et al*., 1997). These receptors have not previously been characterized for *B. fusca*. The present study is the first to generate some sequence data for *aminopeptidase* (isogenes 1 to 6), *cadherin* and *alkaline phosphatase*. More unidentified and uncharacterized Bt toxin-binding receptors, with one or more isogenes, may still exist.

5.5. Sequence analysis of PCR amplicons

5.5.1. *Cytochrome b*

Cytochrome b sequences obtained in this present study showed 99-100% sequence similarity to the same gene from *B. fusca* (Accession numbers AY769586.1 and AY769605.1). Expected (E) values were 0.0, thus signifying that the query sequences showed high similarity to the matched sequences in GenBank. Sequences from susceptible and resistant samples matched the *cytochrome b* gene of *B. fusca* haplotypes hapl51-k2 (Accession number AY769586.1) and hapl70-k2 (Accession number AY769605.1), respectively. These results were as expected, since Sezonlin *et al*. (2006) designed this primer set (Section 3.4) specifically for the *cytochrome b* gene of *B. fusca*.

These two matched haplotypes correspond to the KII clade produced in phylogenetic analysis done by Sezonlin *et al*. (2006). In this study, *B. fusca* populations from Central to East Africa clustered together in this clade. It is then reasonable for the susceptible samples to match the haplotype in clade KII, since these samples originated from Kenya (Section 3.1). It is, however, unusual that the resistant samples, which originated from the Vaalharts area, also match a haplotype in this same clade. A possible reason for the similar match obtained with the susceptible and resistant samples, may be the absence of

southern African *B. fusca* population representatives in the phylogenetic study done by Sezonlin *et al*. (2006).

The *cytochrome b* sequences generated in the present study are further discussed in the phylogenetic analysis section (Section 5.6.1).

5.5.2 Cry receptor genes

Sequences of PCR amplicons confirmed that these were indeed regions of the respective Cry receptor genes from lepidopteran species. Overall, 70-100% sequence similarity and Expected (E) values of <0-1.1 were obtained for these sequences (Table 4.2). This signifies that some query sequences showed a high similarity to the matched sequences on GenBank (for E values below 0), whereas others showed only moderate similarity (for E values between 0 and 1.1).

For *APN1*, fragment 3 from the susceptible *H. armigera* samples showed 99-100% sequence similarity to the *APN1* gene of the same species. These results were as expected. Similarities to *APN1* were, however, found with both the susceptible and resistant *H. armigera* strain. This implies that the amplified region does not extend over the mutation in *APN1* that has been described by Zhang *et al*. (2009). Percentage query coverage was low (11%) for the forward sequence of fragment 3, but higher (84%) for the reverse sequence of the same fragment. An E value of 0.084 was obtained with the forward sequence of fragment 3, which indicates that similarities between this sequence and the matched sequences are low. Fragments (4, 5 and 6) from the susceptible *B. fusca* samples showed 74-99% sequence similarity to *APN1* from both the susceptible and resistant *H. armigera* strain. Query coverage was higher (34-81%) for the sequences of fragment 5. All the sequences discussed in this section showed closest similarities to those from the Zhang *et al*. (2009) study.

For fragments 6 and 7, query coverage of 15%-28% was obtained. Fragment 7 from the resistant *B. fusca* sample showed a 79% sequence similarity to *APN1* from *Spodoptera exigua* (Herrero *et al*., 2005). This species also belong to the same order (Lepidoptera) and

family (Noctuidae) as *B. fusca*. Query coverage for this sequence was only 28%. According to Herrero *et al*. (2005) this *S. exigua* strain was Cry1Ca-resistant and lacked the expression of one of four *APN* genes.

For *APN2*, only fragment 10 from the susceptible *H. armigera* samples showed similarity to *APN*. Sequence similarity was, however, found with the *APN4* isogene of *H. armigera* (Wang *et al*., 2002). Sequence similarity and query coverage for fragment 10 were 87% and 57% for the forward sequence, and 99% and 70% for the reverse sequence, respectively. High values like these are expected for the *H. armigera* samples, because sequence data of receptor protein genes in this species are available on GenBank.

For *APN3*, only fragment 22 from the susceptible *H. armigera* samples showed similarity to *APN*. Very high sequence similarity (91% and 96%) was found with the same isogene of *H. armigera* (Su *et al*., 2003), as expected. Query coverage was, however, quite low (18% and 30%). This implies either that the primer set employed only amplified a small region of the *APN3* gene, or that introns are present between the priming sites of these primers. Sequences for *APN2* and *APN3* were not obtained for *B. fusca*.

For *APN4*, fragments from both susceptible (fragment 23) and resistant (fragment 24) *B. fusca* samples showed sequence similarity to *APN*. These sequences were similar to *APN1* in *H. armigera* (Zhang *et al*., 2009), *APN8* in *Mamestra configurata* (Toprak *et al*., 2010) and *APN4* in *Bombyx mori* (Wang *et al*., 2011). These species also belong to the same order and family as *B. fusca* (Lepidoptera: Noctuidae). Although sequence similarities were high for both samples (70%-93%), query coverage was intermediate (56%) and very low (6%-14%) for the susceptible (fragment 23) and resistant (fragment 24) *B. fusca* samples, respectively. A high E value (0.68) was obtained for the forward sequence of fragment 24, indicating that the similarity found with isogene *APN8* from *M. configurata* (Toprak *et al*., 2010) was low. These findings also indicate either that this gene was only partially amplified or that introns were present between the priming sites of this particular primer set.

For *APN5*, fragment 25 failed to sequence. Sequencing of fragment 26 was successful, but no similarities to *APN* were found. Thus no results are indicated for this gene in Table 4.2.

For *APN6*, two fragments (27 and 28) showed sequence similarity to the same isogene from *H. armigera*. Fragment 27 (from susceptible *H. armigera* samples) showed 96%-98% sequence similarity to the same isogene in *H. armigera* (Angelucci *et al*., 2008). Query coverage was 64%-66%, indicating that quite a large region of this isogene was amplified. These high values are expected for the *H. armigera* samples. Fragment 28 (from susceptible *B. fusca* samples) also showed sequence similarity (79%-82%) to the same isogene from *H. armigera* (Angelucci *et al*., 2008). Query coverage was moderate (44%) for the forward sequence, but low (22%) for the reverse sequence of this fragment. These values indicate that at least a partial region of *APN6* was amplified for both *H. armigera* and *B. fusca*. According to Angelucci *et al*. (2008) this isogene (*APN6*) of *H. armigera* was expressed at slightly lower levels than *APN1-4*. However, a study by Tiewsiri and Wang (2011) showed that *APN1* was down-regulated in *Trichoplusia ni*, while *APN6* was up-regulated. The study of Tiewsiri and Wang (2011) indicated that Cry1Ac resistance in *Trichoplusia ni* (reared in greenhouses) was, however, associated with the down-regulation of *APN1*, but not with the up-regulation of *APN6*.

Re-amplification, and thus also sequencing, of fragments obtained with the amplification of *cadherin* failed many times. The only sequence data that could be generated are indicated in Table 4.2. Sequences of three fragments (39, 46 and 62) from susceptible and resistant *B. fusca* samples did not match the *cadherin* gene from any lepidopteran species. These sequences did, however, show similarities to microsatellite sequences from *B. fusca* (AY884601.1: Faure & Silvain, 2005; DQ393654.1: Meglecz *et al*., 2006). Sequence similarity was 97%-100%, while query coverage was only 9%-21%. At the least, this indicates that these particular primer sets bound successfully to genomic DNA from *B. fusca*. Faure and Silvain (2005) successfully characterized eight microsatellite loci in *B. fusca*, which were used as markers to study the genetic variability within and between *B. fusca* populations in sub-Saharan Africa. The authors (Faure & Silvain, 2005) did not indicate from which geographical region the specific *B. fusca* population, containing the

microsatellite sequence (AY884601.1), was collected. These BLAST results, however, indicate that the resistant *B. fusca* samples (from the Vaalharts area) used in the present study, are similar to other *B. fusca* populations in sub-Saharan Africa.

Alkaline phosphatase was successfully re-amplified and sequenced. Sequences of several fragments from susceptible *H. armigera* and susceptible and resistant *B. fusca* samples showed high similarities to two *alkaline phosphatase* isogenes. Most similarities were found with *ALP1* and *ALP2* from *H. armigera* (Ning *et al*., 2010). Ning *et al*. (2010) successfully characterized two *ALP* isogenes in the midgut of *H. armigera*. *Alkaline phosphatase* (*ALP*) has previously only been proposed as a receptor for Cry toxins (Jurat-Fuentes & Adang, 2004; McNall & Adang, 2003). Ning *et al*. (2010) identified ALP as a receptor for Cry1Ac toxins (based on their study as well as other studies), and suggested that ALP is involved in the mode of action of Cry1Ac. There were also similarities to *ALP* in *B. mori* (Nishimura, *et al*., 2008) and *M. configurata* (Toprak *et al*., 2010). For all the sequences, high sequence similarities (74%-98%) were obtained. Query coverage was very low (12%-30%) for all the sequences, except for sequences of fragment 91. For this fragment (from susceptible *H. armigera* samples) query coverage was high (89%-100%) together with high percentage similarity (78%-84%). Thus a large region of the *ALP* gene was amplified in *H. armigera*. Sequences of fragment 87 showed similarities (74%-75%) to cadherin of *H. armigera* (Su & Shen, 2004). Query coverage was low (25%-30%) for these sequences.

The Cry receptor gene sequences generated in this present study (Table 4.2) thus illustrate that the degenerate primers were successful for the amplification of several Cry receptor genes. These genes include six *aminopeptidase* isogenes, *cadherin* and *alkaline phosphatase*. Even though query coverage was low for most of these sequences, high sequence similarities were obtained. Sequence data can now be used to design primers for these Cry receptor genes that are specific for *B. fusca*. Even though several Cry receptor genes exist, *APN*, *cadherin* and *ALP* are the major genes that have been implicated in resistance development among pests (Jurat-Fuentes *et al*., 2011; Morin *et al*., 2003; Zhang *et al*., 2009). Subsequently only these genes were investigated in the present study. Cry

receptor gene sequences generated in this study were further subjected to phylogenetic analysis (Section 5.6.2).

5.6. Phylogenetic analysis

5.6.1. *Cytochrome b*

According to Hampl *et al*. (2001) a phylogenetic tree reflects the evolution of a particular gene, based on molecular data. In molecular phylogenetics sequence information is converted to an evolutionary tree. This is based on similarities and/or differences between the sequences (Wright, 2011). In the present study, the *cytochrome b* gene was investigated to determine the phylogenetic relationship between *B. fusca* and other lepidopteran species. Figure 4.9 shows a bootstrap neighbor-joining phylogenetic tree that was constructed with the obtained *cytochrome b* sequences from *B. fusca* (Section 4.4) and 56 other lepidopteran species. Bootstrapping tests the reliability of a dataset, while neighbor-joining creates an unrooted tree (Aravind *et al*., 1998). This phylogenetic tree (Figure 4.9) thus indicates the evolutionary relationship of the obtained sequences and *cytochrome b* gene sequences of various lepidopteran species.

Species of the same genus formed single clusters, as illustrated in Figure 4.9. Even though no outgroups were specified in this analysis, *Bombyx mori* (gi: 33694269) and *Chelonus insularis* (gi: 255762233) seemed to be outgroups compared to the other lepidopteran species. Separate sub-clusters were produced by the susceptible and resistant *B. fusca* samples. These sub-clusters are supported by bootstrap values between 49% and 91%. The *B. fusca cytochrome b* gene sequence from GenBank (gi: 82623153) clustered together with the sub-cluster of resistant samples with a 41% bootstrap support. This pattern of clustering was thought to be odd. According to a phylogenetic study done by Sezonlin *et al*. (2006), *B. fusca* populations from sub-Saharan Africa clustered into three main groups according to their geographical differences. These groups were W (populations from West Africa), KI (East Africa) and KII (Central to East Africa). Thus it was expected that the *B. fusca cytochrome b* gene sequence from GenBank (gi: 82623153) would cluster together with the susceptible samples (which originated from Kenya).

Sezonlin *et al*. (2006), however, did not include *B. fusca* population representatives from southern Africa. This explains clustering patterns observed in the present study. The phylogenetic relationship of the susceptible and resistant *B. fusca* representatives is supported by a 100% bootstrap value.

The rate of evolution in silent codon positions of the *cytochrome b* gene is relatively fast (Irwin *et al*., 1991). This makes the gene useful for studying genetic variation between insect populations (Boudabous *et al*., 2011). Lohman *et al*. (2008) used this gene to study genetic variation in the lepidopteran species *Lampides boeticus*. This gene was also used by Arias *et al*. (2005) to study genetic variation in the dipteran species *Anopheles albimanus*. Even though deeper evolutionary studies are limited by base compositional biases and limited variation in the first two codon positions of the *cytochrome b* gene, this gene is still useful for recent evolutionary inferences (Farias *et al*., 2001).

Phylogenetic analysis performed on the *cytochrome b* gene in this present study demonstrated that *B. fusca* is more closely related to species of the genus *Helicoverpa*. It is suggested that phylogenetic analysis be repeated with a bigger sample size from different geographic areas to determine molecular variations that arise in geographically separated populations. To strengthen this inferred phylogenetic relationship between *B. fusca* and *H. armigera*, it is suggested that more genes (such as actin and cytochrome c oxidase I and II) are also included.

5.6.2 Cry receptor genes

In the present study, receptor protein genes were investigated in order to detect possible mutations. This was achieved by designing degenerate primers that would amplify *aminopeptidase* (isogenes 1 to 6), *cadherin* and *alkaline phosphatase* receptor protein genes. Phylogenetic analysis made use of the sequences obtained for these genes from *H. armigera* and *B. fusca* to evaluate the phylogenetic relationship of these genes between *B. fusca* and other lepidopteran species. Figure 4.11 indicates bootstrap neighbor-joining phylogenetic trees of the respective receptor protein genes.

The unexpected clustering of 3F (Figure 4.11a) may be due to the low query coverage (11%) of this sequence (Table 4.2) compared to that (84%) of 3R, which clustered with *H. armigera* as expected. A similar clustering pattern was observed for the consensus sequence of fragment 22 (Figure 4.11c). Query coverage was, again, very low (18%-30%) for this consensus sequence (Table 4.2). However, the clustering pattern observed for sequences (5F, 5R and 7F) of *B. fusca* indicate a phylogenetic relationship (with bootstrap support of 47%) between *B. fusca* and *S. exigua* for the *APN1* receptor protein gene (Figure 4.11a). Similar results were observed for *APN4* (Figure 4.11d), where the relationship between a *B. fusca APN4* sequence (Consensus 24) and that of *P. xylostella* was supported by a bootstrap value of 92%.

For *APN2* and *APN3*, sequences could not be generated for *B. fusca* and thus no phylogenetic relationships could be inferred. Even though sequences could be generated for *APN6* from *B. fusca*, the clustering of these sequences with those of other lepidopterans was inaccurate (Figure 4.11e). These results were a bit puzzling, because query coverage was generally high (44%-66%) for three of the four sequences. Query coverage was only low (21%) for 28R (Table 4.2). The clustering pattern observed for *cadherin* (Figure 4.11f) was expected, because the sequences used did not show any homology with *cadherin* from any other lepidopteran species. These sequences did, however, show sequence similarity to *B. fusca* microsatellite sequences, which is plausible since these fragments were derived from amplification of *B. fusca* genomic DNA.

A similar study was done by Wang *et al*. (2005), wherein degenerate primers were used to amplify *APN3* in *H. armigera*. In that study, cDNA encoding *APN3* was cloned using degenerate PCR and RACE (Rapid Amplification of cDNA Ends) techniques. These authors (Wang *et al*., 2005) compared mutations and expression levels of the *APN3* gene from both Bt-susceptible and -resistant *H. armigera* strains. First, a multiple sequence alignment was prepared by using *APN3* amino acid sequences of six lepidopteran insects (*H. virescens*, *H. armigera*, *L. dispar*, *S. litura*, *M. sexta* and *B. mori*). Degenerate primers were then designed according to the characteristics of conservative regions.

These degenerate primers were used to amplify complementary DNA (cDNA) synthesized from *H. armigera* messenger RNA (mRNA). PCR sequences were determined and subsequently used to design specific primers to amplify fragments of the *APN3* gene. Sequences of the subsequent PCR products were, again, determined and used to design specific primers for RACE. Finally, specific primers were designed based on RACE products. These specific primers could amplify the full-length open reading frame (ORF) of *APN3* from *H. armigera*.

This approach can be used for the investigation of receptor protein genes from *B. fusca*. However, this is a lengthy process, but not unattainable. With this approach full-length sequence data can thus be generated for each receptor protein gene investigated in the present study, and even more. If mutations are then present in any of these genes, these could be detected by comparing the full-length receptor protein gene sequences of Bt-susceptible and -resistant *B. fusca* strains. However, mutations in receptor protein genes are not the only mechanism of resistance in pests. For resistance caused by down- or up-regulation of receptor protein genes, transcriptomic and proteomic analyses are essential. Ultimately, genome sequencing can provide the much-needed molecular data to study genomic and transcriptomic variations in Bt-susceptible and -resistant *B. fusca* populations.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The aim of this study was to investigate possible mutations in Cry receptor genes from *Busseola fusca* potentially involved in Bt-resistance by using a PCR method.

In order to achieve the aim of this study, five specific objectives were formulated. For each objective trends and conclusions will be subsequently discussed.

6.1.1. DNA isolation

DNA was successfully isolated from *H. armigera* and *B. fusca* larvae using the NucleoSpin® Tissue Kit (Macherey-Nagel, Germany). Good quality DNA could be isolated in high quantities, which was sufficient for PCR amplification. It was, however, observed that use of liquid nitrogen resulted in DNA fragmentation. Thus methods employing liquid nitrogen are too harsh for isolation of DNA from *B. fusca* larvae.

6.1.2. Design of primers for Cry receptor genes

Degenerate primers were designed for the Cry receptor genes *aminopeptidase N* (isogenes 1 to 6), *cadherin* and *alkaline phosphatase*. Primers were designed based on conserved regions observed in the protein sequence alignments of the Cry receptor genes (Appendix A). These primers successfully amplified Cry receptor genes from *H. armigera* and *B. fusca*.

6.1.3. Amplification of Cry receptor genes

Cry receptor genes were successfully amplified from *H. armigera* and *B. fusca* using the degenerate primers. Multiple fragments were, however, obtained due to non-specific binding of these primers. Nevertheless, degenerate primers were required in order to take

into consideration variable regions present in Cry receptor genes across several lepidopteran species (Appendices A and B).

6.1.4. Sequence analysis of PCR amplicons

Sequences of some Cry receptor gene PCR amplicons were successfully determined. High sequence similarities were obtained between the query sequences and the matched sequences on GenBank. Thus new molecular data regarding Cry receptor genes were generated for *B. fusca*, which is not available elsewhere.

6.1.5. Investigation of mutations

Identification of mutations was not possible at this stage due to the difficulties experienced with the detection of the Cry receptor genes in *B. fusca*. In order to identify mutations, complete gene sequences of Cry receptor genes are required. It is also not certain that mutations are indeed present in the Cry receptor genes from *B. fusca*, since resistance can result from many mechanisms. It is, however, important to investigate for potential mutations in order to determine the mechanism by which *B. fusca* resistance to Cry1Ab toxin is conferred.

Due to failed attempts to amplify Cry receptor genes from *B. fusca*, a *cytochrome b* PCR was included as a control to verify that the isolated DNA was indeed amplifiable. The expected fragment size of 1000 bp was obtained for both susceptible and resistant *B. fusca* samples. These fragments were subsequently sequenced and BLAST searches were performed. BLAST results confirmed that the obtained sequences were indeed partial regions of the *cytochrome b* gene of *B. fusca*. These sequences were used in phylogenetic analysis. Phylogenetic analysis demonstrated that *B. fusca* was more closely related to *Helicoverpa* spp., which supports the approach of this present study. The approach of this study was based on the mechanism of *H. armigera* resistance to Cry1Ac. These additional results also provide novel information regarding the relationship between *B. fusca* and other lepidopteran species, which was previously not available.

6.2 Recommendations

The outcomes of the present study have resulted in the following recommendations:

(1) It is suggested that larvae should first be preserved through fixing in absolute alcohol and then incubated with lysis buffer for less than one hour. If mechanical shearing and fragmentation of DNA still occur, an alternative DNA isolation kit, such as the QAIGEN kit, should be investigated.

(2) Sequence data obtained for the Cry receptor genes from *B. fusca* can now be used to design primers that are specific for *B. fusca*. In that way more complete sequences of the respective Cry receptor genes may be determined. The approach of Wang *et al.* (2005) may possibly be replicated for *B. fusca.*

(3) Optimization of the PCR composition and cycling conditions for the amplification of Cry receptor genes may thus result in more specific DNA fragments that could be used for the mutation study.

(4) Once complete gene sequences of Cry receptor genes are determined, possible mutations can be identified by comparing these genes between susceptible and resistant *B. fusca* strains.

(5) If possible mutations are identified; allele-specific PCR processes can be developed to distinguish between susceptible and resistant *B. fusca* strains. Such processes can also be used to determine the frequency of resistance in field populations.

(6) If the determination of complete Cry receptor gene sequences is still not adequate for the identification of possible mutations, the alternative will be to sequence the whole genome of *B. fusca*.

(7) A future study using *cytochrome b* sequences to analyze the molecular diversity of *B. fusca* populations in different geographical areas can be conducted. To achieve

this, a bigger sample size will be essential. It is also suggested that additional genes (such as actin and cytochrome c oxidase I and II) are included to strengthen population and phylogenetic relationship data.

(8) Whole genome sequence data will create various new possibilities for future studies on *B. fusca*. These include: (i) identification of genes involved in resistance pathways; (ii) proteomic and transcriptomic analyses of these and other genes in the biology of this pest; (iii) the development of strain-specific probes for routine detection of resistance alleles as well as (iv) linkage mapping to resistance loci.

(9) The information provided in the present study can be usefully employed in the development of an Integrated Pest Management (IPM) plan. This can be achieved by firstly determining the mechanism by which *B. fusca* has developed resistance to Cry1Ab. Strategies to combat development or spread of resistance in this pest can then be formulated. One such example is intelligent toxin stacking, where the same crop encodes for several Cry toxins. In this way resistant stem borers feeding on Bt crops can be eliminated effectively.

Four of the five objectives were successfully executed. Even though mutations could not be identified at this stage, new molecular data were generated for *B. fusca* which were not available previously. Significant phylogenetic relationships between *B. fusca* and other lepidopteran species were also established. The lack in molecular data for *B. fusca* is, however, a great hindrance in the control of this pest. Repetition of *cytochrome b* phylogenetic analysis may establish improved phylogenetic relationships for *B. fusca*. Replication of the approach of Wang *et al*. (2005) for *B. fusca* may possibly generate full-length sequences for the receptor genes that were investigated in this present study, which may then be used for mutation studies.

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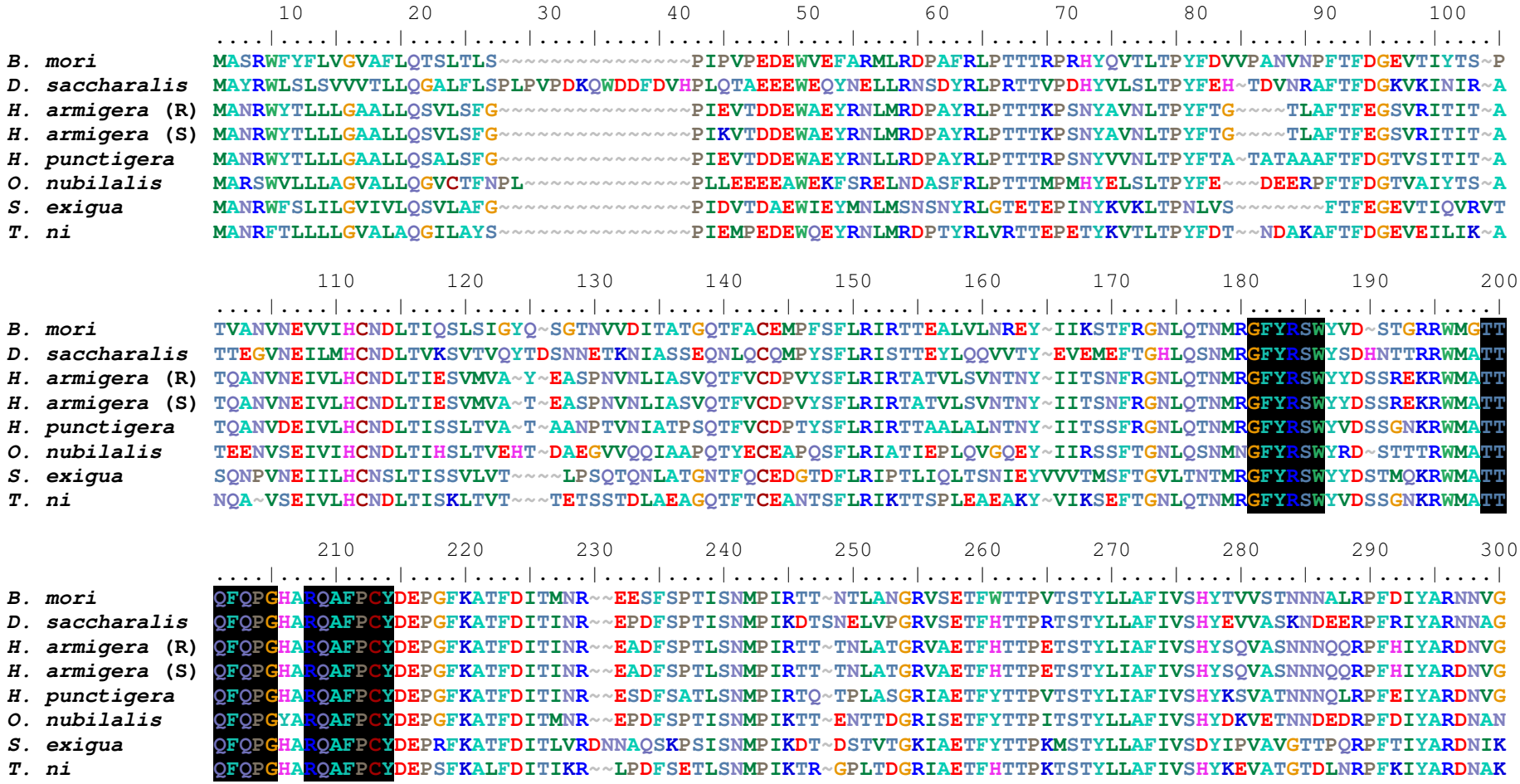
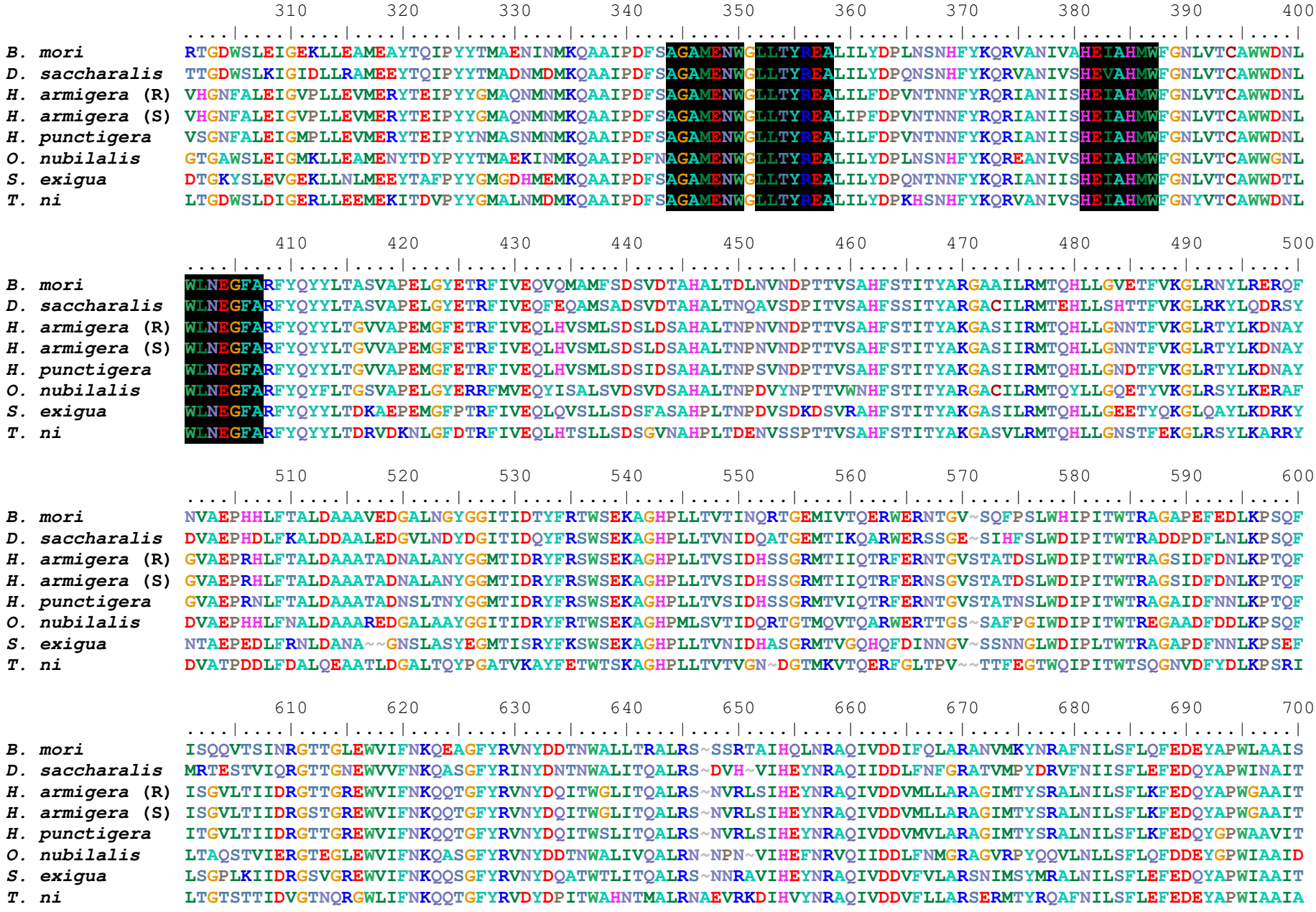
APPENDIX A

A.1. *Aminopeptidase N1* (*APN1*)




310 320 330 340 350 360 370 380 390 400
B. mori RTGDWSLEIGEKLLEAMEAYTQIPYYTMAENINMKQAAIPDFSAGAMENWGLLTYREALILYDPLNSNHFYKQRVANIVAHEIAHMWFGNLVTCAWWDNL
D. saccharalis TTGDWSLKIGIDLLRAMEEYTQIPYYTMADNMDMKQAAIPDFSAGAMENWGLLTYREALILYDPQNSNHFYKQRVANIVSHEVAHMWFGNLVTCAWWDNL
H. armigera (R) VHGNFALEIGVPLLEVMERYTEIPYYGMAQNMNMKQAAIPDFSAGAMENWGLLTYREALILFDPVNTNNFYRQRIANIISHEIAHMWFGNLVTCAWWDNL
H. armigera (S) VHGNFALEIGVPLLEVMERYTEIPYYGMAQNMNMKQAAIPDFSAGAMENWGLLTYREALIPFDPVNTNNFYRQRIANIISHEIAHMWFGNLVTCAWWDNL
H. punctigera VSGNFALEIGMPLLEVMERYTEIPYYNMASNMNMKQAAIPDFSAGAMENWGLLTYREALILFDPVNTNNFYKQRIANIISHEIAHMWFGNLVTCAWWDNL
O. nubilalis GTGAWSLEIGMKLLEAMENYTDYPYYTMAEKINMKQAAIPDFNAGAMENWGLLTYREALILYDPLNSNHFYKQREANIVSHEIAHMWFGNLVTCAWWGNL
S. exigua DTGKYSLEVGEKLLNLMEEYTAFPYYGMGDHMEMKQAAIPDFSAGAMENWGLLTYREALILYDPQNTNNFYKQRIANIISHEIAHMWFGNLVTCAWWDTL
T. ni LTGDWSLDIGERLLEEMEKITDVPYYGMALNMDMKQAAIPDFSAGAMENWGLLTYREALILYDPKHSNHFYKQRVANIVSHEIAHMWFGNYVTCAWWDNL
410 420 430 440 450 460 470 480 490 500
B. mori WLNEGFARFYQYYLTASVAPELGYETRFIVEQVQMAMFSDSVDTAHALTDLNVNDPTTVSAHFSTITYARGAAILRMTQHLLGVETFVKGLRNYLRERQF
D. saccharalis WLNEGFARFYQYYLTASVAPELGYETRFIVEQFEQAMSADSVDTAHALTNQAVSDPITVSAHFSSITYARGACILRMTEHLLSHTTFVKGLRKYLQDRSY
H. armigera (R) WLNEGFARFYQYYLTGVVAPEMGFETRFIVEQLHVSMLSDSLDSAHALTNPNVNDPTTVSAHFSTITYAKGASIIRMTQHLLGNNTFVKGLRTYLKDNAY
H. armigera (S) WLNEGFARFYQYYLTGVVAPEMGFETRFIVEQLHVSMLSDSLDSAHALTNPNVNDPTTVSAHFSTITYAKGASIIRMTQHLLGNNTFVKGLRTYLKDNAY
H. punctigera WLNEGFARFYQYYLTGVVAPEMGFETRFIVEQLHVSMLSDSIDSAHALTNPSVNDPTTVSAHFSTITYAKGASIIRMTQHLLGNDTFVKGLRTYLKDNAY
O. nubilalis WLNEGFARFYQYFLTGSVAPELGYERRFMVEQYISALSVDSVDSAHALTNPDVYNPTTVWNHFSTITYARGACILRMTQYLLGQETYVKGLRSYLKERAF
S. exigua WLNEGFARFYQYYLTDKAEPEMGFPTRFIVEQLQVSLLSDSFASAHPLTNPDVSDKDSVRAHFSTITYAKGASILRMTQHLLGEETYQKGLQAYLKDRKY
T. ni WLNEGFARFYQYYLTDRVDKNLGFDTRFIVEQLHTSLLSDSGVNAHPLTDENVSSPTTVSAHFSTITYAKGASVLRMTQHLLGNSTFEKGLRSYLKARRY
510 520 530 540 550 560 570 580 590 600
B. mori NVAEPHHLFTALDAAAVEDGALNGYGGITIDTYFRTWSEKAGHPLLTVTINQRTGEMIVTQERWERNTGV~SQFPSLWHIPITWTRAGAPEFEDLKPSQF
D. saccharalis DVAEPHDLFKALDDAALEDGVLNDYDGITIDQYFRSWSEKAGHPLLTVNIDQATGEMTIKQARWERSSGE~SIHFSLWDIPITWTRADDPDFLNLKPSQF
H. armigera (R) GVAEPRHLFTALDAAATADNALANYGGMTIDRYFRSWSEKAGHPLLTVSIDHSSGRMTIIQTRFERNTGVSTATDSLWDIPITWTRAGSIDFDNLKPTQF
H. armigera (S) GVAEPRHLFTALDAAATADNALANYGGMTIDRYFRSWSEKAGHPLLTVSIDHSSGRMTIIQTRFERNSGVSTATDSLWDIPITWTRAGSIDFDNLKPTQF
H. punctigera GVAEPRNLFTALDAAATADNSLTNYGGMTIDRYFRSWSEKAGHPLLTVSIDHSSGRMTVIQTRFERNTGVSTATNSLWDIPITWTRAGAIDFNNLKPTQF
O. nubilalis DVAEPHHLFNALDAAARDEDGALAAYGGITIDRYFRTWSEKAGHPMLSVTIDQRTGTMQVTQARWERTTGS~SAFPGIWDIPITWTREGAADFDDLKPSQF
S. exigua NTAEPEDLFRNLDANA~~GNSLASYEGMTISRYFKSWSEKAGHPLLTVNIDHASGRMTVGQHQFDINNGV~SSNNGLWDIPLTWTRAGAPDFNNLKPSEF
T. ni DVATPDDLFDALQEAATLDGALTQYPGATVKAYFETWTSKAGHPLLTVTVGN~DGTMKVTQERFGLTPV~~TTFEGTWQIPITWTSQGNVDFYDLKPSRI
610 620 630 640 650 660 670 680 690 700
B. mori ISQQVTSINRGTTGLEWVIFNKQEAGFYRVNYDDTNWALLTRALRS~SSRTAIHQLNRAQIVDDIFQLARANVMKYNRAFNILSFLQFEDEYAPWLAAIS
D. saccharalis MRTESTVIQRGTTGNEWVVFNKQASGFYRINYDNTNWALITQALRS~DVH~VIHEYNRAQIIDDLFNFGRATVMPYDRVFNIISFLEFEDQYAPWINAIT
H. armigera (R) ISGVLTIIDRGTTGREWVIFNKQQTGFYRVNYDQITWGLITQALRS~NVRLSIHEYNRAQIVDDVMLLARAGIMTYSRALNILSFLKFEDQYAPWGAAIT
H. armigera (S) ISGVLTIIDRGSTGREWVIFNKQQTGFYRVNYDQITWGLITQALRS~NVRLSIHEYNRAQIVDDVMLLARAGIMTYSRALNILSFLKFEDQYAPWGAAIT
H. punctigera ITGVLTIIDRGTTGREWVIFNKQQTGFYRVNYDQITWSLITQALRS~NVRLSIHEYNRAQIVDDVMVLARAGIMTYSRALNILSFLKFEDQYGPWAAVIT
O. nubilalis LTAQSTVIERGTEGLEWVIFNKQASGFYRVNYDDTNWALIVQALRN~NPN~VIHEFNRVQIIDDLFNMGRAGVRPYQQVLNLLSFLQFDDEYGPWIAAID
S. exigua LSGPLKIIDRGSVGREWVIFNKQQSGFYRVNYDQATWTLITQALRS~NNRAVIHEYNRAQIVDDVFVLARSNIMSYMRALNILSFLEFEDQYAPWIAAIT
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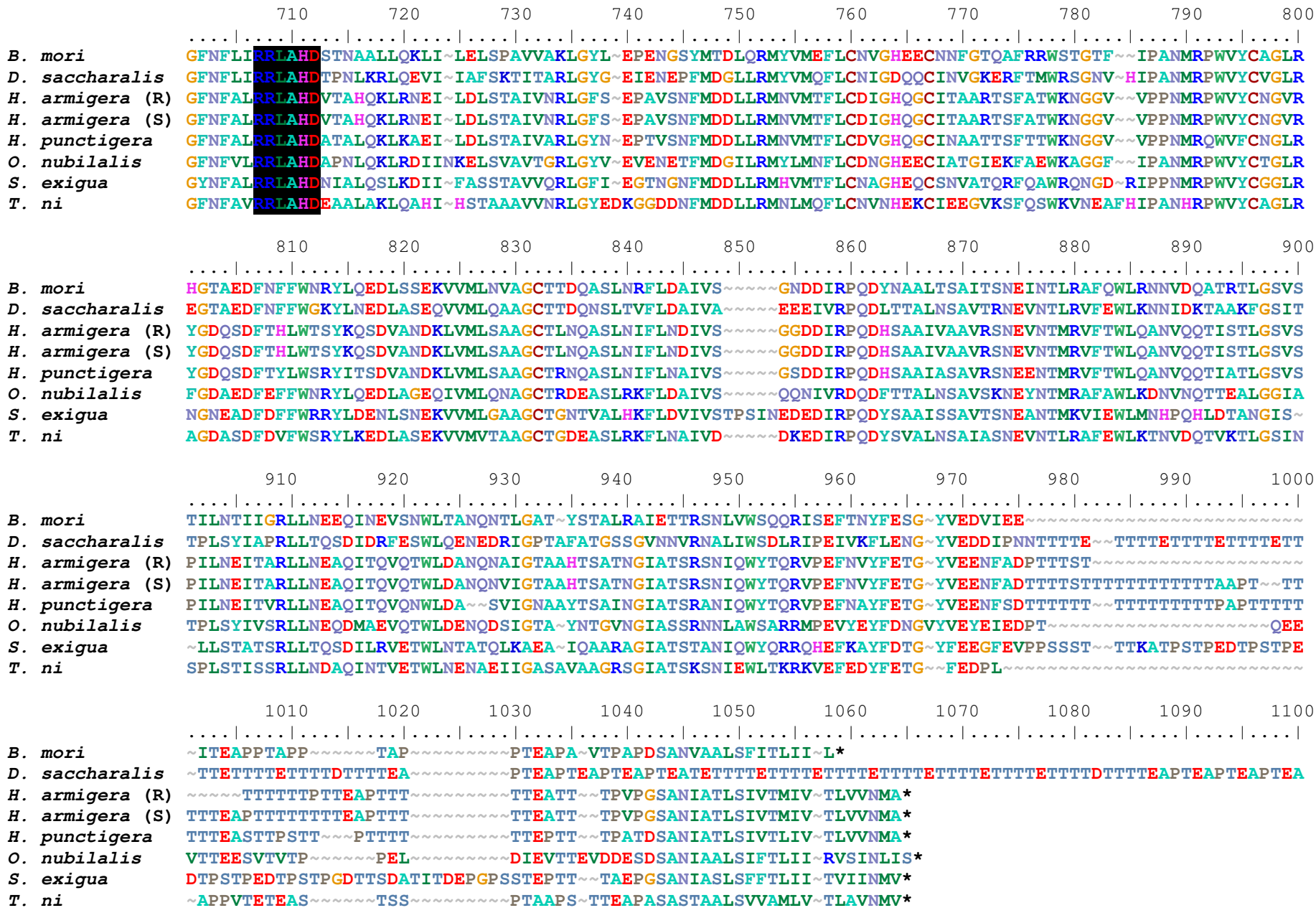
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D. saccharalis GFNFLIRRLAHDTPNLKRLQEVI~IAFSKTITARLGYG~EIENEPFMDGLLRMYVMQFLCNIGDQQCINVGKERFTMWRSGNV~HIPANMRPWVYCVGLR
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H. armigera (S) GFNFALRRLAHDVTAHQKLRNEI~LDLSTAIVNRLGFS~EPAVSNFMDDLLRMNVMTFLCDIGHQGCITAARTSFATWKNGGV~~VPPNMRPWVYCNGVR
H. punctigera GFNFALRRLAHDATALQKLKAEI~LDLSTAIVARLGYN~EPTVSNFMDDLLRMNVMTFLCDVGHQGCINAATTSFTTWKNGGV~~VPPNMRQWVFCNGLR
O. nubilalis GFNFVLRRLAHDAPNLQKLRDIINKELSVAVTGRLGYV~EVENETFMDGILRMYLMNFLCDNGHEECIATGIEKFAEWKAGGF~~IPANMRPWVYCTGLR
S. exigua GYNFALRRLAHDNIALQSLKDII~FASSTAVVQRLGFI~EGTNGNFMDDLLRMHVMTFLCNAGHEQCSNVATQRFQAWRQNGD~RIPPNMRPWVYCGGLR
T. ni GFNFAVRRLAHDEAALAKLQAHI~HSTAAAVVNRLGYEDKGGDDNFMDDLLRMNLMQFLCNVNHEKCIEEGVKSFQSWKVNEAFHIPANHRPWVYCAGLR
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B. mori HGTAEDFNFFWNRYLQEDLSSEKVVMLNVAGCTTDQASLNRFLDAIVS~~~~~GNDDIRPQDYNAALTSAITSNEINTLRAFQWLRNNVDQATRTLGSVS
D. saccharalis EGTAEDFNFFWGKYLNEDLASEQVVMLQAAGCTTDQNSLTVFLDAIVA~~~~~EEEIVRPQDLTTALNSAVTRNEVNTLRVFEWLKNNIDKTAAKFGSIT
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H. armigera (S) YGDQSDFTHLWTSYKQSDVANDKLVMLSAAGCTLNQASLNIFLNDIVS~~~~~GGDDIRPQDHSAAIVAAVRSNEVNTMRVFTWLQANVQQTISTLGSVS
H. punctigera YGDQSDFTYLWSRYITSDVANDKLVMLSAAGCTRNQASLNIFLNAIVS~~~~~GSDDIRPQDHSAAIASAVRSNEENTMRVFTWLQANVQQTIATLGSVS
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S. exigua NGNEADFDFFWRRYLDENLSNEKVVMLGAAGCTGNTVALHKFLDVIVSTPSINEDEDIRPQDYSAAISSAVTSNEANTMKVIEWLMNHPQHLDTANGIS~
T. ni AGDASDFDVFWSRYLKEDLASEKVVMVTAAGCTGDEASLRKFLNAIVD~~~~~DKEDIRPQDYSVALNSAIASNEVNTLRAFEWLKTNVDQTVKTLGSIN
910 920 930 940 950 960 970 980 990 1000
B. mori TILNTIIGRLLNEEQINEVSNWLTANQNTLGAT~YSTALRAIETTRSNLVWSQQRISEFTNYFESG~YVEDVIEE~~~~~~~~~~~~~~~~~~~~~~~~~~~~
D. saccharalis TPLSYIAPRLLTQSDIDRFESWLQENEDRIGPTAFATGSSGVNNVRNALIWSDLRIPEIVKFLENG~YVEDDIPNNTTTTE~~TTTTETTTTETTTTETT
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H. armigera (S) PILNEITARLLNEAQITQVQTWLDANQNVIGTAAHTSATNGIATSRSNIQWYTQRVPEFNVYFETG~YVEENFADTTTTSTTTTTTTTTTTTTAAPT~~TT
H. punctigera PILNEITVRLLNEAQITQVQNWLDA~~SVIGNAAYTSAINGIATSRANIQWYTQRVPEFNAYFETG~YVEENFSDTTTTTT~~TTTTTTTTTPAPTTTTT
O. nubilalis TPLSYIVSRLLNEQDMAEVQTWLDENQDSIGTA~YNTGVNGIASSRNNLAWSARRMPEVYEYFDNGVYVEYEIEDPT~~~~~~~~~~~~~~~~~~~~QEE
S. exigua ~LLSTATSRLLTQSDILRVETWLNTATQLKAEA~IQAARAGIATSTANIQWYQRRQHEFKAYFDTG~YFEEGFEVPPSSST~~TTKATPSTPEDTPSTPE
T. ni SPLSTISSRLLNDAQINTVETWLNENAEIIGASAVAAGRSGIATSKSNIEWLTKRKVEFEDYFETG~~FEDPL~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
B. mori ~ITEAPPTAPP~~~~~~TAP~~~~~~~~~PTEAPA~VTPAPDSANVAALSFITLII~L*
D. saccharalis ~TTETTTTETTTTDTTTTEA~~~~~~~~~PTEAPTEAPTEAPTEATETTTTETTTTETTTTETTTTETTTTETTTTETTTTDTTTTEAPTEAPTEAPTEA
H. armigera (R) ~~~~~TTTTTTPTTEAPTTT~~~~~~~~~TTEATT~~TPVPGSANIATLSIVTMIV~TLVVNMA*
H. armigera (S) TTTEAPTTTTTTTTTEAPTTT~~~~~~~~~TTEATT~~TPVPGSANIATLSIVTMIV~TLVVNMA*
H. punctigera TTTEASTTPSTT~~~PTTTT~~~~~~~~~TTEPTT~~TPATDSANIATLSIVTLIV~TLVVNMA*
O. nubilalis VTTEESVTVTP~~~~~~PEL~~~~~~~~~DIEVTTEVDDESDSANIAALSIFTLII~RVSINLIS*
S. exigua DTPSTPEDTPSTPGDTTSDATITDEPGPSSTEPTT~~TAEPGSANIASLSFFTLII~TVIINMV*
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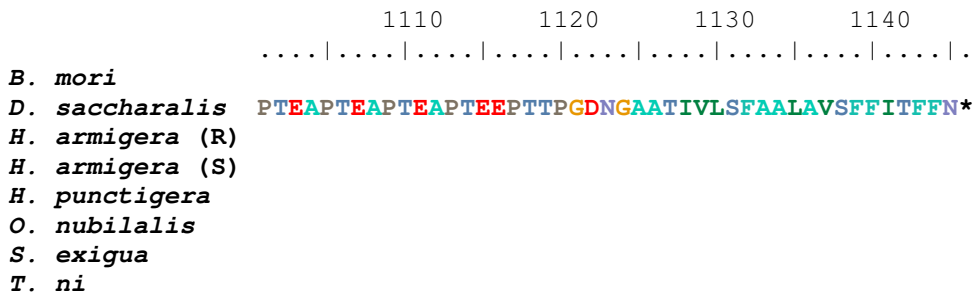
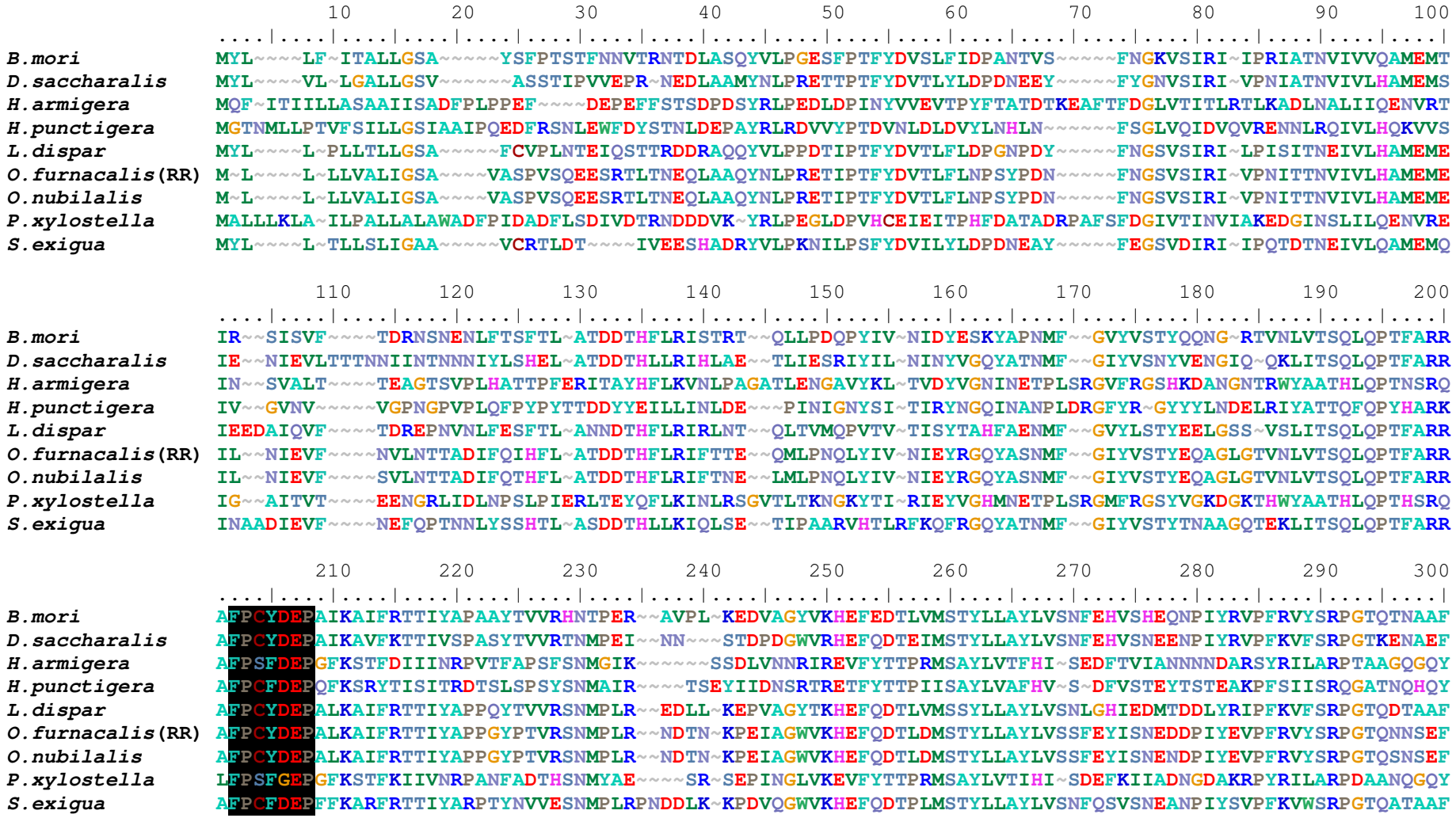
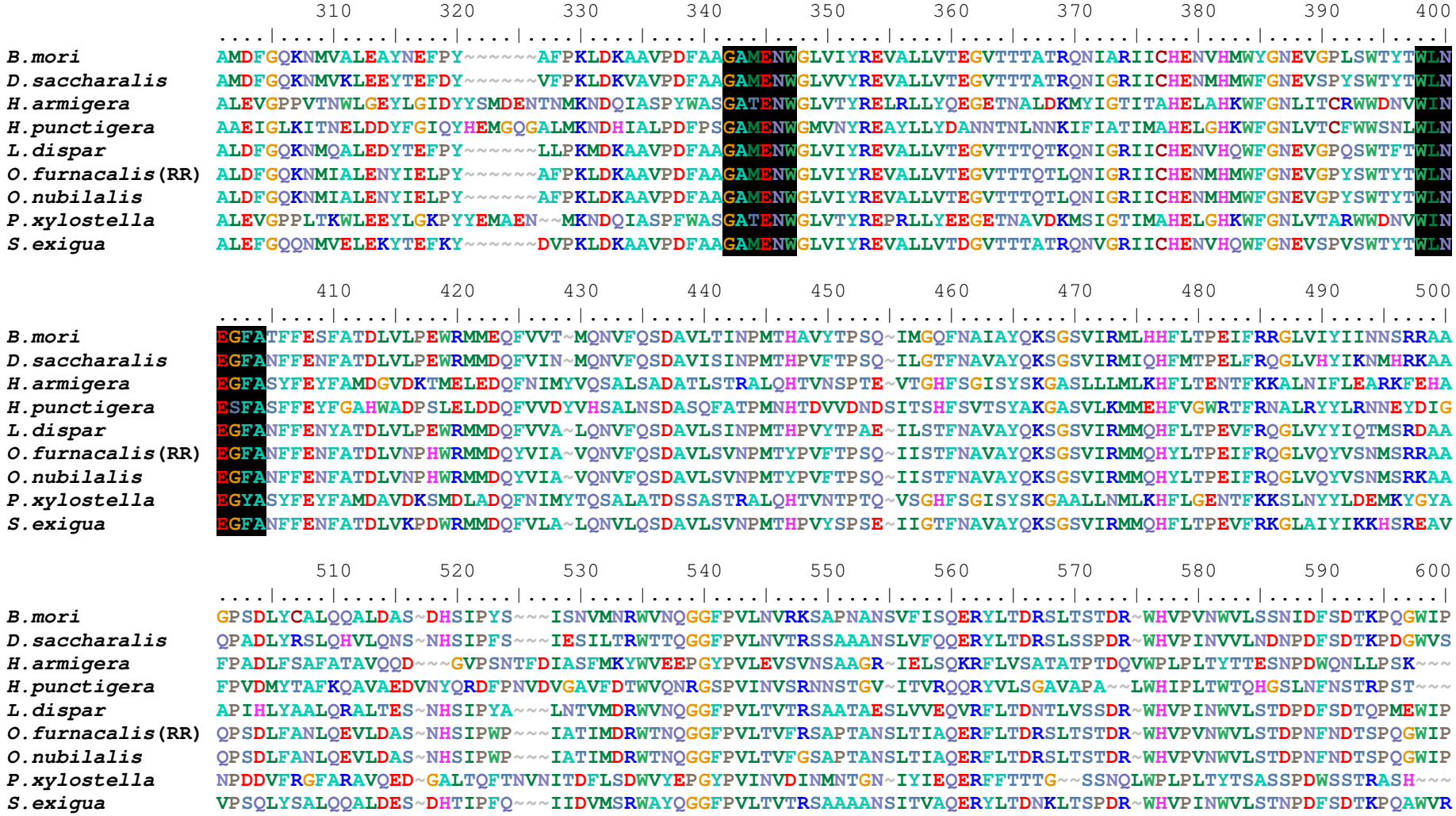


Figure A.1: Protein sequence alignment of *aminopeptidase N1* (*APN1*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (112983237),*Diatraea saccharalis* (302403438), *Helicoverpa armigera* (R) (184161311), *Helicoverpa armigera* (S) (184161309), *Helicoverpa punctigera* (7158839), *Ostrinia nubilalis* (215261001), *Spodoptera exigua* (37788335) and *Trichoplusia ni* (61200970).

A.2. *Aminopeptidase N2* (*APN2*)

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D.saccharalis AMDFGQKNMVKLEEYTEFDY~~~~~~VFPKLDKVAVPDFAAGAMENWGLVVYREVALLVTEGVTTTATRQNIGRIICHENMHMWFGNEVSPYSWTYTWLN
H.armigera ALEVGPPVTNWLGEYLGIDYYSMDENTNMKNDQIASPYWASGATENWGLVTYRELRLLYQEGETNALDKMYIGTITAHELAHKWFGNLITCRWWDNVWIN
H.punctigera AAEIGLKITNELDDYFGIQYHEMGQGALMKNDHIALPDFPSGAMENWGMVNYREAYLLYDANNTNLNNKIFIATIMAHELGHKWFGNLVTCFWWSNLWLN
L.dispar ALDFGQKNMQALEDYTEFPY~~~~~~LLPKMDKAAVPDFAAGAMENWGLVIYREVALLVTEGVTTTQTKQNIGRIICHENVHQWFGNEVGPQSWTFTWLN
O.furnacalis(RR) ALDFGQKNMIALENYIELPY~~~~~~AFPKLDKAAVPDFAAGAMENWGLVIYREVALLVTEGVTTTQTLQNIGRIICHENMHMWFGNEVGPYSWTYTWLN
O.nubilalis ALDFGQKNMIALENYIELPY~~~~~~AFPKLDKAAVPDFAAGAMENWGLVIYREVALLVTEGVTTTQTLQNIGRIICHENMHMWFGNEVGPYSWTYTWLN
P.xylostella ALEVGPPLTKWLEEYLGKPYYEMAEN~~MKNDQIASPFWASGATENWGLVTYREPRLLYEEGETNAVDKMSIGTIMAHELGHKWFGNLVTARWWDNVWIN
S.exigua ALEFGQQNMVELEKYTEFKY~~~~~~DVPKLDKAAVPDFAAGAMENWGLVIYREVALLVTDGVTTTATRQNVGRIICHENVHQWFGNEVSPVSWTYTWLN
410 420 430 440 450 460 470 480 490 500
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D.saccharalis EGFANFFENFATDLVLPEWRMMDQFVIN~MQNVFQSDAVISINPMTHPVFTPSQ~ILGTFNAVAYQKSGSVIRMIQHFMTPELFRQGLVHYIKNMHRKAA
H.armigera EGFASYFEYFAMDGVDKTMELEDQFNIMYVQSALSADATLSTRALQHTVNSPTE~VTGHFSGISYSKGASLLLMLKHFLTENTFKKALNIFLEARKFEHA
H.punctigera ESFASFFEYFGAHWADPSLELDDQFVVDYVHSALNSDASQFATPMNHTDVVDNDSITSHFSVTSYAKGASVLKMMEHFVGWRTFRNALRYYLRNNEYDIG
L.dispar EGFANFFENYATDLVLPEWRMMDQFVVA~LQNVFQSDAVLSINPMTHPVYTPAE~ILSTFNAVAYQKSGSVIRMMQHFLTPEVFRQGLVYYIQTMSRDAA
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P.xylostella EGYASYFEYFAMDAVDKSMDLADQFNIMYTQSALATDSSASTRALQHTVNTPTQ~VSGHFSGISYSKGAALLNMLKHFLGENTFKKSLNYYLDEMKYGYA
S.exigua EGFANFFENFATDLVKPDWRMMDQFVLA~LQNVLQSDAVLSVNPMTHPVYSPSE~IIGTFNAVAYQKSGSVIRMMQHFLTPEVFRKGLAIYIKKHSREAV
510 520 530 540 550 560 570 580 590 600
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D.saccharalis QPADLYRSLQHVLQNS~NHSIPFS~~~IESILTRWTTQGGFPVLNVTRSSAAANSLVFQQERYLTDRSLSSPDR~WHVPINVVLNDNPDFSDTKPDGWVS
H.armigera FPADLFSAFATAVQQD~~~GVPSNTFDIASFMKYWVEEPGYPVLEVSVNSAAGR~IELSQKRFLVSATATPTDQVWPLPLTYTTESNPDWQNLLPSK~~~
H.punctigera FPVDMYTAFKQAVAEDVNYQRDFPNVDVGAVFDTWVQNRGSPVINVSRNNSTGV~ITVRQQRYVLSGAVAPA~~LWHIPLTWTQHGSLNFNSTRPST~~~
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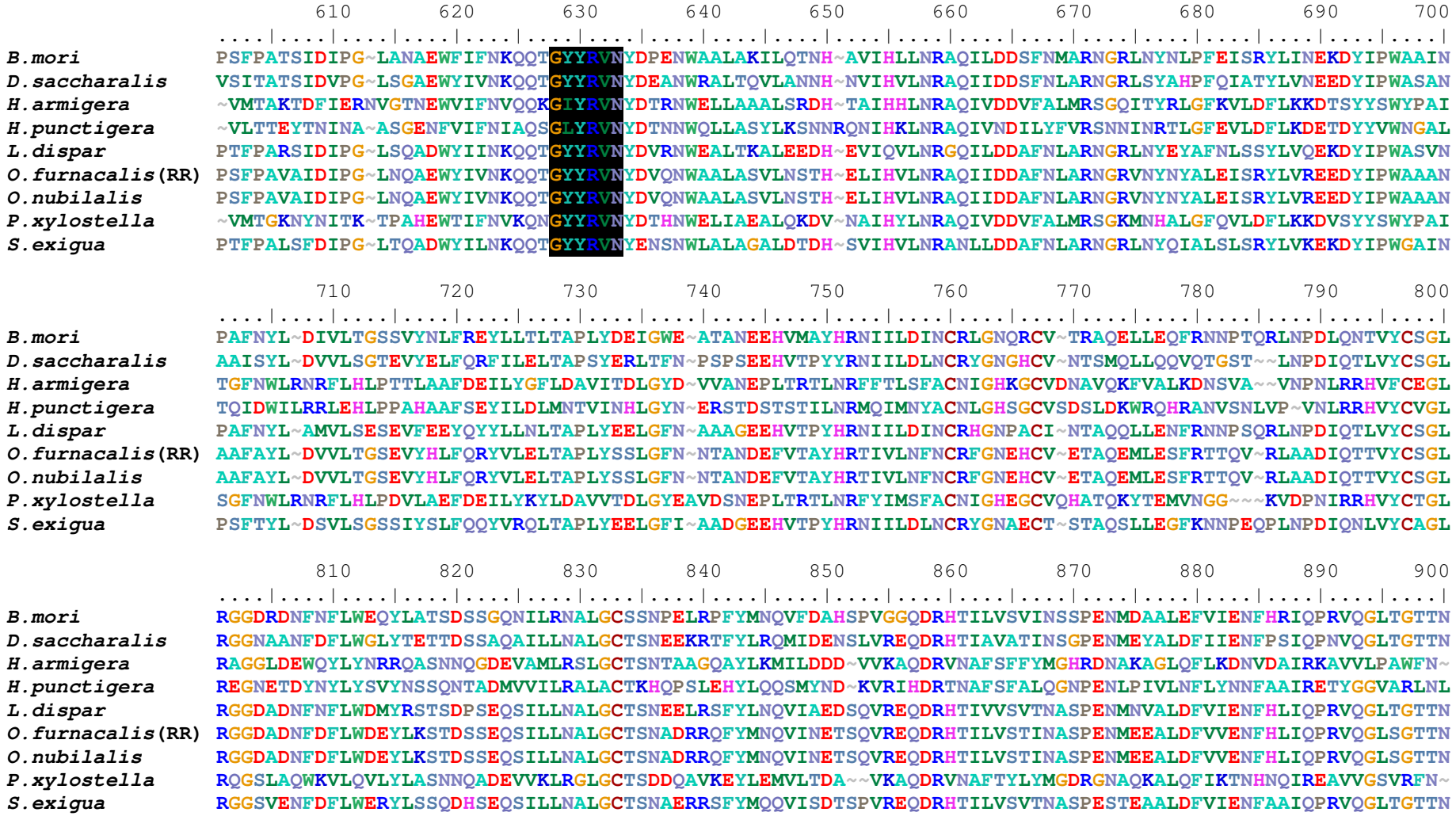
O.nubilalis QPSDLFANLQEVLDAS~NHSIPWP~~~IATIMDRWTNQGGFPVLTVFGSAPTANSLTIAQERFLTDRSLTSTDR~WHVPVNWVLSTDPNFNDTSPQGWIP
P.xylostella NPDDVFRGFARAVQED~GALTQFTNVNITDFLSDWVYEPGYPVINVDINMNTGN~IYIEQERFFTTTG~~SSNQLWPLPLTYTSASSPDWSSTRASH~~~
S.exigua VPSQLYSALQQALDES~DHTIPFQ~~~IIDVMSRWAYQGGFPVLTVTRSAAAANSITVAQERYLTDNKLTSPDR~WHVPINWVLSTNPDFSDTKPQAWVR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D.saccharalis VSITATSIDVPG~LSGAEWYIVNKQQTGYYRVNYDEANWRALTQVLANNH~NVIHVLNRAQIIDDSFNLARNGRLSYAHPFQIATYLVNEEDYIPWASAN
H.armigera ~VMTAKTDFIERNVGTNEWVIFNVQQKGIYRVNYDTRNWELLAAALSRDH~TAIHHLNRAQIVDDVFALMRSGQITYRLGFKVLDFLKKDTSYYSWYPAI
H.punctigera ~VLTTEYTNINA~ASGENFVIFNIAQSGLYRVNYDTNNWQLLASYLKSNNRQNIHKLNRAQIVNDILYFVRSNNINRTLGFEVLDFLKDETDYYVWNGAL
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O.furnacalis(RR) PSFPAVAIDIPG~LNQAEWYIVNKQQTGYYRVNYDVQNWAALASVLNSTH~ELIHVLNRAQIIDDAFNLARNGRVNYNYALEISRYLVREEDYIPWAAAN
O.nubilalis PSFPAVAIDIPG~LNQAEWYIVNKQQTGYYRVNYDVQNWAALASVLNSTH~ELIHVLNRAQIIDDAFNLARNGRVNYNYALEISRYLVREEDYIPWAAAN
P.xylostella ~VMTGKNYNITK~TPAHEWTIFNVKQNGYYRVNYDTHNWELIAEALQKDV~NAIHYLNRAQIVDDVFALMRSGKMNHALGFQVLDFLKKDVSYYSWYPAI
S.exigua PTFPALSFDIPG~LTQADWYILNKQQTGYYRVNYENSNWLALAGALDTDH~SVIHVLNRANLLDDAFNLARNGRLNYQIALSLSRYLVKEKDYIPWGAIN
710 720 730 740 750 760 770 780 790 800
B.mori PAFNYL~DIVLTGSSVYNLFREYLLTLTAPLYDEIGWE~ATANEEHVMAYHRNIILDINCRLGNQRCV~TRAQELLEQFRNNPTQRLNPDLQNTVYCSGL
D.saccharalis AAISYL~DVVLSGTEVYELFQRFILELTAPSYERLTFN~PSPSEEHVTPYYRNIILDLNCRYGNGHCV~NTSMQLLQQVQTGST~~LNPDIQTLVYCSGL
H.armigera TGFNWLRNRFLHLPTTLAAFDEILYGFLDAVITDLGYD~VVANEPLTRTLNRFFTLSFACNIGHKGCVDNAVQKFVALKDNSVA~~VNPNLRRHVFCEGL
H.punctigera TQIDWILRRLEHLPPAHAAFSEYILDLMNTVINHLGYN~ERSTDSTSTILNRMQIMNYACNLGHSGCVSDSLDKWRQHRANVSNLVP~VNLRRHVYCVGL
L.dispar PAFNYL~AMVLSESEVFEEYQYYLLNLTAPLYEELGFN~AAAGEEHVTPYHRNIILDINCRHGNPACI~NTAQQLLENFRNNPSQRLNPDIQTLVYCSGL
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O.nubilalis AAFAYL~DVVLTGSEVYHLFQRYVLELTAPLYSSLGFN~NTANDEFVTAYHRTIVLNFNCRFGNEHCV~ETAQEMLESFRTTQV~RLAADIQTTVYCSGL
P.xylostella SGFNWLRNRFLHLPDVLAEFDEILYKYLDAVVTDLGYEAVDSNEPLTRTLNRFYIMSFACNIGHEGCVQHATQKYTEMVNGG~~~KVDPNIRRHVYCTGL
S.exigua PSFTYL~DSVLSGSSIYSLFQQYVRQLTAPLYEELGFI~AADGEEHVTPYHRNIILDLNCRYGNAECT~STAQSLLEGFKNNPEQPLNPDIQNLVYCAGL
810 820 830 840 850 860 870 880 890 900
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D.saccharalis RGGNAANFDFLWGLYTETTDSSAQAILLNALGCTSNEEKRTFYLRQMIDENSLVREQDRHTIAVATINSGPENMEYALDFIIENFPSIQPNVQGLTGTTN
H.armigera RAGGLDEWQYLYNRRQASNNQGDEVAMLRSLGCTSNTAAGQAYLKMILDDD~VVKAQDRVNAFSFFYMGHRDNAKAGLQFLKDNVDAIRKAVVLPAWFN~
H.punctigera REGNETDYNYLYSVYNSSQNTADMVVILRALACTKHQPSLEHYLQQSMYND~KVRIHDRTNAFSFALQGNPENLPIVLNFLYNNFAAIRETYGGVARLNL
L.dispar RGGDADNFNFLWDMYRSTSDPSEQSILLNALGCTSNEELRSFYLNQVIAEDSQVREQDRHTIVVSVTNASPENMNVALDFVIENFHLIQPRVQGLTGTTN
O.furnacalis(RR) RGGDADNFDFLWDEYLKSTDSSEQSILLNALGCTSNADRRQFYMNQVINETSQVREQDRHTILVSTINASPENMEEALDFVVENFHLIQPRVQGLSGTTN
O.nubilalis RGGDADNFDFLWDEYLKSTDSSEQSILLNALGCTSNADRRQFYMNQVINETSQVREQDRHTILVSTINASPENMEEALDFVVENFHLIQPRVQGLSGTTN
P.xylostella RQGSLAQWKVLQVLYLASNNQADEVVKLRGLGCTSDDQAVKEYLEMVLTDA~~VKAQDRVNAFTYLYMGDRGNAQKALQFIKTNHNQIREAVVGSVRFN~
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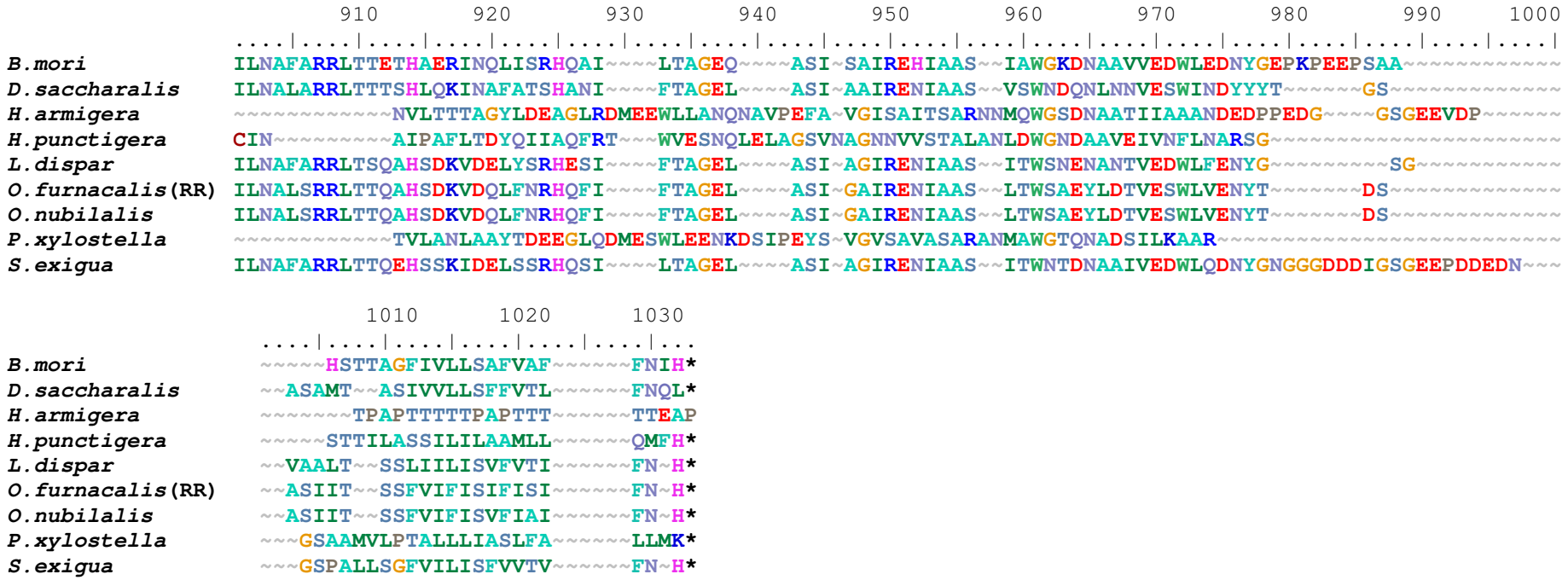
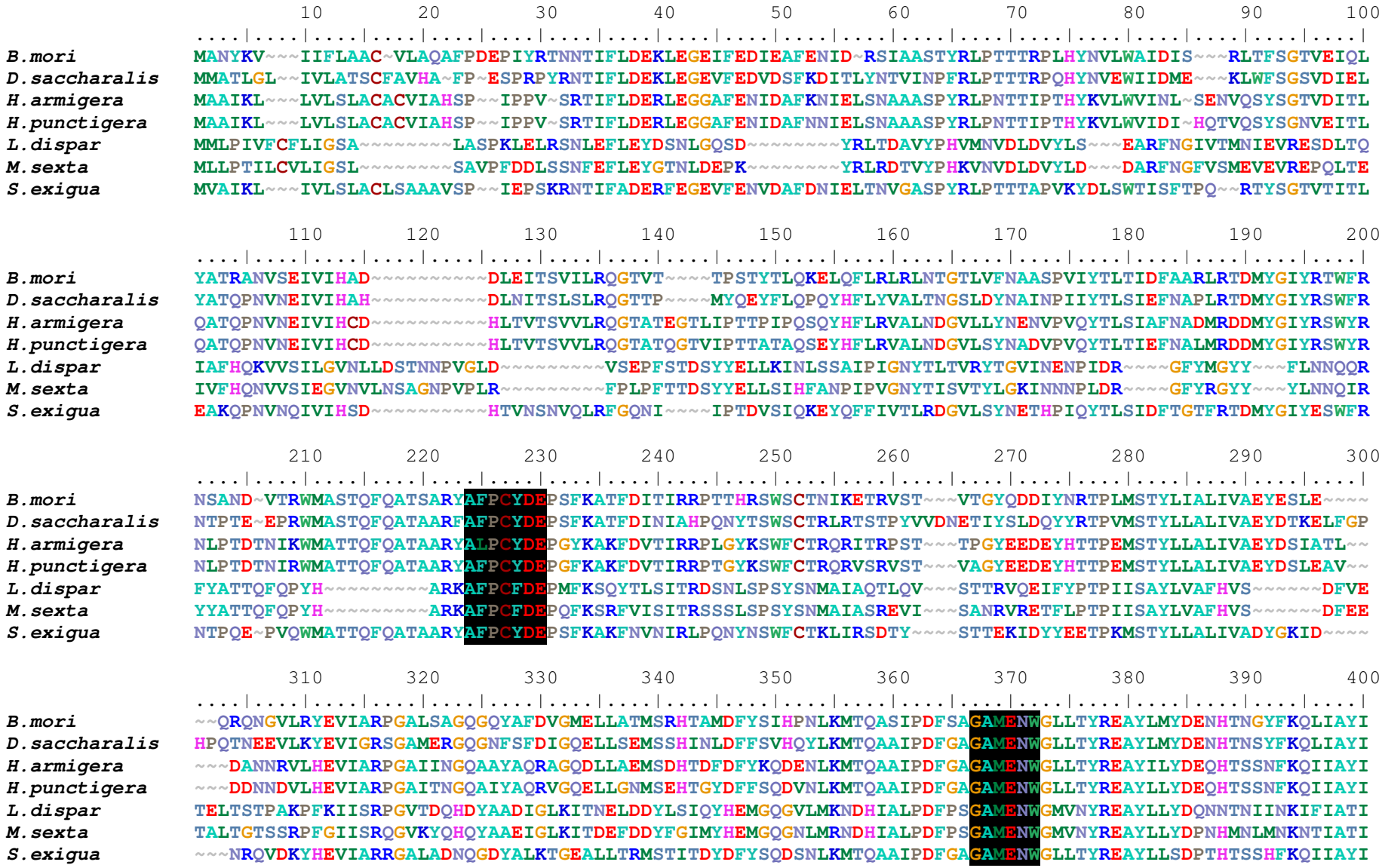
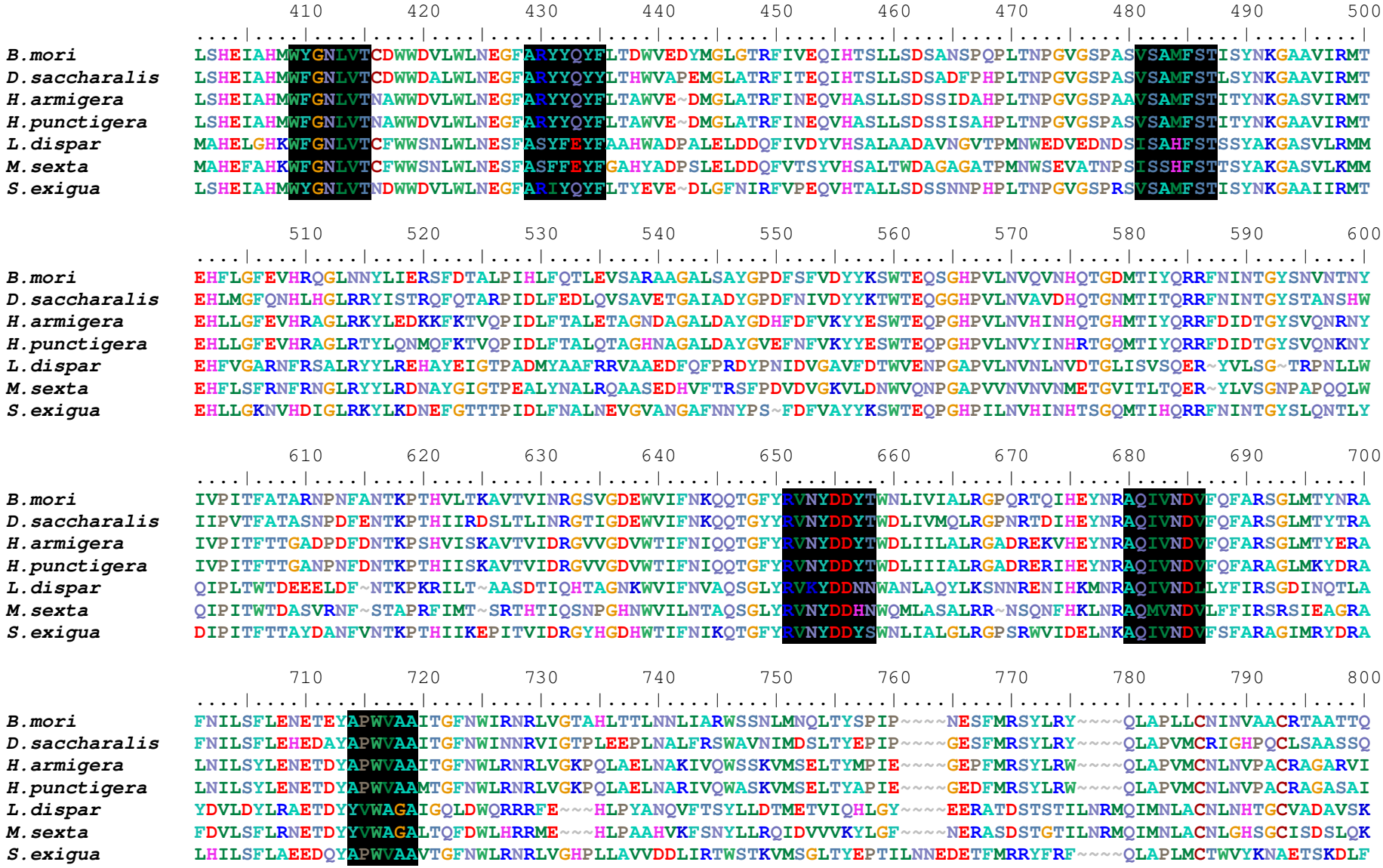


Figure A.2: Protein sequence alignment of *aminopeptidase N2* (*APN2*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (112983995),*Diatraea saccharalis* (302403440), *Helicoverpa armigera* (33641858), *Helicoverpa punctigera* (7158841), *Lymantria dispar* (4868146), *Ostrinia furnacalis* (RR) (194220239), *Ostrinia nubilalis* (215261003), *Plutella xylostella* (48526295) and *Spodoptera exigua* (37788337).

A.3. *Aminopeptidase N3* (*APN3*)

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H.punctigera LSHEIAHMWFGNLVTNAWWDVLWLNEGFARYYQYFLTAWVE~DMGLATRFINEQVHASLLSDSSISAHPLTNPGVGSPASVSAMFSTITYNKGAAVIRMT
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S.exigua LSHEIAHMWYGNLVTNDWWDVLWLNEGFARIYQYFLTYEVE~DLGFNIRFVPEQVHTALLSDSSNNPHPLTNPGVGSPRSVSAMFSTISYNKGAAIIRMT
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D.saccharalis EHLMGFQNHLHGLRRYISTRQFQTARPIDLFEDLQVSAVETGAIADYGPDFNIVDYYKTWTEQGGHPVLNVAVDHQTGNMTITQRRFNINTGYSTANSHW
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H.punctigera EHLLGFEVHRAGLRTYLQNMQFKTVQPIDLFTALQTAGHNAGALDAYGVEFNFVKYYESWTEQPGHPVLNVYINHRTGQMTIYQRRFDIDTGYSVQNKNY
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S.exigua EHLLGKNVHDIGLRKYLKDNEFGTTTPIDLFNALNEVGVANGAFNNYPS~FDFVAYYKSWTEQPGHPILNVHINHTSGQMTIHQRRFNINTGYSLQNTLY
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D.saccharalis IIPVTFATASNPDFENTKPTHIIRDSLTLINRGTIGDEWVIFNKQQTGYYRVNYDDYTWDLIVMQLRGPNRTDIHEYNRAQIVNDVFQFARSGLMTYTRA
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H.punctigera IVPITFTTTGANPNFDNTKPTHIISKAVTVIDRGVVGDVWTIFNIQQTGFYRVNYDDYTWDLIIIALRGADRERIHEYNRAQIVNDVFQFARAGLMKYDRA
L.dispar QIPLTWTDEEELDF~NTKPKRILT~AASDTIQHTAGNKWVIFNVAQSGLYRVKYDDNNWANLAQYLKSNNRENIHKMNRAQIVNDLLYFIRSGDINQTLA
M.sexta QIPITWTDASVRNF~STAPRFIMT~SRTHTIQSNPGHNWVILNTAQSGLYRVNYDDHNWQMLASALRR~NSQNFHKLNRAQMVNDVLFFIRSRSIEAGRA
S.exigua DIPITFTTAYDANFVNTKPTHIIKEPITVIDRGYHGDHWTIFNIKQTGFYRVNYDDYSWNLIALGLRGPSRWVIDELNKAQIVNDVFSFARAGIMRYDRA
710 720 730 740 750 760 770 780 790 800
B.mori FNILSFLENETEYAPWVAAITGFNWIRNRLVGTAHLTTLNNLIARWSSNLMNQLTYSPIP~~~~NESFMRSYLRY~~~~QLAPLLCNINVAACRTAATTQ
D.saccharalis FNILSFLEHEDAYAPWVAAITGFNWINNRVIGTPLEEPLNALFRSWAVNIMDSLTYEPIP~~~~GESFMRSYLRY~~~~QLAPVMCRIGHPQCLSAASSQ
H.armigera LNILSYLENETDYAPWVAAITGFNWLRNRLVGKPQLAELNAKIVQWSSKVMSELTYMPIE~~~~GEPFMRSYLRW~~~~QLAPVMCNLNVPACRAGARVI
H.punctigera LNILSYLENETDYAPWVAAMTGFNWLRNRLVGKPQLAELNARIVQWASKVMSELTYAPIE~~~~GEDFMRSYLRW~~~~QLAPVMCNLNVPACRAGASAI
L.dispar YDVLDYLRAETDYYVWAGAIGQLDWQRRRFE~~~HLPYANQVFTSYLLDTMETVIQHLGY~~~~EERATDSTSTILNRMQIMNLACNLNHTGCVADAVSK
M.sexta FDVLSFLRNETDYYVWAGALTQFDWLHRRME~~~HLPAAHVKFSNYLLRQIDVVVKYLGF~~~~NERASDSTGTILNRMQIMNLACNLGHSGCISDSLQK
S.exigua LHILSFLAEEDQYAPWVAAVTGFNWLRNRLVGHPLLAVVDDLIRTWSTKVMSGLTYEPTILNNEDETFMRRYFRF~~~~QLAPLMCTWVYKNAETSKDLF

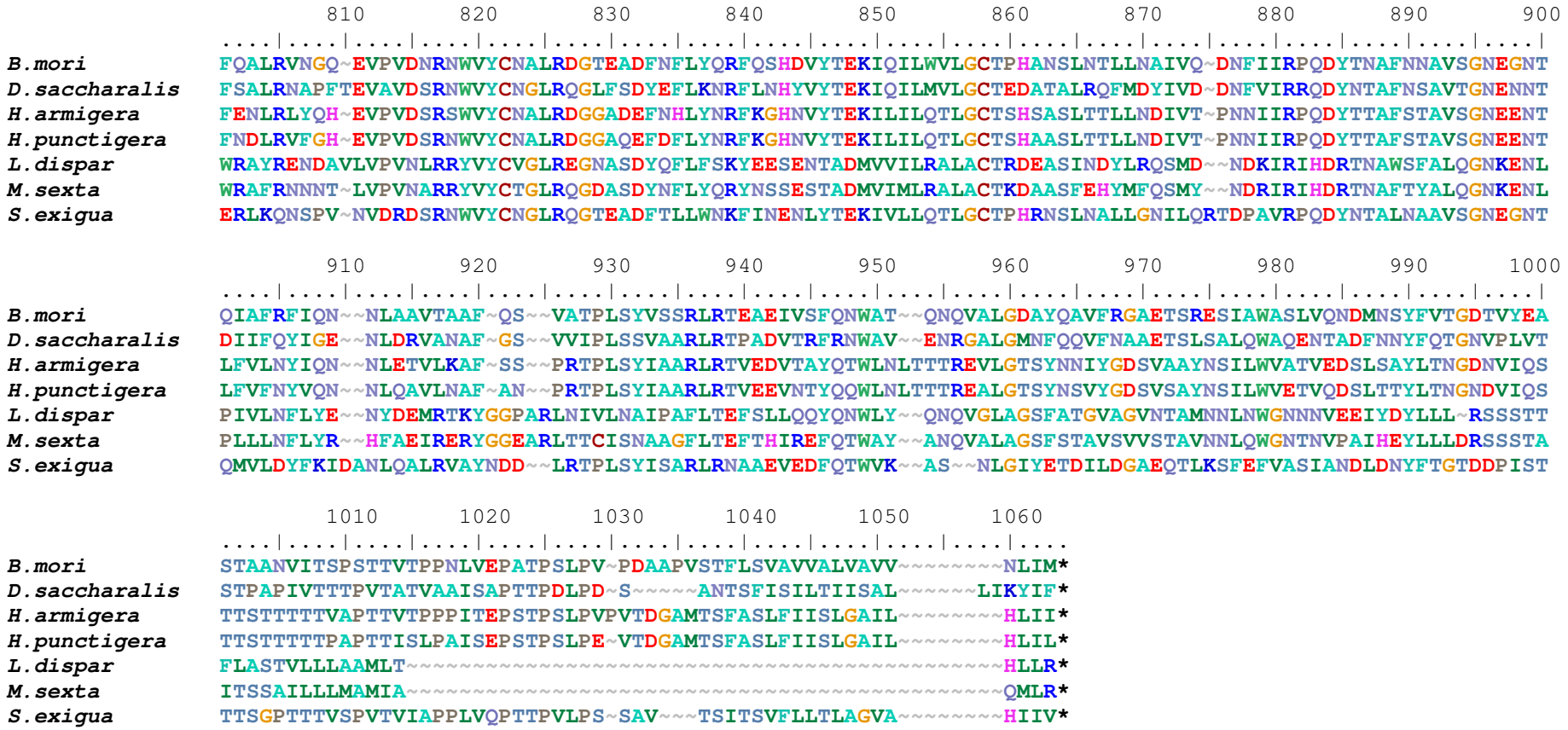
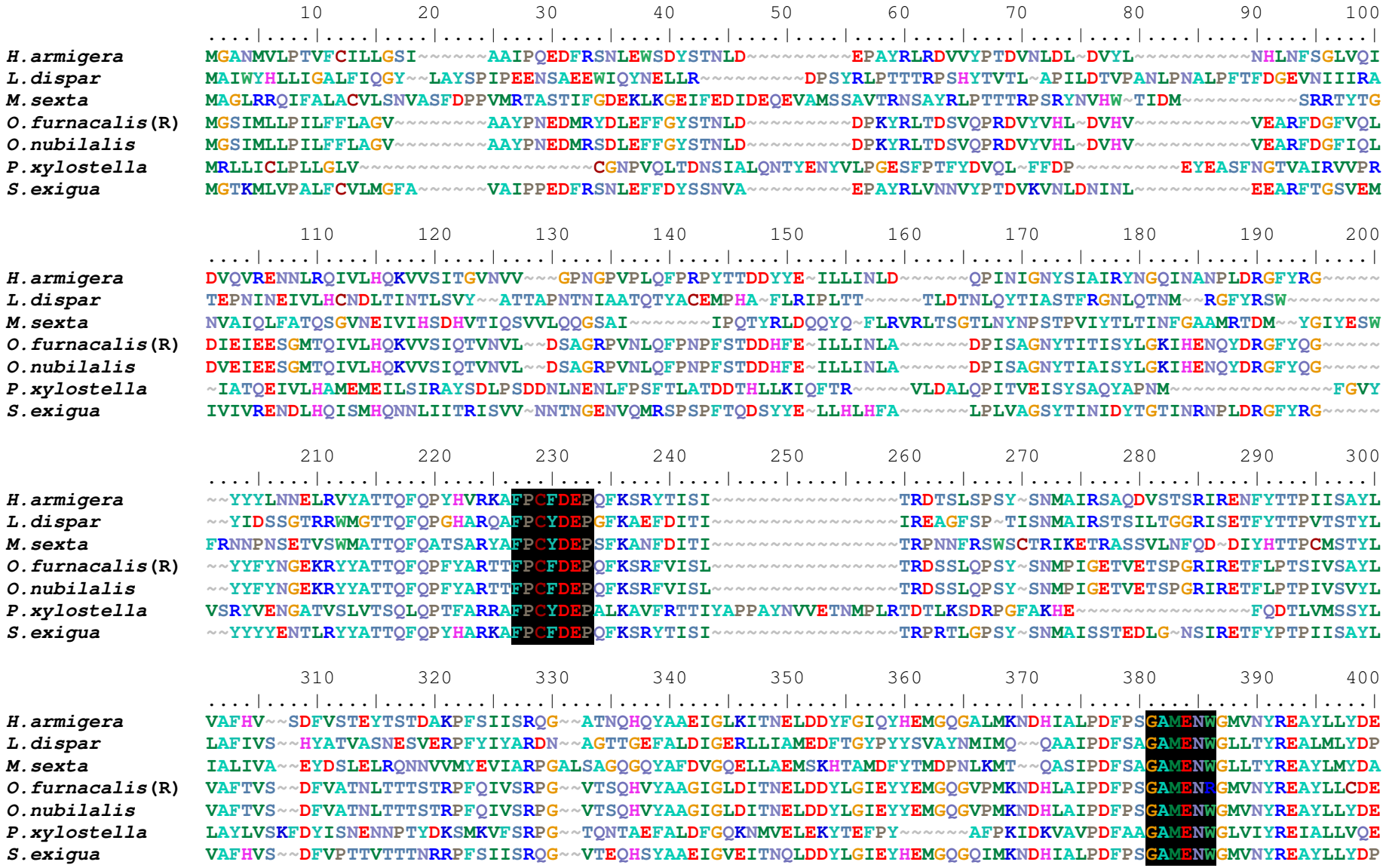
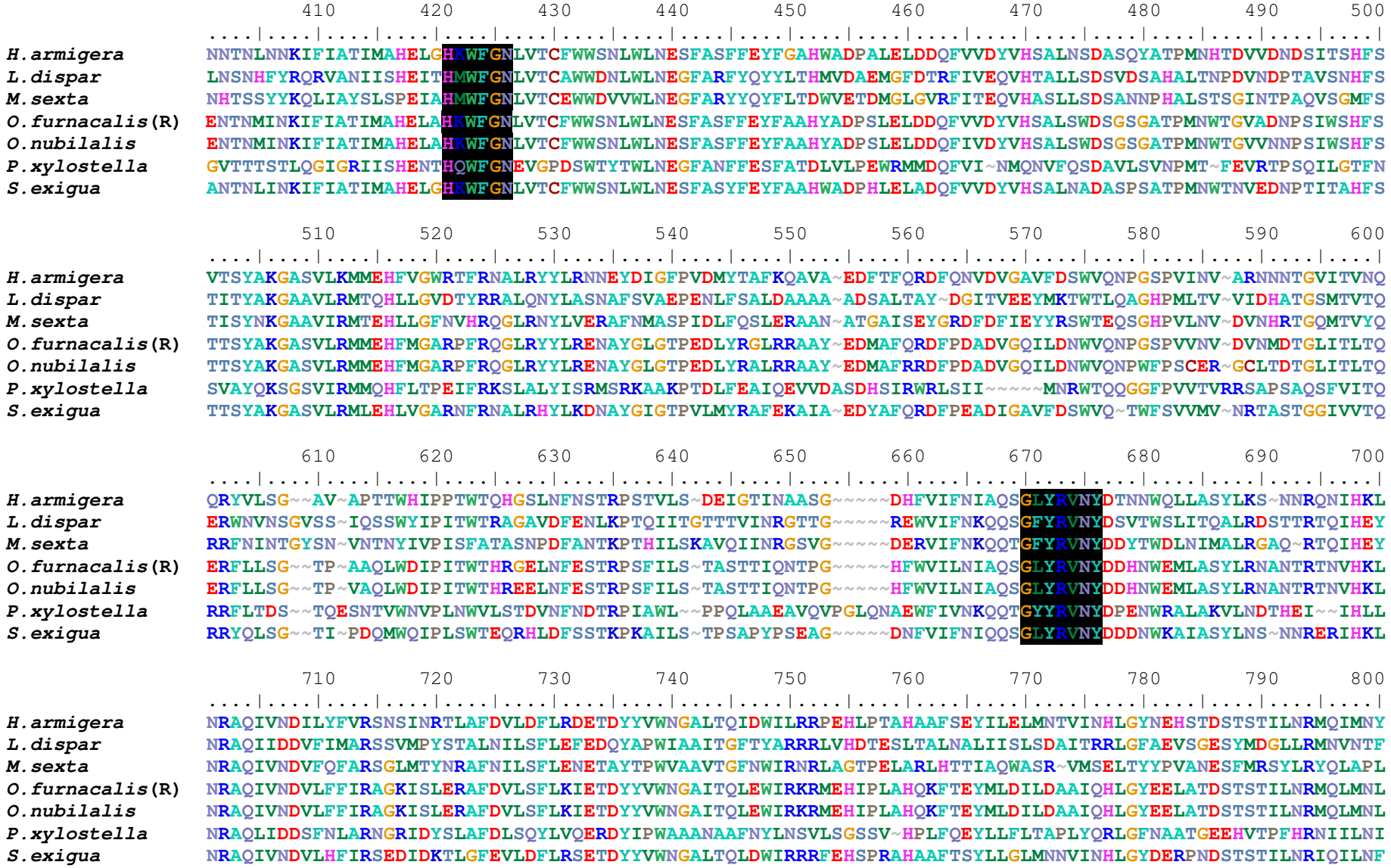


Figure A.3: Protein sequence alignment of *aminopeptidase N3* (*APN3*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (19070648),*Diatraea saccharalis* (302403442), *Helicoverpa armigera* (30961824), *Helicoverpa punctigera* (7158843), *Lymantria dispar* (16588785), *Manduca sexta* (20279108) and *Spodoptera exigua* (60739178).

A.4. *Aminopeptidase N4* (*APN4*)

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H.armigera NNTNLNNKIFIATIMAHELGHKWFGNLVTCFWWSNLWLNESFASFFEYFGAHWADPALELDDQFVVDYVHSALNSDASQYATPMNHTDVVDNDSITSHFS
L.dispar LNSNHFYRQRVANIISHEITHMWFGNLVTCAWWDNLWLNEGFARFYQYYLTHMVDAEMGFDTRFIVEQVHTALLSDSVDSAHALTNPDVNDPTAVSNHFS
M.sexta NHTSSYYKQLIAYSLSPEIAHMWFGNLVTCEWWDVVWLNEGFARYYQYFLTDWVETDMGLGVRFITEQVHASLLSDSANNPHALSTSGINTPAQVSGMFS
O.furnacalis(R) ENTNMINKIFIATIMAHELAHKWFGNLVTCFWWSNLWLNESFASFFEYFAAHYADPSLELDDQFVVDYVHSALSWDSGSGATPMNWTGVADNPSIWSHFS
O.nubilalis ENTNMINKIFIATIMAHELAHKWFGNLVTCFWWSNLWLNESFASFFEYFAAHYADPSLELDDQFIVDYVHSALSWDSGSGATPMNWTGVVNNPSIWSHFS
P.xylostella GVTTTSTLQGIGRIISHENTHQWFGNEVGPDSWTYTWLNEGFANFFESFATDLVLPEWRMMDQFVI~NMQNVFQSDAVLSVNPMT~FEVRTPSQILGTFN
S.exigua ANTNLINKIFIATIMAHELGHKWFGNLVTCFWWSNLWLNESFASYFEYFAAHWADPHLELADQFVVDYVHSALNADASPSATPMNWTNVEDNPTITAHFS
510 520 530 540 550 560 570 580 590 600
H.armigera VTSYAKGASVLKMMEHFVGWRTFRNALRYYLRNNEYDIGFPVDMYTAFKQAVA~EDFTFQRDFQNVDVGAVFDSWVQNPGSPVINV~ARNNNTGVITVNQ
L.dispar TITYAKGAAVLRMTQHLLGVDTYRRALQNYLASNAFSVAEPENLFSALDAAAA~ADSALTAY~DGITVEEYMKTWTLQAGHPMLTV~VIDHATGSMTVTQ
M.sexta TISYNKGAAVIRMTEHLLGFNVHRQGLRNYLVERAFNMASPIDLFQSLERAAN~ATGAISEYGRDFDFIEYYRSWTEQSGHPVLNV~DVNHRTGQMTVYQ
O.furnacalis(R) TTSYAKGASVLRMMEHFMGARPFRQGLRYYLRENAYGLGTPEDLYRGLRRAAY~EDMAFQRDFPDADVGQILDNWVQNPGSPVVNV~DVNMDTGLITLTQ
O.nubilalis TTSYAKGASVLRMMEHFMGARPFRQGLRYYLRENAYGLGTPEDLYRALRRAAY~EDMAFRRDFPDADVGQILDNWVQNPWFPSCER~GCLTDTGLITLTQ
P.xylostella SVAYQKSGSVIRMMQHFLTPEIFRKSLALYISRMSRKAAKPTDLFEAIQEVVDASDHSIRWRLSII~~~~~MNRWTQQGGFPVVTVRRSAPSAQSFVITQ
S.exigua TTSYAKGASVLRMLEHLVGARNFRNALRHYLKDNAYGIGTPVLMYRAFEKAIA~EDYAFQRDFPEADIGAVFDSWVQ~TWFSVVMV~NRTASTGGIVVTQ
610 620 630 640 650 660 670 680 690 700
H.armigera QRYVLSG~~AV~APTTWHIPPTWTQHGSLNFNSTRPSTVLS~DEIGTINAASG~~~~~DHFVIFNIAQSGLYRVNYDTNNWQLLASYLKS~NNRQNIHKL
L.dispar ERWNVNSGVSS~IQSSWYIPITWTRAGAVDFENLKPTQIITGTTTVINRGTTG~~~~~REWVIFNKQQSGFYRVNYDSVTWSLITQALRDSTTRTQIHEY
M.sexta RRFNINTGYSN~VNTNYIVPISFATASNPDFANTKPTHILSKAVQIINRGSVG~~~~~DERVIFNKQQTGFYRVNYDDYTWDLNIMALRGAQ~RTQIHEY
O.furnacalis(R) ERFLLSG~~TP~AAQLWDIPITWTHRGELNFESTRPSFILS~TASTTIQNTPG~~~~~HFWVILNIAQSGLYRVNYDDHNWEMLASYLRNANTRTNVHKL
O.nubilalis ERFLLSG~~TP~VAQLWDIPITWTHREELNFESTRPSFILS~TASTTIQNTPG~~~~~HFWVILNIAQSGLYRVNYDDHNWEMLASYLRNANTRTNVHKL
P.xylostella RRFLTDS~~TQESNTVWNVPLNWVLSTDVNFNDTRPIAWL~~PPQLAAEAVQVPGLQNAEWFIVNKQQTGYYRVNYDPENWRALAKVLNDTHEI~~IHLL
S.exigua RRYQLSG~~TI~PDQMWQIPLSWTEQRHLDFSSTKPKAILS~TPSAPYPSEAG~~~~~DNFVIFNIQQSGLYRVNYDDDNWKAIASYLNS~NNRERIHKL
710 720 730 740 750 760 770 780 790 800
H.armigera NRAQIVNDILYFVRSNSINRTLAFDVLDFLRDETDYYVWNGALTQIDWILRRPEHLPTAHAAFSEYILELMNTVINHLGYNEHSTDSTSTILNRMQIMNY
L.dispar NRAQIIDDVFIMARSSVMPYSTALNILSFLEFEDQYAPWIAAITGFTYARRRLVHDTESLTALNALIISLSDAITRRLGFAEVSGESYMDGLLRMNVNTF
M.sexta NRAQIVNDVFQFARSGLMTYNRAFNILSFLENETAYTPWVAAVTGFNWIRNRLAGTPELARLHTTIAQWASR~VMSELTYYPVANESFMRSYLRYQLAPL
O.furnacalis(R) NRAQIVNDVLFFIRAGKISLERAFDVLSFLKIETDYYVWNGAITQLEWIRKRMEHIPLAHQKFTEYMLDILDAAIQHLGYEELATDSTSTILNRMQLMNL
O.nubilalis NRAQIVNDVLFFIRAGKISLERAFDVLSFLKIETDYYVWNGAITQLEWIRKRMEHIPLAHQKFTEYMLDILDAAIQHLGYEELATDSTSTILNRMQLMNL
P.xylostella NRAQLIDDSFNLARNGRIDYSLAFDLSQYLVQERDYIPWAAANAAFNYLNSVLSGSSV~HPLFQEYLLFLTAPLYQRLGFNAATGEEHVTPFHRNIILNI
S.exigua NRAQIVNDVLHFIRSEDIDKTLGFEVLDFLRSETDYYVWNGALTQLDWIRRRFEHSPRAHAAFTSYLLGLMNNVINHLGYDERPNDSTSTILNRIQILNF

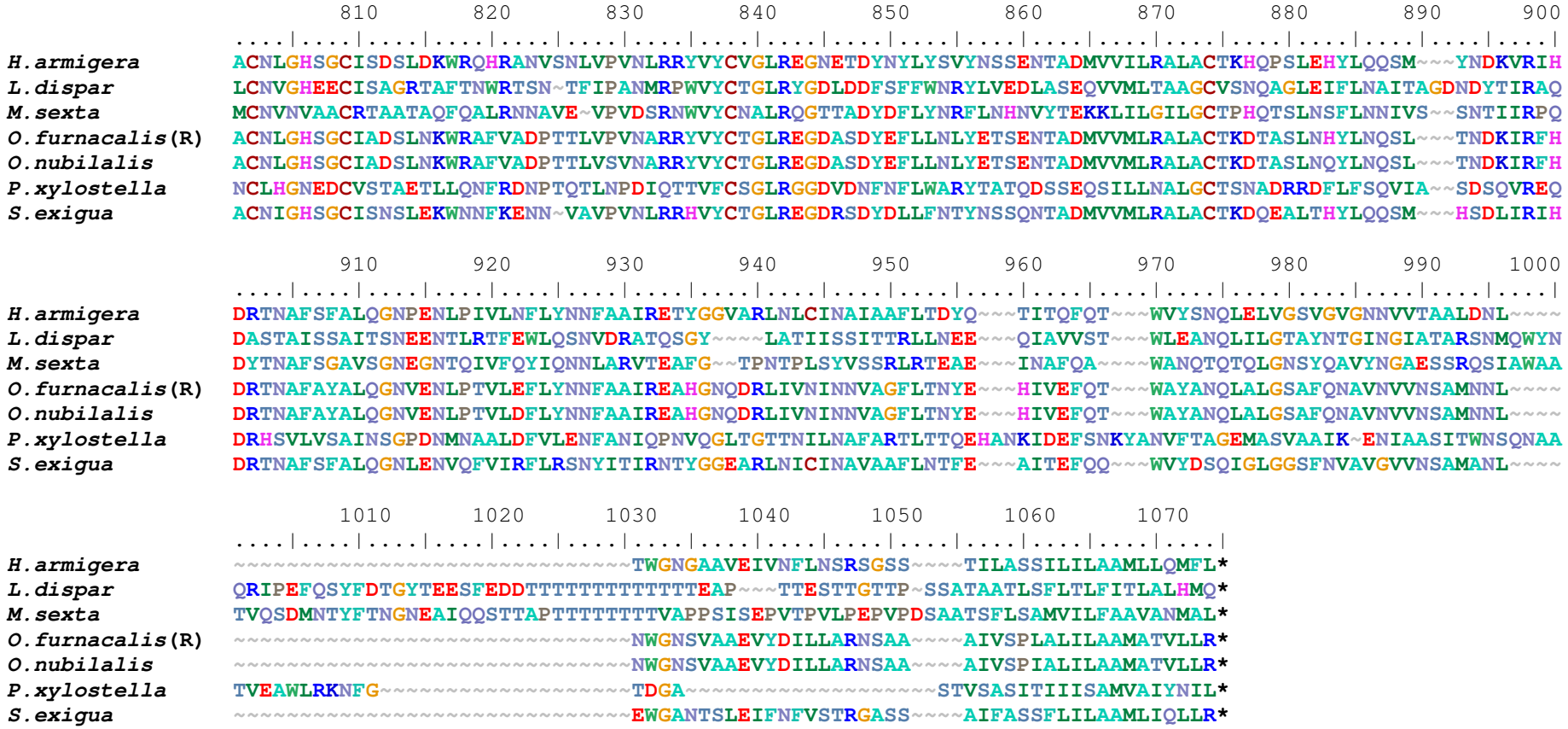

Figure A.4: Protein sequence alignment of *aminopeptidase N4* (*APN4*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Helicoverpa armigera* (27818924), *Lymantria dispar* (16588788), *Manduca sexta* (20260703) *Ostrinia furnacalis* (R) (207091423), *Ostrinia nubilalis* (258547213), *Plutella xylostella* (45685594) and *Spodoptera exigua* (37788343).

A.5. *Aminopeptidase N5* (*APN5*)

```
                         10        20        30        40        50        60        70        80        90       100
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera       MAMNVNADTIRVYSDRVPNSNIYASSTLATDDTHLLRINLNQTMTILQ~~PHAIEIEYVGHYAE~~~~~NMF~GIYLSKYENNGQEQRLITSQLQPTFAR
P.xylostella(S)  MALLLKLAILPALLALAWADFPIDADFLSDIVDTRNDDDVKYRLPESLDPVHCEIEITPHFDATADRPAFSFDGIVTINVIAKEDGINSLILQENVREIG
P.xylostella(R)  MALLLKLAILPALLALAWADFPIDADFLSDIVDTRNDDDVKYRLPESLDPVHCEIEITPHFDATADRPAFSFDGIVTINVIAKEDGINSLILQENVREIG

                        110       120       130       140       150       160       170       180       190       200
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera       RAFPCYDEPALKAVFRTTIFAPASYTVVRSNMPLRTDLLKEDVAGYVKHEFQDTLIMSTYLIA~YLVSNFVAIENNVNP~~LYRVPFRVYSRPGTQNTAE
P.xylostella(S)  AITVTEENGRL~IDLNPSSPFERLTEYQFLKINLRSGVTLSKNGKYTIRIEYVGHMNETPLSRGMFRGSYVGKDG~~~KTHWYAATHLQPTHSRQLFPSF
P.xylostella(R)  AITVTEENGRL~IDLNPSSPFERLTEYQFLKINLRSGVTLSKNGKYTIRIEYVGHMNETPLSRGMFRGSYVGKDG~~~KTHWYAATHLQPTHSRQLFPSF

                        210       220       230       240       250       260       270       280       290       300
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera       FALTFGQQNMAALERY~TEFNY~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
P.xylostella(S)  DEPGFKSTFKIIVNRPANFADTHSNM~~YAESRSEPINGLVKEVFYTTPRMSAYLVTIHISDEFTIIADNGDAKRPYRIL~~~~ARPDAANQGQYALEVG
P.xylostella(R)  DEPGFKSTFKIIVNRPANFADTHSNM~~YAESRSEPINGLVKEVFYTTPRMSAYLVTIHISDEFTIIADNGDAKRPYRIL~~~~ARPDAANQGQYALEVG

                        310       320       330       340       350       360       370       380       390       400
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera       ~~~~~~~~~~~~~~~~~~~EFPKMDKVAVPDFAAGAMENWGLVIYREVALLVTDGVTTTAVRQNVGRIICHENVHQWFGNEVGPLSWTYTWLNEGFANFF
P.xylostella(S)  PPLTKWLEEYLGKPYYEMAENMKNDQIASPFWASGATENWGLVTYRELRLLYEEGETNVVDKMSIGTITAHELGHKWFGNLVTARWWDNVWINEGYASYF
P.xylostella(R)  PPLTKWLEEYLGKPYYEMAESMKNDQIASPFWASGATENWGLVTYRELRLLYEEGETNVVDKMSIGTITAHELGHKWFGNLVTARWWDNVWINEGYASYF

                        410       420       430       440       450       460       470       480       490       500
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera       ENFATDLVRPEWRMMDQFVLALQNVFQSDAVASVNPMT~HEVYTPSQILGTFNAVAYQKSGSVIRMMQHFL~~~TPEVFRQGLVIYIRNNTRDAASPLNL
P.xylostella(S)  EYFAMDAVDKSMDLADQFNIMYTQSALATDSSASTRALQHTVNTPTQVSGHFSGISYSKGAALLNMLKHFLGENTFKK~SLNYYLDEMKYEY~~~~~ANP
P.xylostella(R)  EYFAMDAVDKSMDLADQFNIMYTQSALATDSSASTRALQHTVNTPTQVSGHFSGISYSKGAALLNMLKHFLGENTFKK~SLNYYLDEMKYEY~~~~~ANP

                        510       520       530       540       550       560       570       580       590       600
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera       YAALQQALDQSSHSIGFPVNT~~~~~~IMQRWVNQGGFPVLTVTRSAPTAQSIVVEQERFLTDRSQRLTDRWHV~~~~~~PINWVLSTNPDFSDTSPQDW
P.xylostella(S)  DDVFRGFARAVQEDGALTQFTNVNITDFLSDWVYEPGYPVINVDINMNT~GNIYIEQERFFT~~~~~~~TTGSSNQVWPLPLTYTSASSPDWSNTRASHV
P.xylostella(R)  DDVFRGFARAVQEDGALTQFTNVNITDFLSDWVYEPGYPVINVDINMNT~GNIYIGQERFFT~~~~~~~TTGSSNQVWPLPLTYTSASSPDWSNTRASHV
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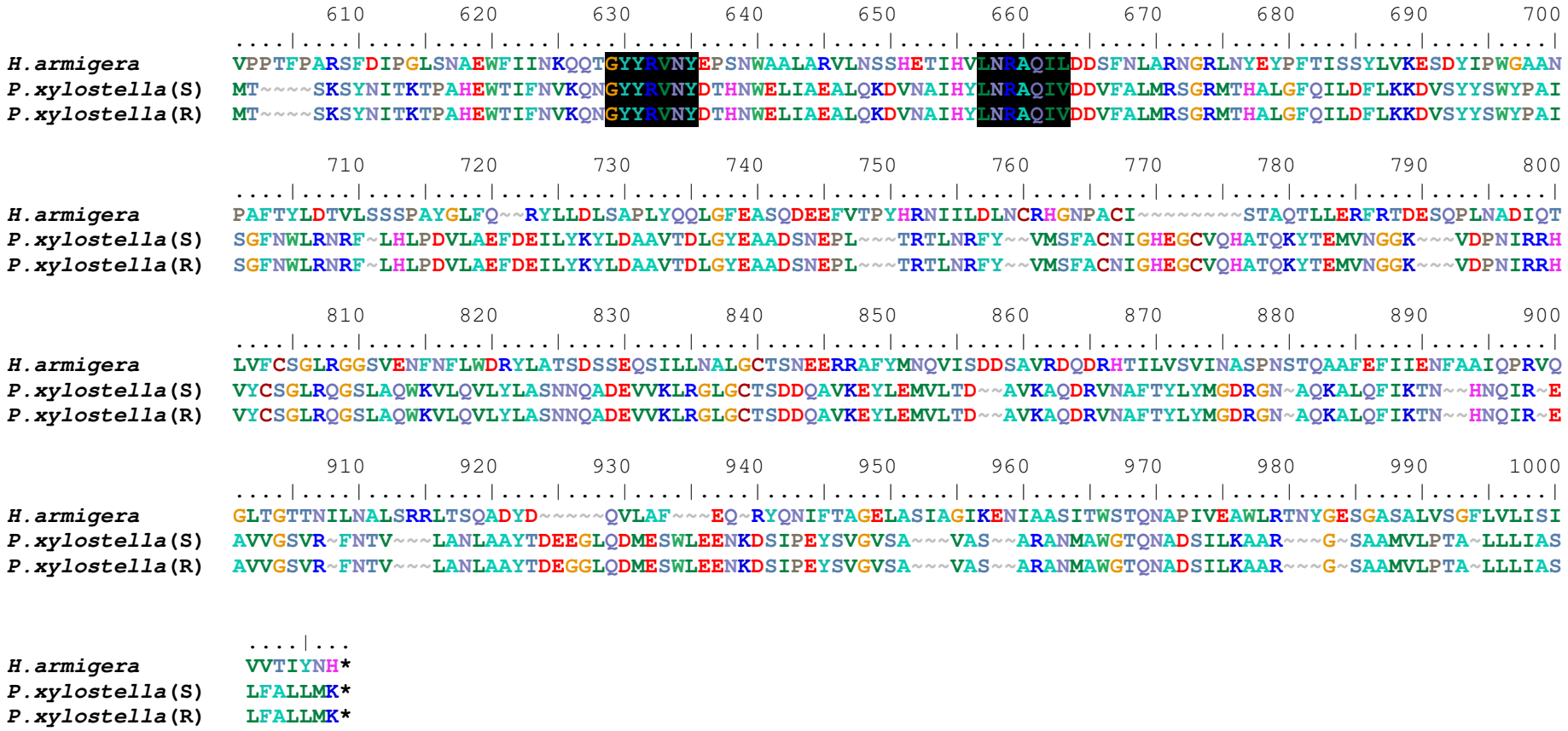


Figure A.5: Protein sequence alignment of *aminopeptidase N5* (*APN5*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Helicoverpa armigera* (126009702), *Plutella xylostella* (S) (281313031) and *Plutella xylostella* (R) (281313029).

A.6. *Aminopeptidase N6* (*APN6*)

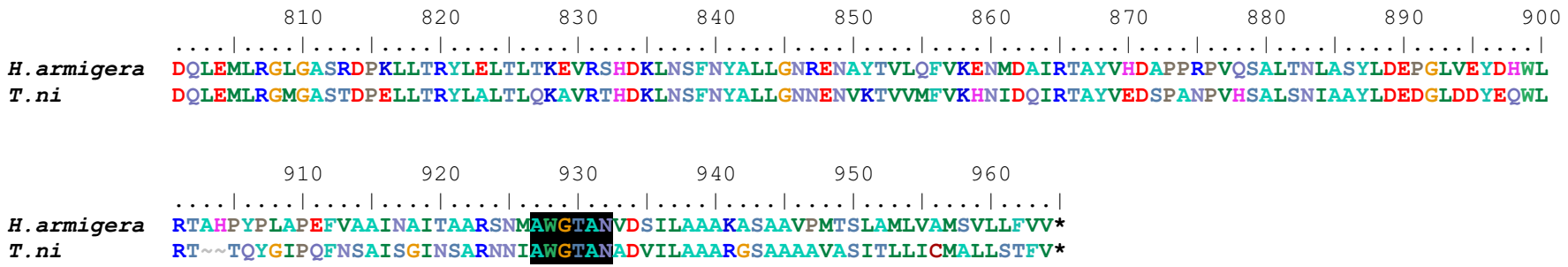
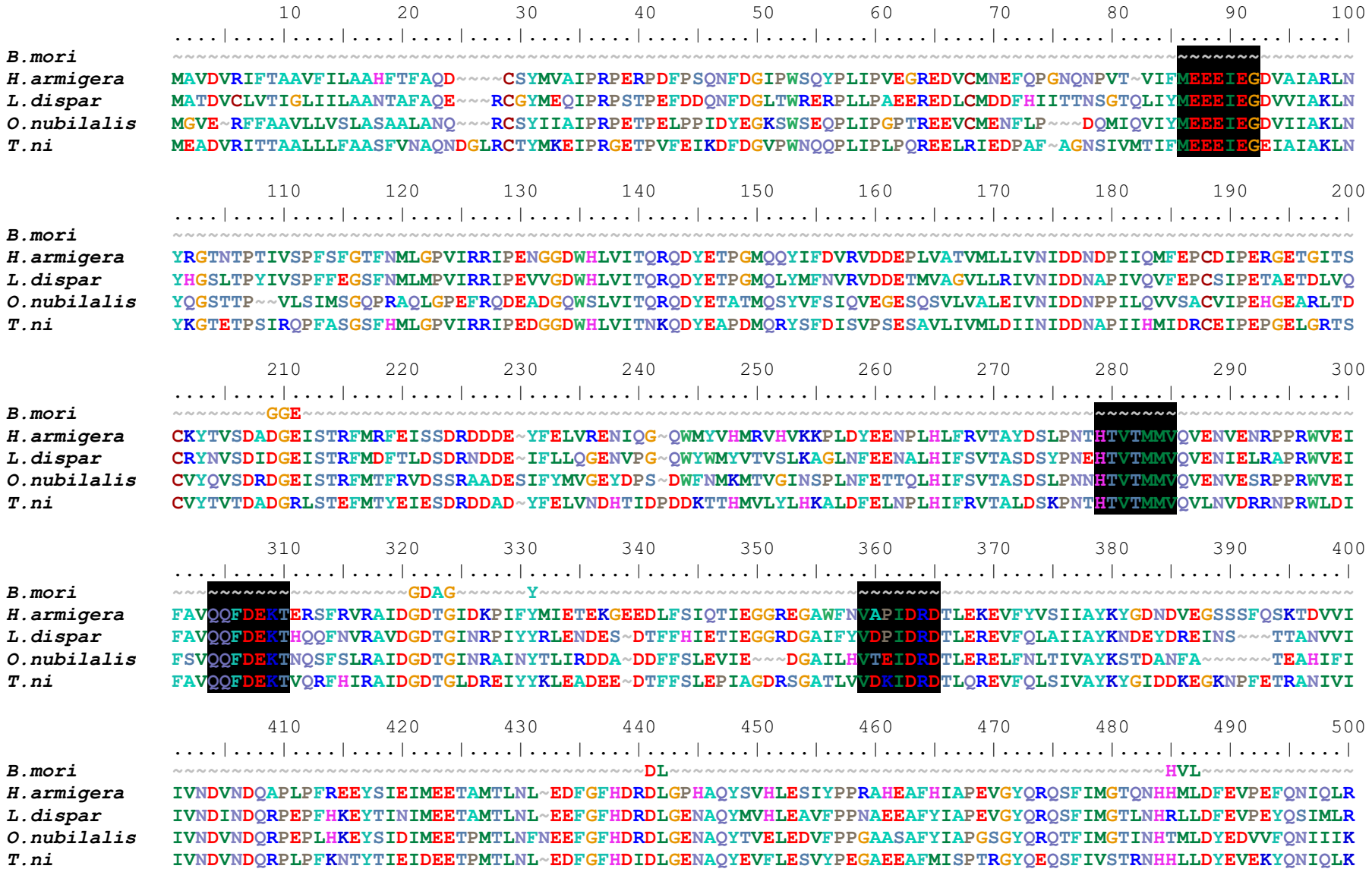
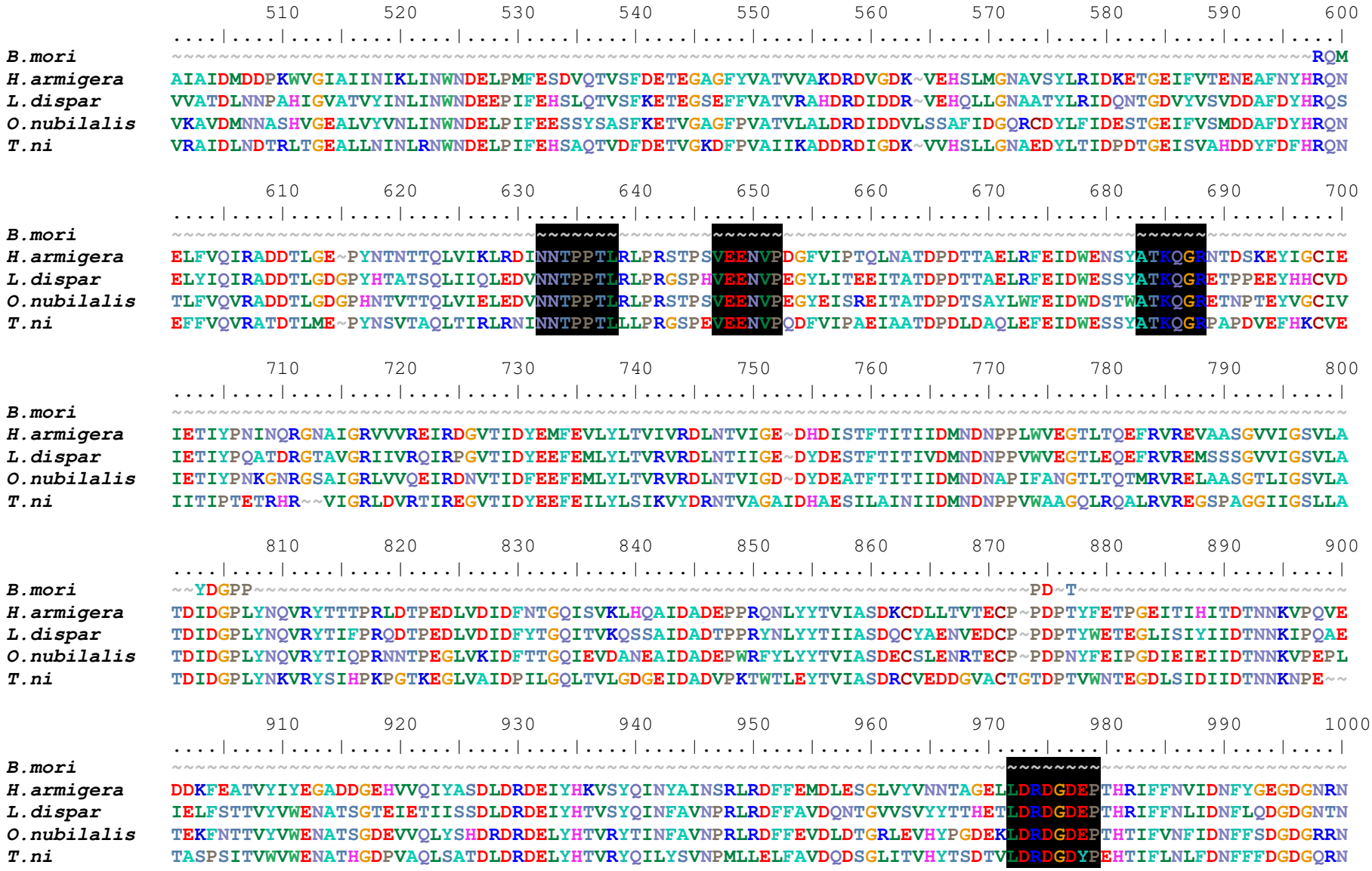
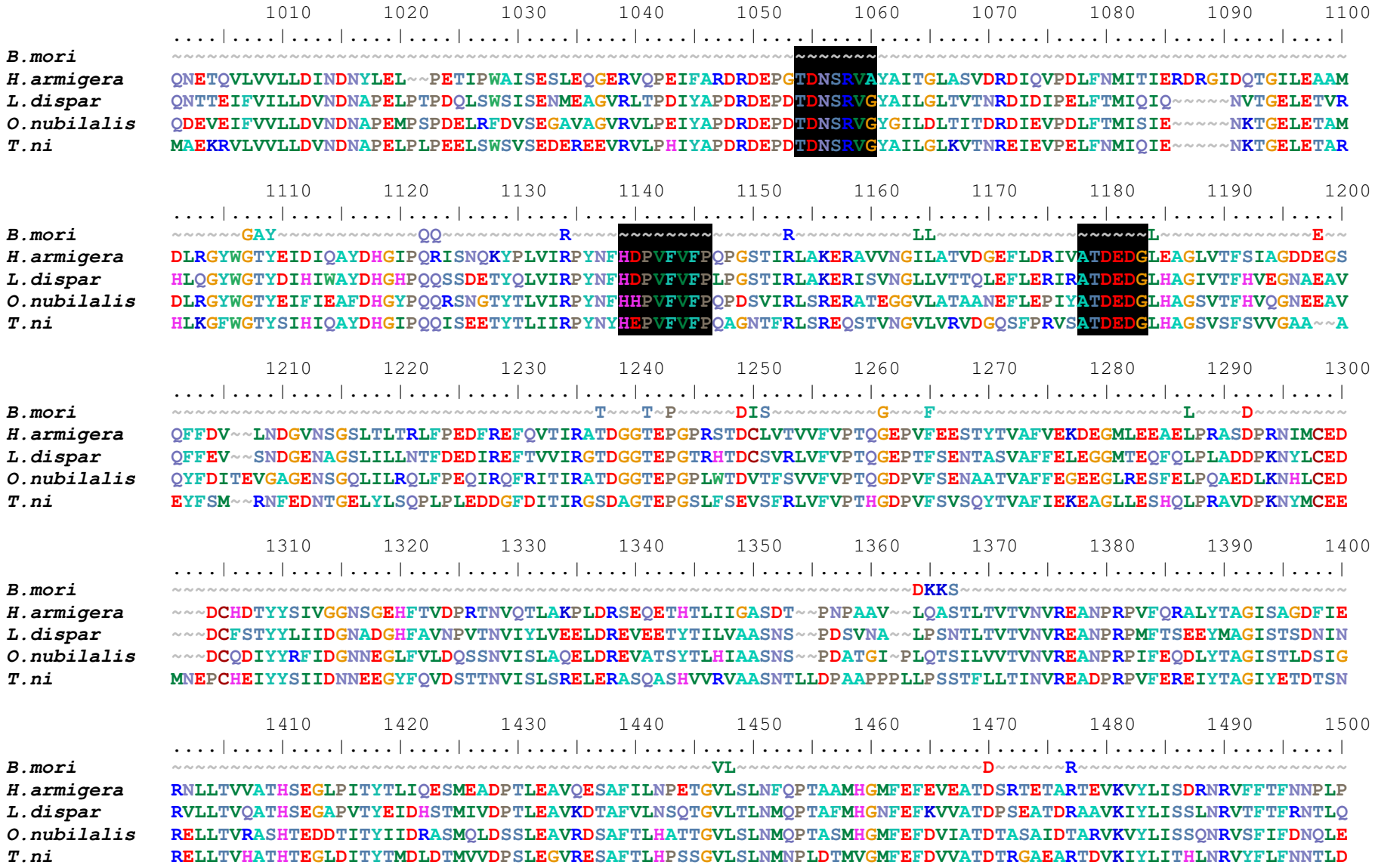


Figure A.6: Protein sequence alignment of *aminopeptidase N6* (*APN6*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Helicoverpa armigera* (170791084) and *Trichoplusia ni* (327082324).

A.7. *Cadherin* (*CAD*)




510 520 530 540 550 560 570 580 590 600
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
610 620 630 640 650 660 670 680 690 700
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
710 720 730 740 750 760 770 780 790 800
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
810 820 830 840 850 860 870 880 890 900
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni
910 920 930 940 950 960 970 980 990 1000
B.mori
H.armigera
L.dispar
O.nubilalis
T.ni


1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
B.mori
H.armigera QNETQVLVVLLDINDNYLEL~~PETIPWAISESLEQGERVQPEIFARDRDEPGTDNSRVAYAITGLASVDRDIQVPDLFNMITIERDRGIDQTGILEAAM
L.dispar QNTTEIFVILLDVNDNAPELPTPDQLSWSISENMEAGVRLTPDIYAPDRDEPDTDNSRVGYAILGLTVTNRDIDIPELFTMIQIQ~~~~~NVTGELETVR
O.nubilalis QDEVEIFVVLLDVNDNAPEMPSPDELRFDVSEGAVAGVRVLPEIYAPDRDEPDTDNSRVGYGILDLTITDRDIEVPDLFTMISIE~~~~~NKTGELETAM
T.ni MAEKRVLVVLLDVNDNAPELPLPEELSWSVSEDEREEVRVLPHIYAPDRDEPDTDNSRVGYAILGLKVTNREIEVPELFNMIQIE~~~~~NKTGELETAR
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
B.mori
H.armigera DLRGYWGTYEIDIQAYDHGIPQRISNQKYPLVIRPYNFHDPVFVFPQPGSTIRLAKERAVVNGILATVDGEFLDRIVATDEDGLEAGLVTFSIAGDDEGS
L.dispar HLQGYWGTYDIHIWAYDHGHPQQSSDETYQLVIRPYNFHDPVFVFPLPGSTIRLAKERISVNGLLVTTQLEFLERIRATDEDGLHAGIVTFHVEGNAEAV
O.nubilalis DLRGYWGTYEIFIEAFDHGYPQQRSNGTYTLVIRPYNFHHPVFVFPQPDSVIRLSRERATEGGVLATAANEFLEPIYATDEDGLHAGSVTFHVQGNEEAV
T.ni HLKGFWGTYSIHIQAYDHGIPQQISEETYTLIIRPYNYHEPVFVFPQAGNTFRLSREQSTVNGVLVRVDGQSFPRVSATDEDGLHAGSVSFSVVGAA~~A
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
B.mori
H.armigera QFFDV~~LNDGVNSGSLTLTRLFPEDFREFQVTIRATDGGTEPGPRSTDCLVTVVFVPTQGEPVFEESTYTVAFVEKDEGMLEEAELPRASDPRNIMCED
L.dispar QFFEV~~SNDGENAGSLILLNTFDEDIREFTVVIRGTDGGTEPGTRHTDCSVRLVFVPTQGEPTFSENTASVAFFELEGGMTEQFQLPLADDPKNYLCED
O.nubilalis QYFDITEVGAGENSGQLILRQLFPEQIRQFRITIRATDGGTEPGPLWTDVTFSVVFVPTQGDPVFSENAATVAFFEGEEGLRESFELPQAEDLKNHLCED
T.ni EYFSM~~RNFEDNTGELYLSQPLPLEDDGFDITIRGSDAGTEPGSLFSEVSFRLVFVPTHGDPVFSVSQYTVAFIEKEAGLLESHQLPRAVDPKNYMCEE
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
B.mori
H.armigera ~~~DCHDTYYSIVGGNSGEHFTVDPRTNVQTLAKPLDRSEQETHTLIIGASDT~~PNPAAV~~LQASTLTVTVNVREANPRPVFQRALYTAGISAGDFIE
L.dispar ~~~DCFSTYYLIIDGNADGHFAVNPVTNVIYLVEELDREVEETYTILVAASNS~~PDSVNA~~LPSNTLTVTVNVREANPRPMFTSEEYMAGISTSDNIN
O.nubilalis ~~~DCQDIYYRFIDGNNEGLFVLDQSSNVISLAQELDREVATSYTLHIAASNS~~PDATGI~PLQTSILVVTVNVREANPRPIFEQDLYTAGISTLDSIG
T.ni MNEPCHEIYYSIIDNNEEGYFQVDSTTNVISLSRELERASQASHVVRVAASNTLLDPAAPPPLLPSSTFLLTINVREADPRPVFEREIYTAGIYETDTSN
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
B.mori
H.armigera RNLLTVVATHSEGLPITYTLIQESMEADPTLEAVQESAFILNPETGVLSLNFQPTAAMHGMFEFEVEATDSRTETARTEVKVYLISDRNRVFFTFNNPLP
L.dispar RVLLTVQATHSEGAPVTYEIDHSTMIVDPTLEAVKDTAFVLNSQTGVLTLNMQPTAFMHGNFEFKVVATDPSEATDRAAVKIYLISSLNRVTFTFRNTLQ
O.nubilalis RELLTVRASHTEDDTITYIIDRASMQLDSSLEAVRDSAFTLHATTGVLSLNMQPTASMHGMFEFDVIATDTASAIDTARVKVYLISSQNRVSFIFDNQLE
T.ni RELLTVHATHTEGLDITYTMDLDTMVVDPSLEGVRESAFTLHPSSGVLSLNMNPLDTMVGMFEFDVVATDTRGAEARTDVKIYLITHLNRVYFLFNNTLD

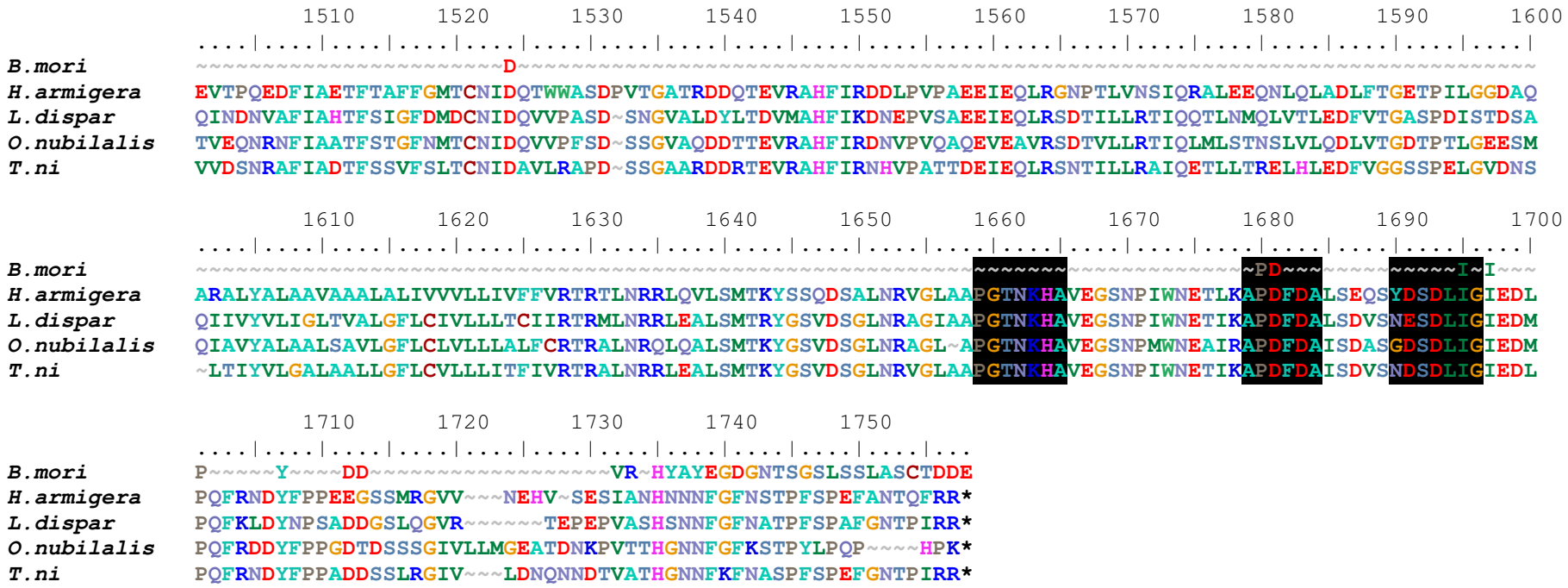
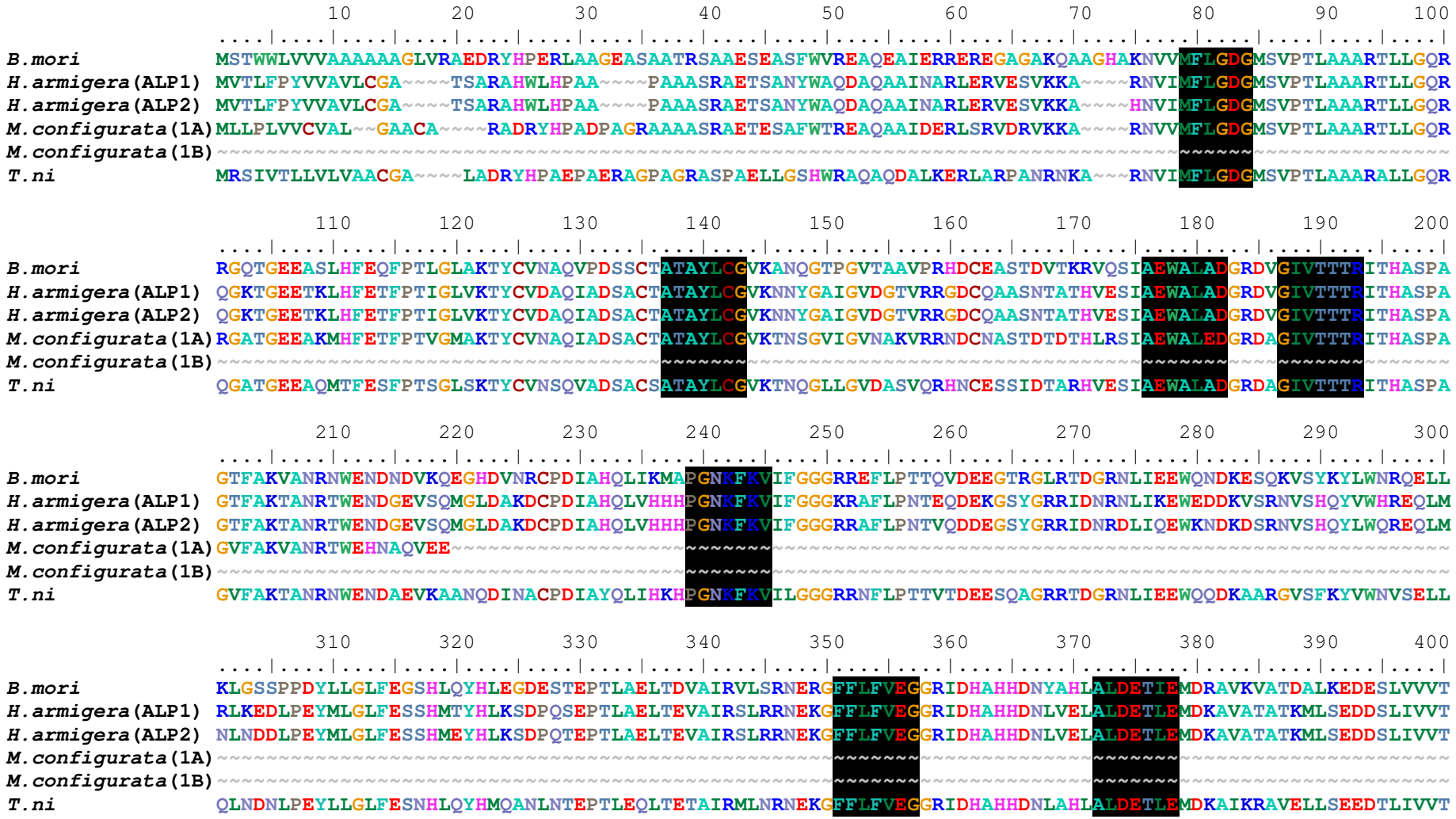


Figure A.7: Protein sequence alignment of *cadherin* for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (2381487), *Helicoverpa armigera* (40548429), *Lymantria dispar* (16588791), *Ostrinia nubilalis* (217315829) and *Trichoplusia ni* (327082289).

A.8. *Alkaline phosphatase* (*ALP*)



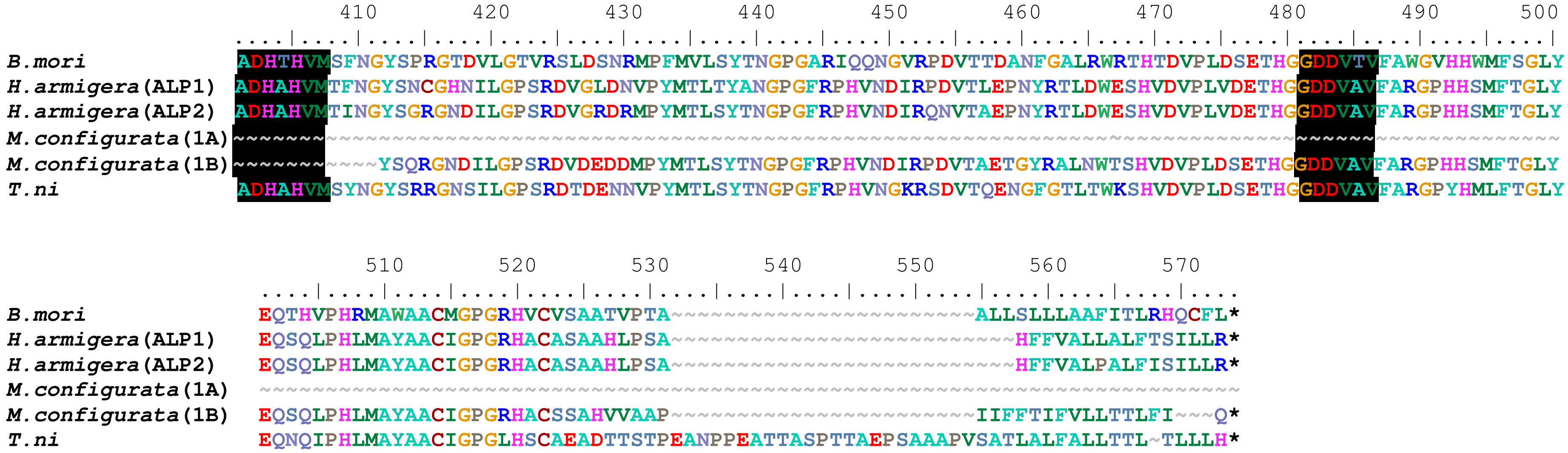
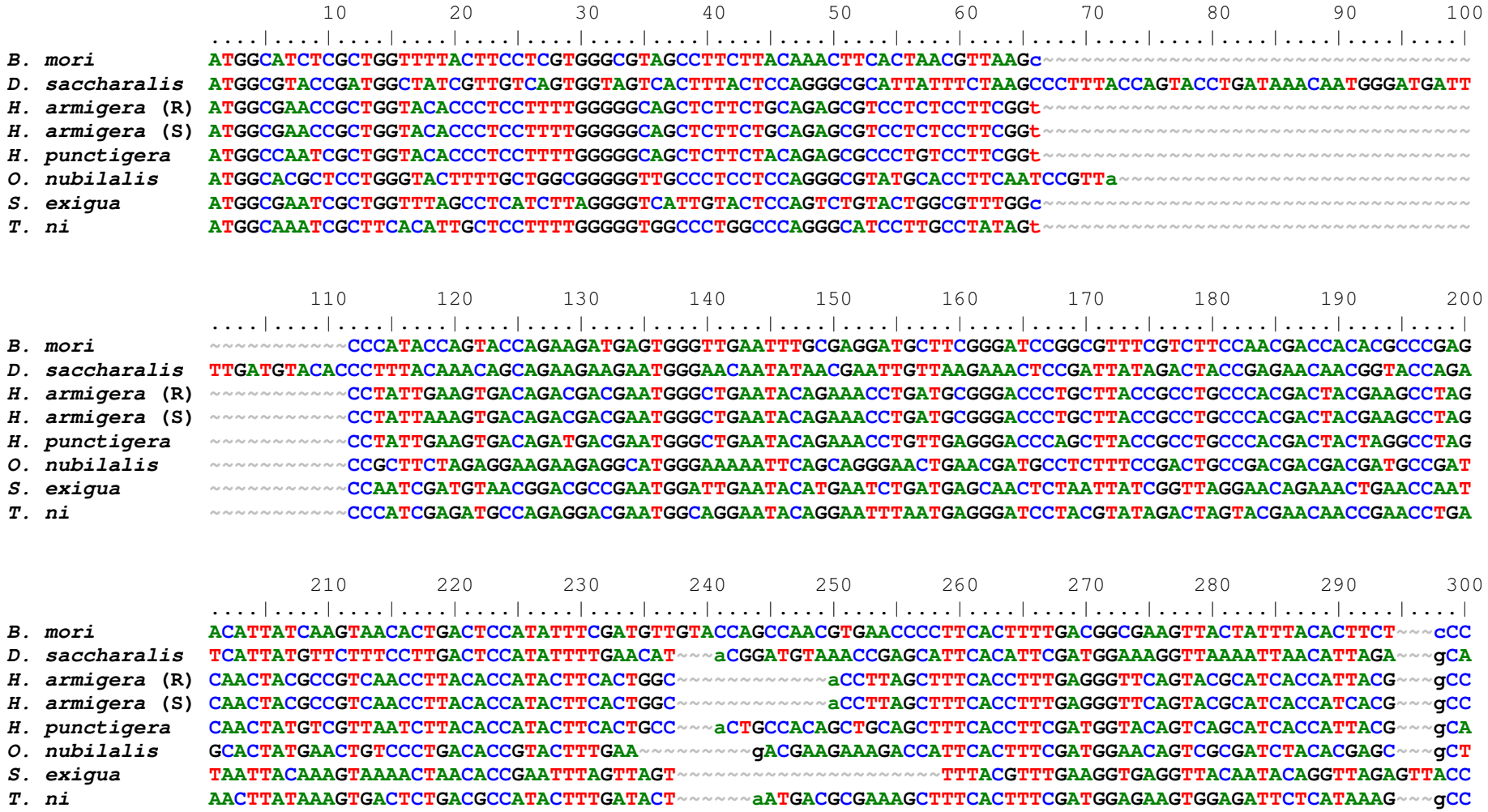
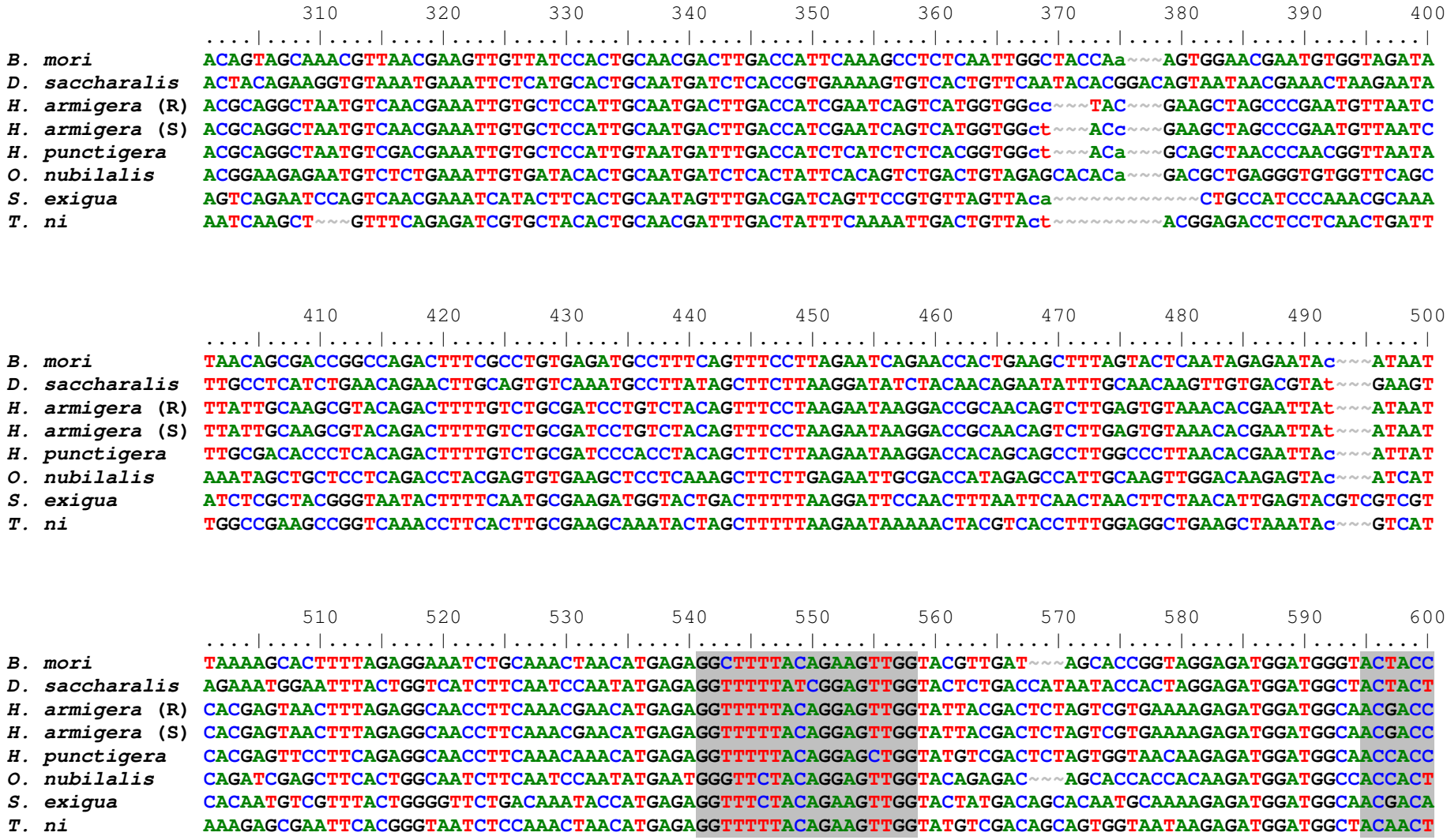


Figure A.8: Protein sequence alignment of *alkaline phosphatase* (*ALP*) for several lepidopteran species. Conserved regions observed in this alignment are highlighted in black. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (113208403), *Helicoverpa armigera* (ALP1) (194295555), *Helicoverpa armigera* (ALP2) (194295557), *Mamestra configurata* (ALP1A) (327420509), *Mamestra configurata* (ALP1B) (327420467) and *Trichoplusia ni* (334562422).

APPENDIX B

B.1. *Aminopeptidase N1* (*APN1*)

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H. armigera (S) ACGCAGGCTAATGTCAACGAAATTGTGCTCCATTGCAATGACTTGACCATCGAATCAGTCATGGTGGct~~~ACc~~~GAAGCTAGCCCGAATGTTAATC
H. punctigera ACGCAGGCTAATGTCGACGAAATTGTGCTCCATTGTAATGATTTGACCATCTCATCTCTCACGGTGGct~~~ACa~~~GCAGCTAACCCAACGGTTAATA
O. nubilalis ACGGAAGAGAATGTCTCTGAAATTGTGATACACTGCAATGATCTCACTATTCACAGTCTGACTGTAGAGCACACa~~~GACGCTGAGGGTGTGGTTCAGC
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T. ni AATCAAGCT~~~GTTTCAGAGATCGTGCTACACTGCAACGATTTGACTATTTCAAAATTGACTGTTAct~~~~~~~~~ACGGAGACCTCCTCAACTGATT
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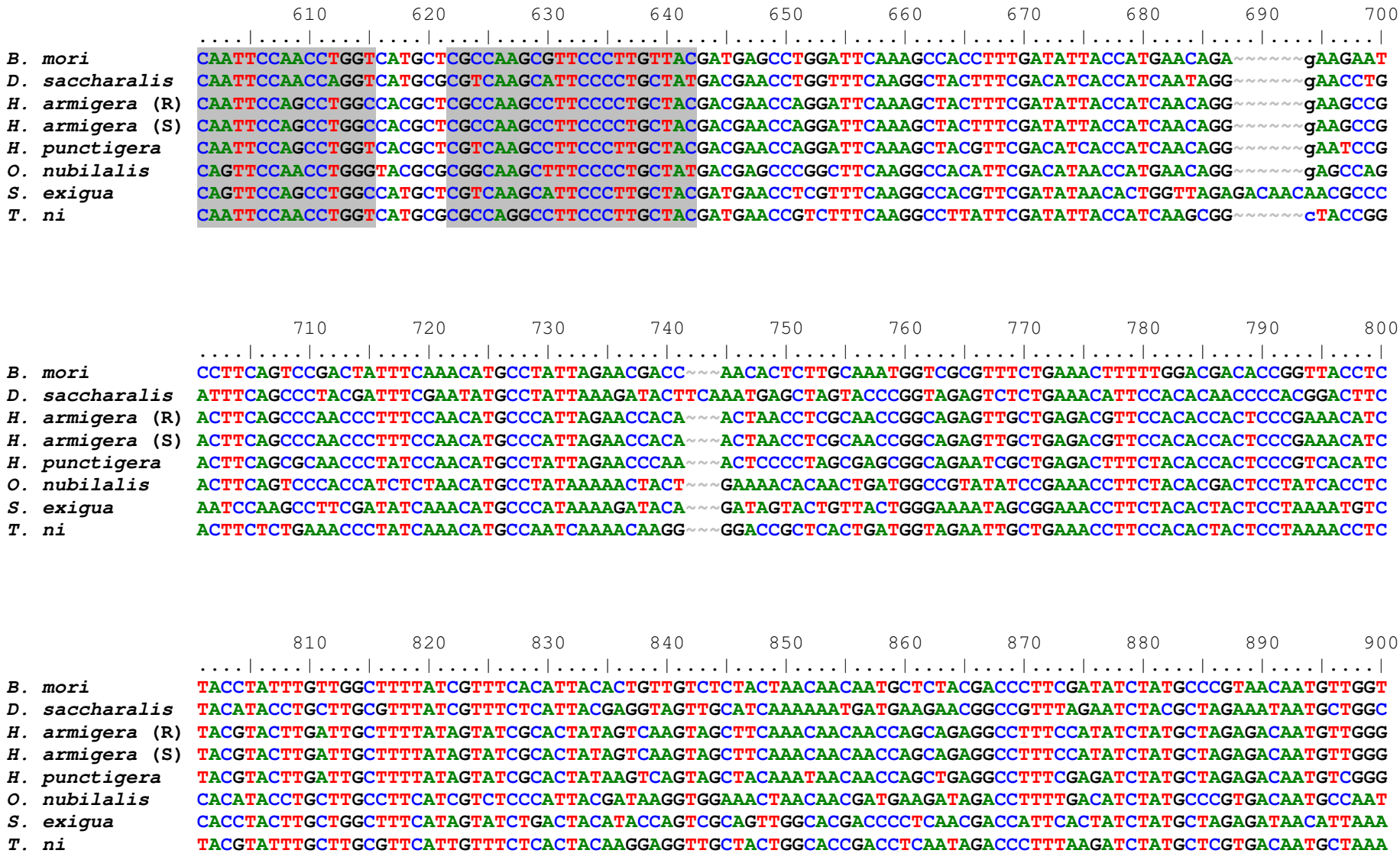
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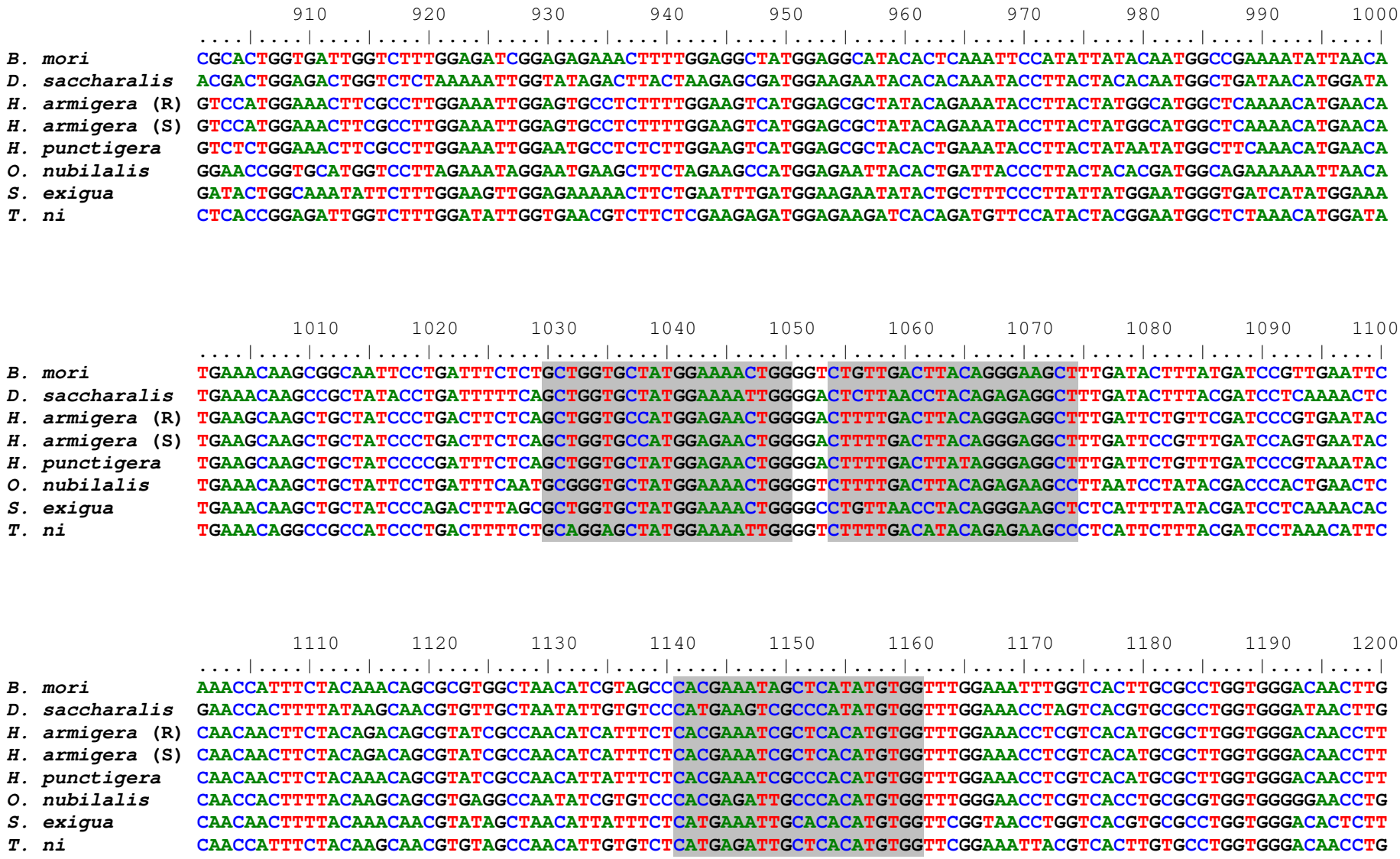
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O. nubilalis CAACCACTTTTTACAAGCAGCGTGAGGCCAATATCGTGTCCCACGAGATTGCCCACATGTGGTTTGGGAACCTCGTCACCTGCGCGTGGTGGGGGAACCTG
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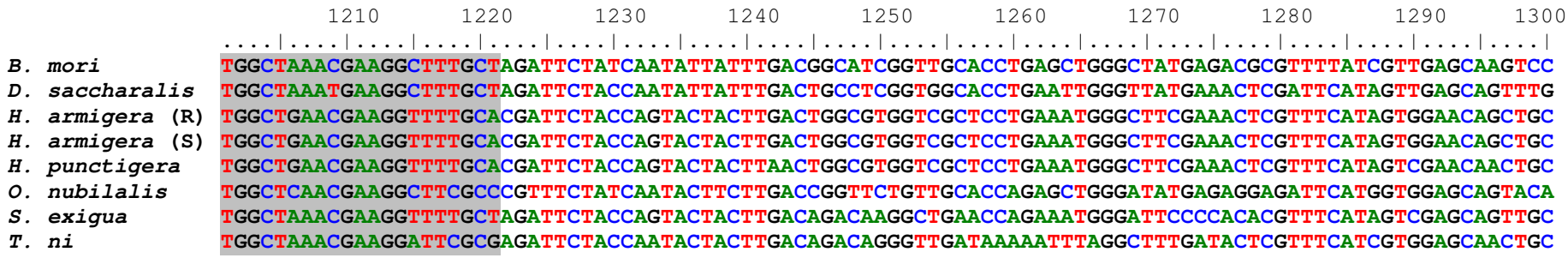
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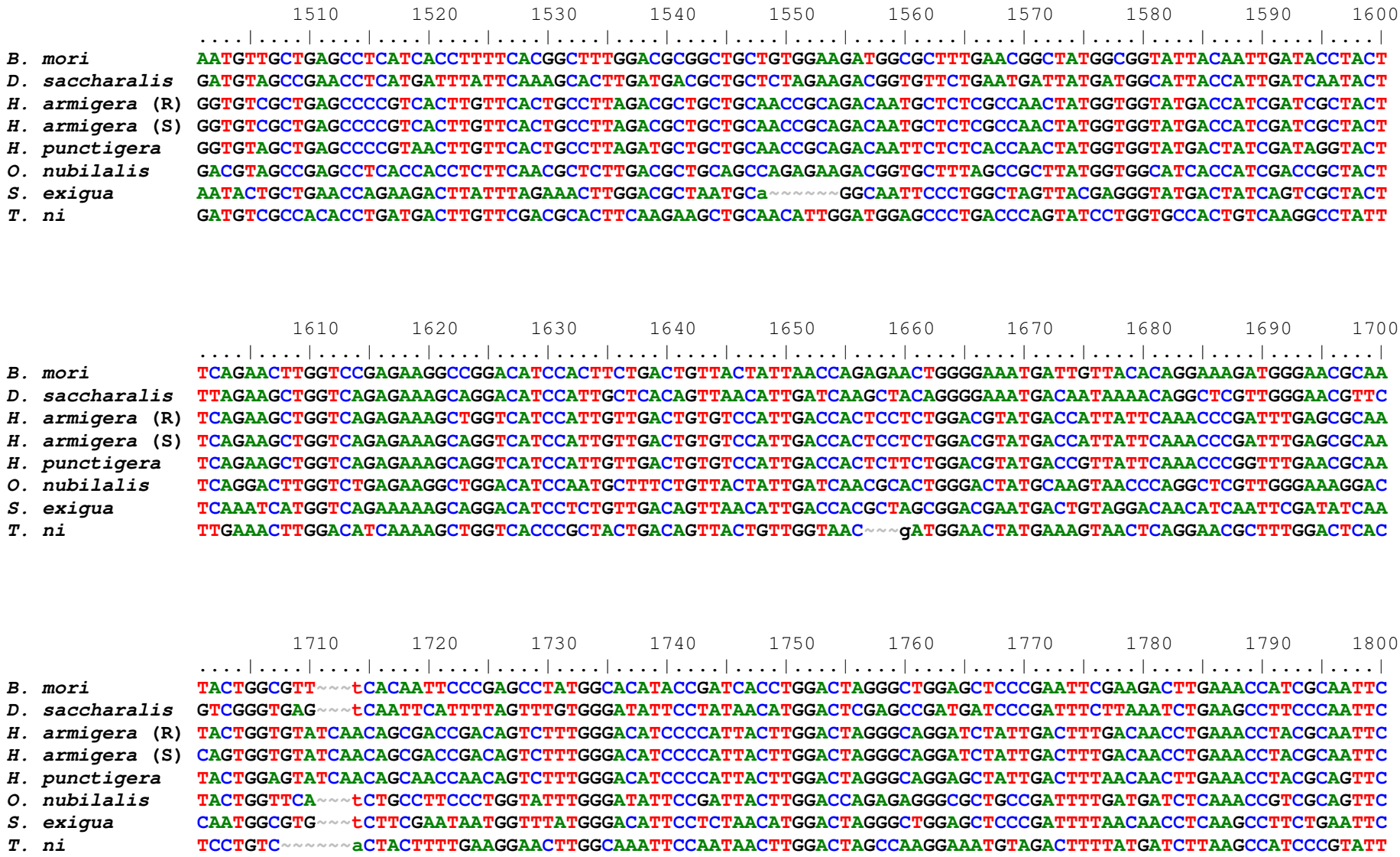
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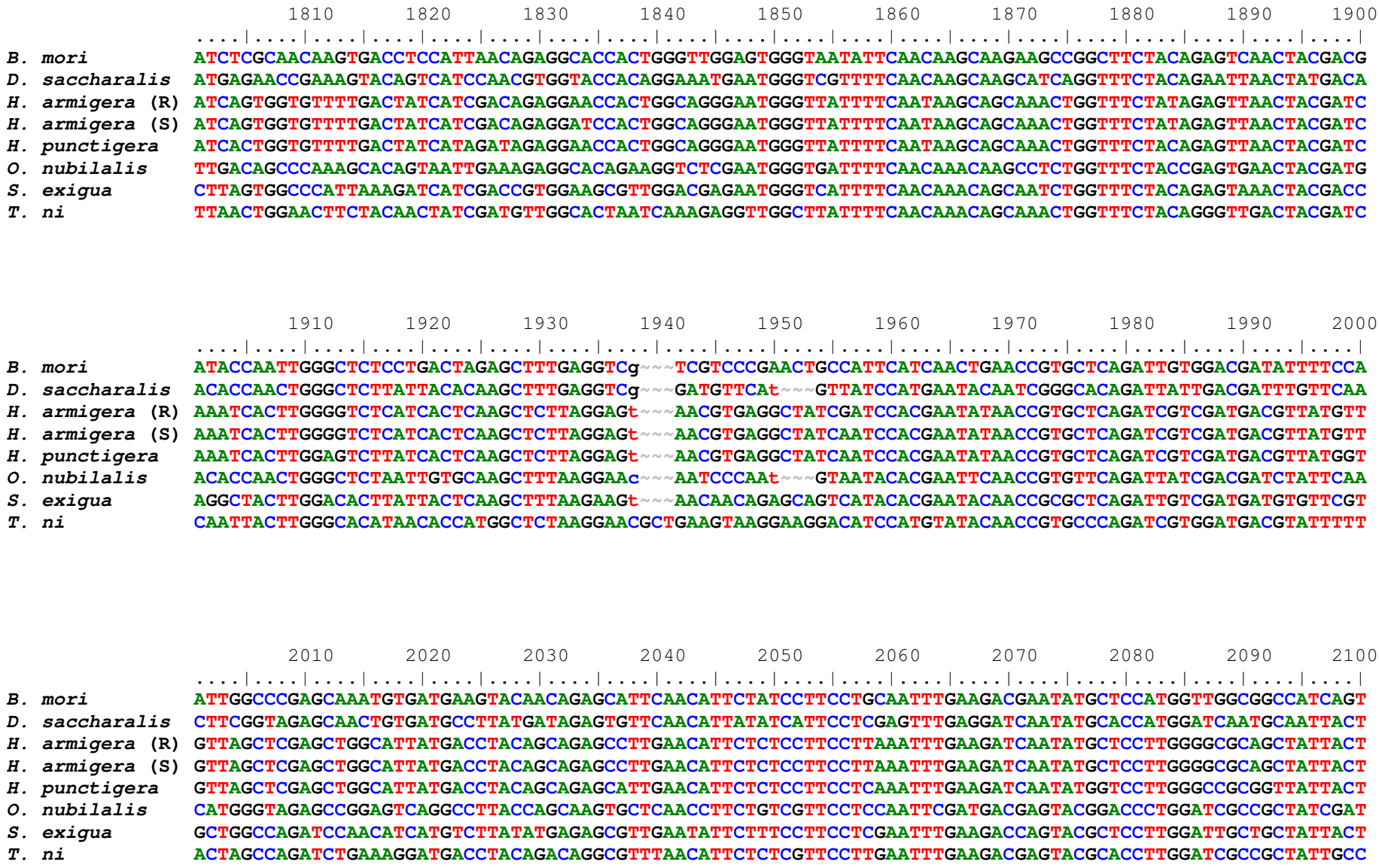
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H. armigera (R) AAATCACTTGGGGTCTCATCACTCAAGCTCTTAGGAGt~~~AACGTGAGGCTATCGATCCACGAATATAACCGTGCTCAGATCGTCGATGACGTTATGTT
H. armigera (S) AAATCACTTGGGGTCTCATCACTCAAGCTCTTAGGAGt~~~AACGTGAGGCTATCAATCCACGAATATAACCGTGCTCAGATCGTCGATGACGTTATGTT
H. punctigera AAATCACTTGGAGTCTTATCACTCAAGCTCTTAGGAGt~~~AACGTGAGGCTATCAATCCACGAATATAACCGTGCTCAGATCGTCGATGACGTTATGGT
O. nubilalis ACACCAACTGGGCTCTAATTGTGCAAGCTTTAAGGAAc~~~AATCCCAAt~~~GTAATACACGAATTCAACCGTGTTCAGATTATCGACGATCTATTCAA
S. exigua AGGCTACTTGGACACTTATTACTCAAGCTTTAAGAAGt~~~AACAACAGAGCAGTCATACACGAATACAACCGCGCTCAGATTGTCGATGATGTGTTCGT
T. ni CAATTACTTGGGCACATAACACCATGGCTCTAAGGAACGCTGAAGTAAGGAAGGACATCCATGTATACAACCGTGCCCAGATCGTGGATGACGTATTTTT
2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
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D. saccharalis CTTCGGTAGAGCAACTGTGATGCCTTATGATAGAGTGTTCAACATTATATCATTCCTCGAGTTTGAGGATCAATATGCACCATGGATCAATGCAATTACT
H. armigera (R) GTTAGCTCGAGCTGGCATTATGACCTACAGCAGAGCCTTGAACATTCTCTCCTTCCTTAAATTTGAAGATCAATATGCTCCTTGGGGCGCAGCTATTACT
H. armigera (S) GTTAGCTCGAGCTGGCATTATGACCTACAGCAGAGCCTTGAACATTCTCTCCTTCCTTAAATTTGAAGATCAATATGCTCCTTGGGGCGCAGCTATTACT
H. punctigera GTTAGCTCGAGCTGGCATTATGACCTACAGCAGAGCATTGAACATTCTCTCCTTCCTCAAATTTGAAGATCAATATGGTCCTTGGGCCGCGGTTATTACT
O. nubilalis CATGGGTAGAGCCGGAGTCAGGCCTTACCAGCAAGTGCTCAACCTTCTGTCGTTCCTCCAATTCGATGACGAGTACGGACCCTGGATCGCCGCTATCGAT
S. exigua GCTGGCCAGATCCAACATCATGTCTTATATGAGAGCGTTGAATATTCTTTCCTTCCTCGAATTTGAAGACCAGTACGCTCCTTGGATTGCTGCTATTACT
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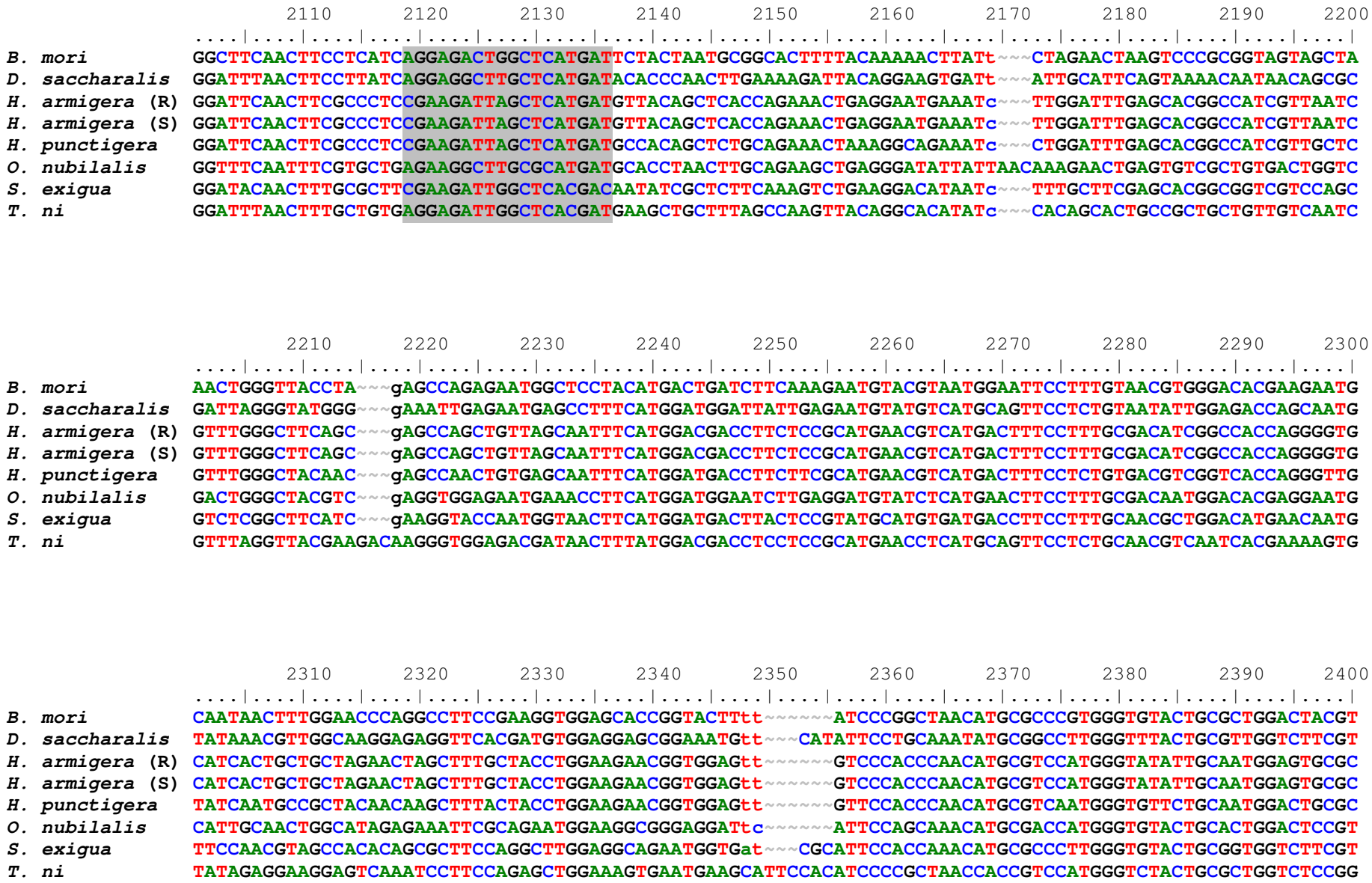
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D. saccharalis GGATTTAACTTCCTTATCAGGAGGCTTGCTCATGATACACCCAACTTGAAAAGATTACAGGAAGTGATt~~~ATTGCATTCAGTAAAACAATAACAGCGC
H. armigera (R) GGATTCAACTTCGCCCTCCGAAGATTAGCTCATGATGTTACAGCTCACCAGAAACTGAGGAATGAAATc~~~TTGGATTTGAGCACGGCCATCGTTAATC
H. armigera (S) GGATTCAACTTCGCCCTCCGAAGATTAGCTCATGATGTTACAGCTCACCAGAAACTGAGGAATGAAATc~~~TTGGATTTGAGCACGGCCATCGTTAATC
H. punctigera GGATTCAACTTCGCCCTCCGAAGATTAGCTCATGATGCCACAGCTCTGCAGAAACTAAAGGCAGAAATc~~~CTGGATTTGAGCACGGCCATCGTTGCTC
O. nubilalis GGTTTCAATTTCGTGCTGAGAAGGCTTGCGCATGATGCACCTAACTTGCAGAAGCTGAGGGATATTATTAACAAAGAACTGAGTGTCGCTGTGACTGGTC
S. exigua GGATACAACTTTGCGCTTCGAAGATTGGCTCACGACAATATCGCTCTTCAAAGTCTGAAGGACATAATc~~~TTTGCTTCGAGCACGGCGGTCGTCCAGC
T. ni GGATTTAACTTTGCTGTGAGGAGATTGGCTCACGATGAAGCTGCTTTAGCCAAGTTACAGGCACATATc~~~CACAGCACTGCCGCTGCTGTTGTCAATC
2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
B. mori AACTGGGTTACCTA~~~gAGCCAGAGAATGGCTCCTACATGACTGATCTTCAAAGAATGTACGTAATGGAATTCCTTTGTAACGTGGGACACGAAGAATG
D. saccharalis GATTAGGGTATGGG~~~gAAATTGAGAATGAGCCTTTCATGGATGGATTATTGAGAATGTATGTCATGCAGTTCCTCTGTAATATTGGAGACCAGCAATG
H. armigera (R) GTTTGGGCTTCAGC~~~gAGCCAGCTGTTAGCAATTTCATGGACGACCTTCTCCGCATGAACGTCATGACTTTCCTTTGCGACATCGGCCACCAGGGGTG
H. armigera (S) GTTTGGGCTTCAGC~~~gAGCCAGCTGTTAGCAATTTCATGGACGACCTTCTCCGCATGAACGTCATGACTTTCCTTTGCGACATCGGCCACCAGGGGTG
H. punctigera GTTTGGGCTACAAC~~~gAGCCAACTGTGAGCAATTTCATGGATGACCTTCTTCGCATGAACGTCATGACTTTCCTCTGTGACGTCGGTCACCAGGGTTG
O. nubilalis GACTGGGCTACGTC~~~gAGGTGGAGAATGAAACCTTCATGGATGGAATCTTGAGGATGTATCTCATGAACTTCCTTTGCGACAATGGACACGAGGAATG
S. exigua GTCTCGGCTTCATC~~~gAAGGTACCAATGGTAACTTCATGGATGACTTACTCCGTATGCATGTGATGACCTTCCTTTGCAACGCTGGACATGAACAATG
T. ni GTTTAGGTTACGAAGACAAGGGTGGAGACGATAACTTTATGGACGACCTCCTCCGCATGAACCTCATGCAGTTCCTCTGCAACGTCAATCACGAAAAGTG
2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
B. mori CAATAACTTTGGAACCCAGGCCTTCCGAAGGTGGAGCACCGGTACTTTt~~~~~~ATCCCGGCTAACATGCGCCCGTGGGTGTACTGCGCTGGACTACGT
D. saccharalis TATAAACGTTGGCAAGGAGAGGTTCACGATGTGGAGGAGCGGAAATGtt~~~~CATATTCCTGCAAATATGCGGCCTTGGGTTTACTGCGTTGGTCTTCGT
H. armigera (R) CATCACTGCTGCTAGAACTAGCTTTGCTACCTGGAAGAACGGTGGAGtt~~~~~~GTCCCACCCAACATGCGTCCATGGGTATATTGCAATGGAGTGCGC
H. armigera (S) CATCACTGCTGCTAGAACTAGCTTTGCTACCTGGAAGAACGGTGGAGtt~~~~~~GTCCCACCCAACATGCGTCCATGGGTATATTGCAATGGAGTGCGC
H. punctigera TATCAATGCCGCTACAACAAGCTTTACTACCTGGAAGAACGGTGGAGtt~~~~~~GTTCCACCCAACATGCGTCAATGGGTGTTCTGCAATGGACTGCGC
O. nubilalis CATTGCAACTGGCATAGAGAAATTCGCAGAATGGAAGGCGGGAGGATTc~~~~~~ATTCCAGCAAACATGCGACCATGGGTGTACTGCACTGGACTCCGT
S. exigua TTCCAACGTAGCCACACAGCGCTTCCAGGCTTGGAGGCAGAATGGTGat~~~CGCATTCCACCAAACATGCGCCCTTGGGTGTACTGCGGTGGTCTTCGT
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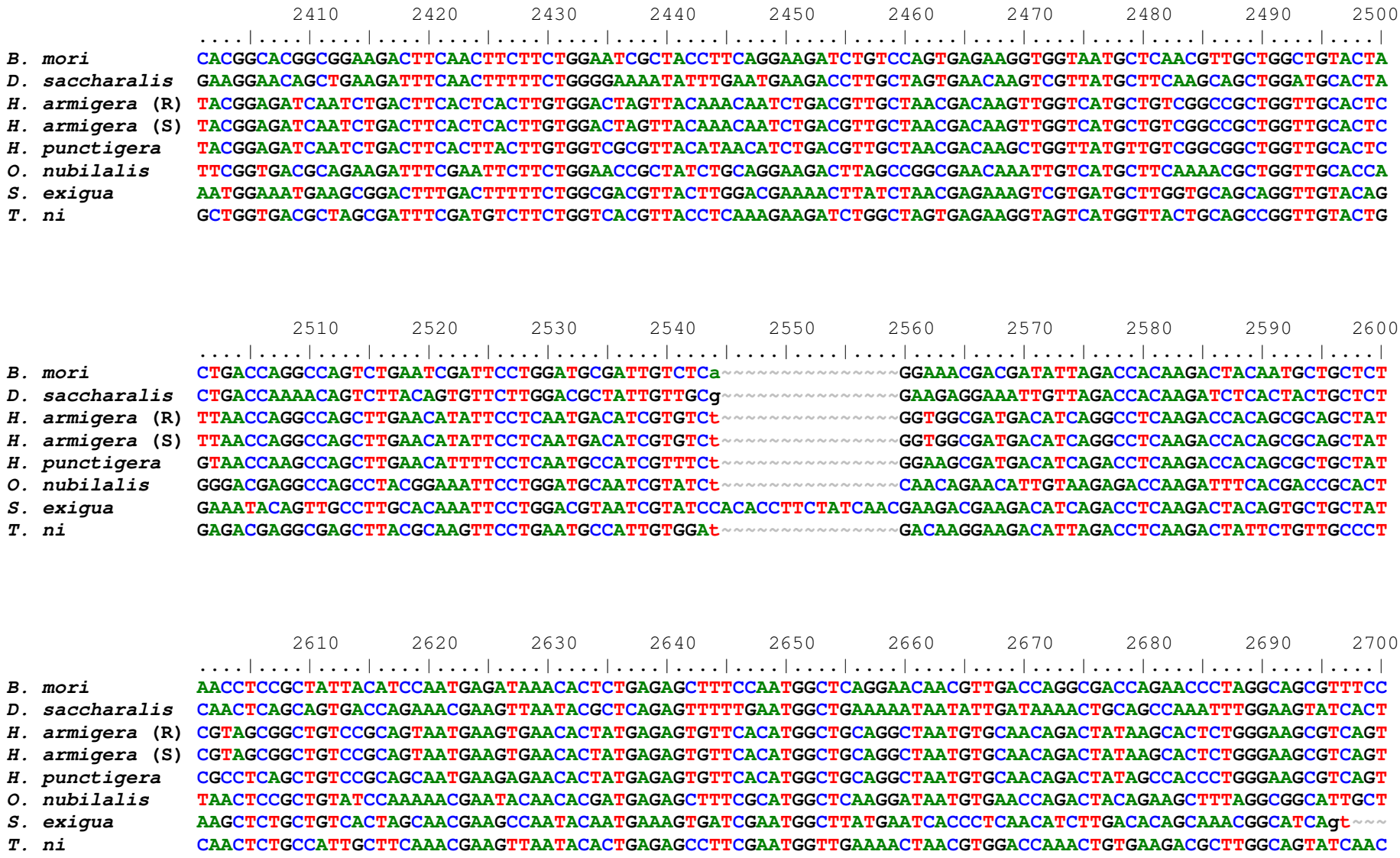
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D. saccharalis GAAGGAACAGCTGAAGATTTCAACTTTTTCTGGGGAAAATATTTGAATGAAGACCTTGCTAGTGAACAAGTCGTTATGCTTCAAGCAGCTGGATGCACTA
H. armigera (R) TACGGAGATCAATCTGACTTCACTCACTTGTGGACTAGTTACAAACAATCTGACGTTGCTAACGACAAGTTGGTCATGCTGTCGGCCGCTGGTTGCACTC
H. armigera (S) TACGGAGATCAATCTGACTTCACTCACTTGTGGACTAGTTACAAACAATCTGACGTTGCTAACGACAAGTTGGTCATGCTGTCGGCCGCTGGTTGCACTC
H. punctigera TACGGAGATCAATCTGACTTCACTTACTTGTGGTCGCGTTACATAACATCTGACGTTGCTAACGACAAGCTGGTTATGTTGTCGGCGGCTGGTTGCACTC
O. nubilalis TTCGGTGACGCAGAAGATTTCGAATTCTTCTGGAACCGCTATCTGCAGGAAGACTTAGCCGGCGAACAAATTGTCATGCTTCAAAACGCTGGTTGCACCA
S. exigua AATGGAAATGAAGCGGACTTTGACTTTTTCTGGCGACGTTACTTGGACGAAAACTTATCTAACGAGAAAGTCGTGATGCTTGGTGCAGCAGGTTGTACAG
T. ni GCTGGTGACGCTAGCGATTTCGATGTCTTCTGGTCACGTTACCTCAAAGAAGATCTGGCTAGTGAGAAGGTAGTCATGGTTACTGCAGCCGGTTGTACTG
2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
B. mori CTGACCAGGCCAGTCTGAATCGATTCCTGGATGCGATTGTCTCa~~~~~~~~~~~~~~~~GGAAACGACGATATTAGACCACAAGACTACAATGCTGCTCT
D. saccharalis CTGACCAAAACAGTCTTACAGTGTTCTTGGACGCTATTGTTGCg~~~~~~~~~~~~~~~~GAAGAGGAAATTGTTAGACCACAAGATCTCACTACTGCTCT
H. armigera (R) TTAACCAGGCCAGCTTGAACATATTCCTCAATGACATCGTGTCt~~~~~~~~~~~~~~~~GGTGGCGATGACATCAGGCCTCAAGACCACAGCGCAGCTAT
H. armigera (S) TTAACCAGGCCAGCTTGAACATATTCCTCAATGACATCGTGTCt~~~~~~~~~~~~~~~~GGTGGCGATGACATCAGGCCTCAAGACCACAGCGCAGCTAT
H. punctigera GTAACCAAGCCAGCTTGAACATTTTCCTCAATGCCATCGTTTCt~~~~~~~~~~~~~~~~GGAAGCGATGACATCAGACCTCAAGACCACAGCGCTGCTAT
O. nubilalis GGGACGAGGCCAGCCTACGGAAATTCCTGGATGCAATCGTATCt~~~~~~~~~~~~~~~~CAACAGAACATTGTAAGAGACCAAGATTTCACGACCGCACT
S. exigua GAAATACAGTTGCCTTGCACAAATTCCTGGACGTAATCGTATCCACACCTTCTATCAACGAAGACGAAGACATCAGACCTCAAGACTACAGTGCTGCTAT
T. ni GAGACGAGGCGAGCTTACGCAAGTTCCTGAATGCCATTGTGGAt~~~~~~~~~~~~~~~~GACAAGGAAGACATTAGACCTCAAGACTATTCTGTTGCCCT
2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
B. mori AACCTCCGCTATTACATCCAATGAGATAAACACTCTGAGAGCTTTCCAATGGCTCAGGAACAACGTTGACCAGGCGACCAGAACCCTAGGCAGCGTTTCC
D. saccharalis CAACTCAGCAGTGACCAGAAACGAAGTTAATACGCTCAGAGTTTTTGAATGGCTGAAAAATAATATTGATAAAACTGCAGCCAAATTTGGAAGTATCACT
H. armigera (R) CGTAGCGGCTGTCCGCAGTAATGAAGTGAACACTATGAGAGTGTTCACATGGCTGCAGGCTAATGTGCAACAGACTATAAGCACTCTGGGAAGCGTCAGT
H. armigera (S) CGTAGCGGCTGTCCGCAGTAATGAAGTGAACACTATGAGAGTGTTCACATGGCTGCAGGCTAATGTGCAACAGACTATAAGCACTCTGGGAAGCGTCAGT
H. punctigera CGCCTCAGCTGTCCGCAGCAATGAAGAGAACACTATGAGAGTGTTCACATGGCTGCAGGCTAATGTGCAACAGACTATAGCCACCCTGGGAAGCGTCAGT
O. nubilalis TAACTCCGCTGTATCCAAAAACGAATACAACACGATGAGAGCTTTCGCATGGCTCAAGGATAATGTGAACCAGACTACAGAAGCTTTAGGCGGCATTGCT
S. exigua AAGCTCTCTGCTGTCACTAGCAACGAAGCCAATACAATGAAAGTGATCGAATGGCTTATGAATCACCCTCAACATCTTGACACAGCAAACGGCATCAgt~~~
T. ni CAACTCTCTGCCATTGCTTCAAACGAAGTTAATACACTGAGAGCCTTCGAATGGTTGAAAACTAACGTGGACCAAACTGTGAAGACGCTTGGCAGTATCAAC

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                             2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
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B. mori             ACGATCTTGAACACAATAATTGGACGTCTCCTCAACGAGGAACAGATAAATGAGGTTTCGAACTGGTTGACGGCCAACCAAAACACCCTGGGAGCCACA~
D. saccharalis      ACACCTCTCAGCTATATTGCACCACGACTTTTGACTCAAAGTGACATAGACCGGTTTGAAAGTTGGTTGCAAGAAAATGAAGATAGAATTGGTCCAACGG
H. armigera (R)     CCTATTTTGAACGAAATCACAGCGCGCCTATTGAATGAAGCCCAAATCACTCAGGTTCAAACTTGGTTGGATGCAAACCAGAACGCAATTGGTACTGCAG
H. armigera (S)     CCTATTTTGAACGAAATCACAGCGCGCCTATTGAATGAAGCCCAAATCACTCAGGTTCAAACATGGTTGGATGCAAACCAGAACGTAATTGGTACTGCAG
H. punctigera       CCTATTTTGAACGAAATCACAGTTCGTCTATTGAATGAAGCCCAAATCACTCAGGTCCAAAATTGGTTGGATGCA~~~~~~AGCGTAATTGGTAATGCAG
O. nubilalis        ACTCCATTGAGCTACATTGTTTCACGGCTGTTGAATGAACAGGACATGGCAGAGGTTCAAACTTGGCTCGACGAGAACCAAGACTCGATTGGAACAGCG~
S. exigua           ~~~CTTTTGTCGACTGCTACAAGCCGATTGTTGACCCAGAGTGATATTCTAAGGGTCGAAACCTGGTTGAATACTGCTACCCAACTCAAAGCGGAGGCC~
T. ni               TCTCCTCTCAGCACAATTAGCAGTCGACTGTTGAATGACGCTCAGATTAATACTGTCGAAACCTGGTTGAATGAAAACGCTGAAATTATCGGTGCATCAG
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                             2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B. mori             ~~TACAGCACGGCGCTACGAGCTATCGAGACCACGCGGTCGAACCTCGTCTGGTCACAGCAGAGAATTTCAGAATTCACGAACTACTTCGAGTCAGGC~~
D. saccharalis      CTTTCGCAACGGGCAGCAGTGGTGTAAATAACGTCAGAAACGCTCTCATTTGGTCAGATCTACGAATTCCAGAAATTGTTAAATTCCTTGAAAATGGT~~
H. armigera (R)     CTCACACTAGTGCCACAAATGGCATCGCCACGTCTAGGTCCAACATTCAGTGGTACACACAGAGGGTTCCCGAATTCAACGTATACTTTGAAACTGGA~~
H. armigera (S)     CTCACACTAGCGCCACAAATGGCATCGCCACGTCTAGGTCCAACATTCAGTGGTACACACAGAGGGTTCCCGAATTCAACGTATACTTTGAAACTGGA~~
H. punctigera       CTTATACTAGCGCCATAAATGGCATCGCAACGTCTAGGGCCAACATTCAGTGGTACACACAGAGGGTTCCCGAATTCAACGCATACTTTGAAACTGGA~~
O. nubilalis        ~~TACAATACCGGTGTTAACGGGATCGCCTCATCCAGAAACAATCTGGCGTGGTCGGCCAGACGCATGCCTGAAGTCTACGAGTATTTCGACAACGGAGT
S. exigua           ~~ATTCAAGCGGCTAGAGCCGGTATTGCTACATCAACGGCAAACATTCAATGGTATCAAAGAAGGCAACATGAGTTCAAAGCTTACTTCGACACAGGA~~
T. ni               CTGTAGCCGCTGGCAGATCTGGTATTGCGACTTCTAAGAGCAATATCGAGTGGTTGACTAAGAGGAAGGTTGAATTCGAAGATTACTTCGAAACCGGg~~
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                             2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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D. saccharalis      ~TATGTAGAAGATGATATTCCCAATAATACTACAACTACTGAA~~~~~~aCTACTACCACTGAAACTACTACCACTGAAACTACTACCACTGAAACTACt
H. armigera (R)     ~TATGTTGAAGAGAACTTCGCTGATCCTACAACTACCAGTACG~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H. armigera (S)     ~TATGTTGAAGAGAACTTCGCTGACACTACAACTACCAGTACGACTACGACTACGACAACTACTACGACCACTACAGCAGCTCCTACG~~~~~~ACTACG
H. punctigera       ~TACGTCGAAGAGAACTTCTCTGACACTACGACTACGACAACT~~~~~~aCTACGACGACTACTACGACAACTACACCAGCTCCTACGACAACAACTACG
O. nubilalis        ATATGTGGAATACGAAATCGAAGACCCCACA~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~cAAGAAGAG
S. exigua           ~TACTTTGAAGAAGGTTTTGAAGTTCCTCCTTCCTCCAGTACT~~~~~~aCAACTAAAGCTACCCCTTCCACACCTGAAGATACCCCTTCCACACCTGAA
T. ni               ~~~~TTCGAAGATCCCTTg~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
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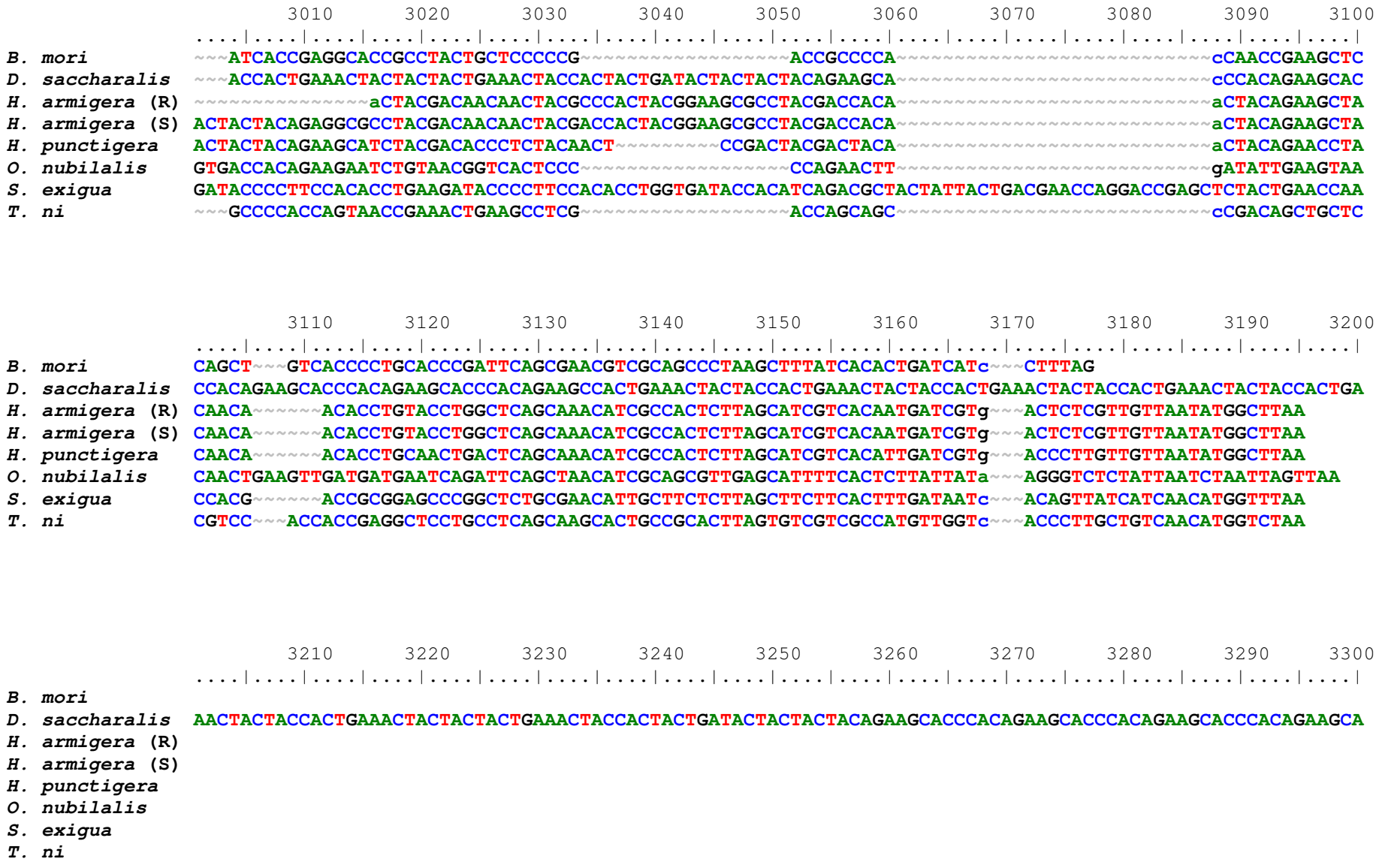
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D. saccharalis ~~~ACCACTGAAACTACTACTACTGAAACTACCACTACTGATACTACTACTACAGAAGCA~~~~~~~~~~~~~~~~~~~~~~~~~~~~cCCACAGAAGCAC
H. armigera (R) ~~~~~~~~~~~~~~~~aCTACGACAACAACTACGCCCACTACGGAAGCGCCTACGACCACA~~~~~~~~~~~~~~~~~~~~~~~~~~~~aCTACAGAAGCTA
H. armigera (S) ACTACTACAGAGGCGCCTACGACAACAACTACGACCACTACGGAAGCGCCTACGACCACA~~~~~~~~~~~~~~~~~~~~~~~~~~~~aCTACAGAAGCTA
H. punctigera ACTACTACAGAAGCATCTACGACACCCTCTACAACT~~~~~~~~~CCGACTACGACTACA~~~~~~~~~~~~~~~~~~~~~~~~~~~~aCTACAGAACCTA
O. nubilalis GTGACCACAGAAGAATCTGTAACGGTCACTCCC~~~~~~~~~~~~~~~~~~~~CCAGAACTT~~~~~~~~~~~~~~~~~~~~~~~~~~~~gATATTGAAGTAA
S. exigua GATACCCCTTCCACACCTGAAGATACCCCTTCCACACCTGGTGATACCACATCAGACGCTACTATTACTGACGAACCAGGACCGAGCTCTACTGAACCAA
T. ni ~~~GCCCCACCAGTAACCGAAACTGAAGCCTCG~~~~~~~~~~~~~~~~~~~~ACCAGCAGC~~~~~~~~~~~~~~~~~~~~~~~~~~~~cCGACAGCTGCTC
3110 3120 3130 3140 3150 3160 3170 3180 3190 3200
B. mori CAGCT~~~GTCACCCCTGCACCCGATTCAGCGAACGTCGCAGCCCTAAGCTTTATCACACTGATCATc~~~CTTTAG
D. saccharalis CCACAGAAGCACCCACAGAAGCACCCACAGAAGCCACTGAAACTACTACCACTGAAACTACTACCACTGAAACTACTACCACTGAAACTACTACCACTGA
H. armigera (R) CAACA~~~~~~ACACCTGTACCTGGCTCAGCAAACATCGCCACTCTTAGCATCGTCACAATGATCGTg~~~ACTCTCGTTGTTAATATGGCTTAA
H. armigera (S) CAACA~~~~~~ACACCTGTACCTGGCTCAGCAAACATCGCCACTCTTAGCATCGTCACAATGATCGTg~~~ACTCTCGTTGTTAATATGGCTTAA
H. punctigera CAACA~~~~~~ACACCTGCAACTGACTCAGCAAACATCGCCACTCTTAGCATCGTCACATTGATCGTg~~~ACCCTTGTTGTTAATATGGCTTAA
O. nubilalis CAACTGAAGTTGATGATGAATCAGATTCAGCTAACATCGCAGCGTTGAGCATTTTCACTCTTATTATa~~~AGGGTCTCTATTAATCTAATTAGTTAA
S. exigua CCACG~~~~~~ACCGCGGAGCCCGGCTCTGCGAACATTGCTTCTCTTAGCTTCTTCACTTTGATAATc~~~ACAGTTATCATCAACATGGTTTAA
T. ni CGTCC~~~ACCACCGAGGCTCCTGCCTCAGCAAGCACTGCCGCACTTAGTGTCGTCGCCATGTTGGTc~~~ACCCTTGCTGTCAACATGGTCTAA
3210 3220 3230 3240 3250 3260 3270 3280 3290 3300
B. mori
D. saccharalis AACTACTACCACTGAAACTACTACTACTGAAACTACCACTACTGATACTACTACTACAGAAGCACCCACAGAAGCACCCACAGAAGCACCCACAGAAGCA
H. armigera (R)
H. armigera (S)
H. punctigera
O. nubilalis
S. exigua
T. ni

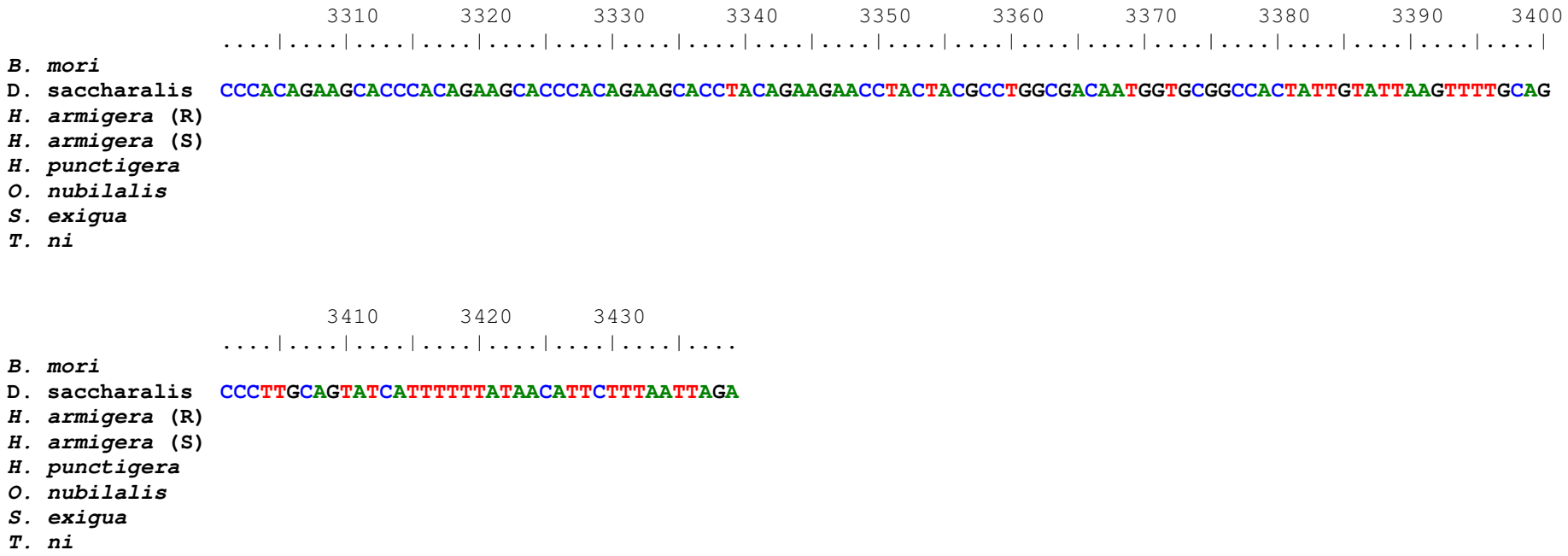
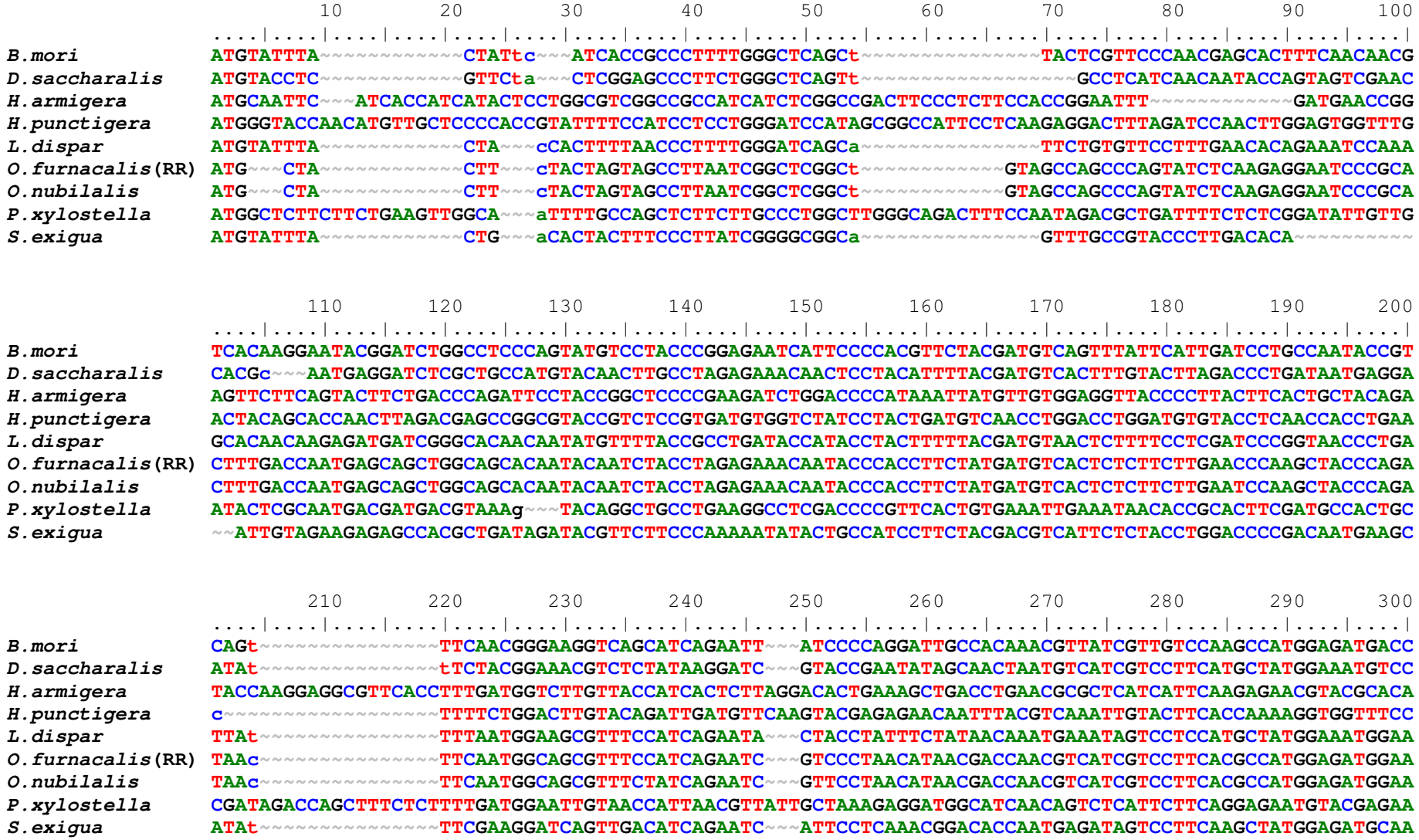
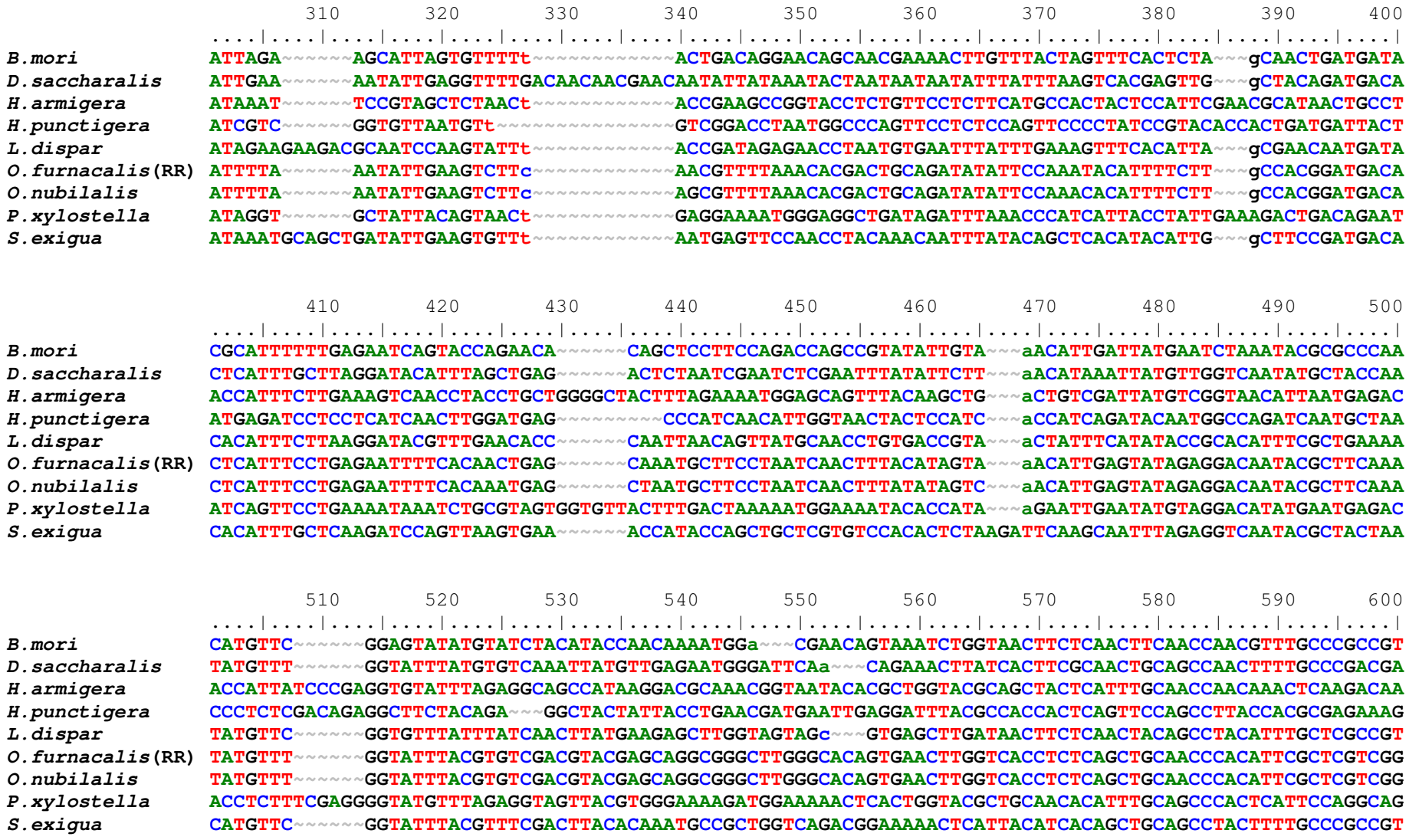


Figure B.1: DNA sequence alignment of *aminopeptidase N1* (*APN1*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (112983237),*Diatraea saccharalis* (302403438), *Helicoverpa armigera* (R) (184161311), *Helicoverpa armigera* (S) (184161309), *Helicoverpa punctigera* (7158839), *Ostrinia nubilalis* (215261001), *Spodoptera exigua* (37788335) and *Trichoplusia ni* (61200970).

B.2. *Aminopeptidase N2* (*APN2*)

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D.saccharalis ATTGAA~~~~~~AATATTGAGGTTTTGACAACAACGAACAATATTATAAATACTAATAATAATATTTATTTAAGTCACGAGTTG~~~gCTACAGATGACA
H.armigera ATAAAT~~~~~~TCCGTAGCTCTAACt~~~~~~~~~~~~~~ACCGAAGCCGGTACCTCTGTTCCTCTTCATGCCACTACTCCATTCGAACGCATAACTGCCT
H.punctigera ATCGTC~~~~~~GGTGTTAATGTt~~~~~~~~~~~~~~~~~GTCGGACCTAATGGCCCAGTTCCTCTCCAGTTCCCCTATCCGTACACCACTGATGATTACT
L.dispar ATAGAAGAAGACGCAATCCAAGTATTt~~~~~~~~~~~~~~ACCGATAGAGAACCTAATGTGAATTTATTTGAAAGTTTCACATTA~~~gCGAACAATGATA
O.furnacalis(RR) ATTTTA~~~~~~AATATTGAAGTCTTc~~~~~~~~~~~~~~AACGTTTTAAACACGACTGCAGATATATTCCAAATACATTTTCTT~~~gCCACGGATGACA
O.nubilalis ATTTTA~~~~~~AATATTGAAGTCTTc~~~~~~~~~~~~~~AGCGTTTTAAACACGACTGCAGATATATTCCAAACACATTTTCTT~~~gCCACGGATGACA
P.xylostella ATAGGT~~~~~~GCTATTACAGTAACt~~~~~~~~~~~~~~GAGGAAAATGGGAGGCTGATAGATTTAAACCCATCATTACCTATTGAAAGACTGACAGAAT
S.exigua ATAAATGCAGCTGATATTGAAGTGTTt~~~~~~~~~~~~~~AATGAGTTCCAACCTACAAACAATTTATACAGCTCACATACATTG~~~gCTTCCGATGACA
410 420 430 440 450 460 470 480 490 500
B.mori CGCATTTTTTGAGAATCAGTACCAGAACA~~~~~~CAGCTCCTTCCAGACCAGCCGTATATTGTA~~~aACATTGATTATGAATCTAAATACGCGCCCAA
D.saccharalis CTCATTTGCTTAGGATACATTTAGCTGAG~~~~~~ACTCTAATCGAATCTCGAATTTATATTCTT~~~aACATAAATTATGTTGGTCAATATGCTACCAA
H.armigera ACCATTTCTTGAAAGTCAACCTACCTGCTGGGGCTACTTTAGAAAATGGAGCAGTTTACAAGCTG~~~aCTGTCGATTATGTCGGTAACATTAATGAGAC
H.punctigera ATGAGATCCTCCTCATCAACTTGGATGAG~~~~~~~~~CCCATCAACATTGGTAACTACTCCATC~~~aCCATCAGATACAATGGCCAGATCAATGCTAA
L.dispar CACATTTCTTAAGGATACGTTTGAACACC~~~~~~CAATTAACAGTTATGCAACCTGTGACCGTA~~~aCTATTTCATATACCGCACATTTCGCTGAAAA
O.furnacalis(RR) CTCATTTCCTGAGAATTTTCACAACTGAG~~~~~~CAAATGCTTCCTAATCAACTTTACATAGTA~~~aACATTGAGTATAGAGGACAATACGCTTCAAA
O.nubilalis CTCATTTCCTGAGAATTTTCACAAATGAG~~~~~~CTAATGCTTCCTAATCAACTTTATATAGTC~~~aACATTGAGTATAGAGGACAATACGCTTCAAA
P.xylostella ATCAGTTCCTGAAAATAAATCTGCGTAGTGGTGTTACTTTGACTAAAAATGGAAAATACACCATA~~~aGAATTGAATATGTAGGACATATGAATGAGAC
S.exigua CACATTTGCTCAAGATCCAGTTAAGTGAA~~~~~~ACCATACCAGCTGCTCGTGTCCACACTCTAAGATTCAAGCAATTTAGAGGTCAATACGCTACTAA
510 520 530 540 550 560 570 580 590 600
B.mori CATGTTC~~~~~~GGAGTATATGTATCTACATACCAACAAAATGGa~~~CGAACAGTAAATCTGGTAACTTCTCAACTTCAACCAACGTTTGCCCGCCGT
D.saccharalis TATGTTT~~~~~~GGTATTTATGTGTCAAATTATGTTGAGAATGGGATTCAa~~~CAGAAACTTATCACTTCGCAACTGCAGCCAACTTTTGCCCGACGA
H.armigera ACCATTATCCCGAGGTGTATTTAGAGGCAGCCATAAGGACGCAAACGGTAATACACGCTGGTACGCAGCTACTCATTTGCAACCAACAAACTCAAGACAA
H.punctigera CCCTCTCGACAGAGGCTTCTACAGA~~~GGCTACTATTACCTGAACGATGAATTGAGGATTTACGCCACCACTCAGTTCCAGCCTTACCACGCGAGAAAG
L.dispar TATGTTC~~~~~~GGTGTTTATTTATCAACTTATGAAGAGCTTGGTAGTAGc~~~GTGAGCTTGATAACTTCTCAACTACAGCCTACATTTGCTCGCCGT
O.furnacalis(RR) TATGTTT~~~~~~GGTATTTACGTGTCGACGTACGAGCAGGCGGGCTTGGGCACAGTGAACTTGGTCACCTCTCAGCTGCAACCCACATTCGCTCGTCGG
O.nubilalis TATGTTT~~~~~~GGTATTTACGTGTCGACGTACGAGCAGGCGGGCTTGGGCACAGTGAACTTGGTCACCTCTCAGCTGCAACCCACATTCGCTCGTCGG
P.xylostella ACCTCTTTCGAGGGGTATGTTTAGAGGTAGTTACGTGGGAAAAGATGGAAAAACTCACTGGTACGCTGCAACACATTTGCAGCCCACTCATTCCAGGCAG
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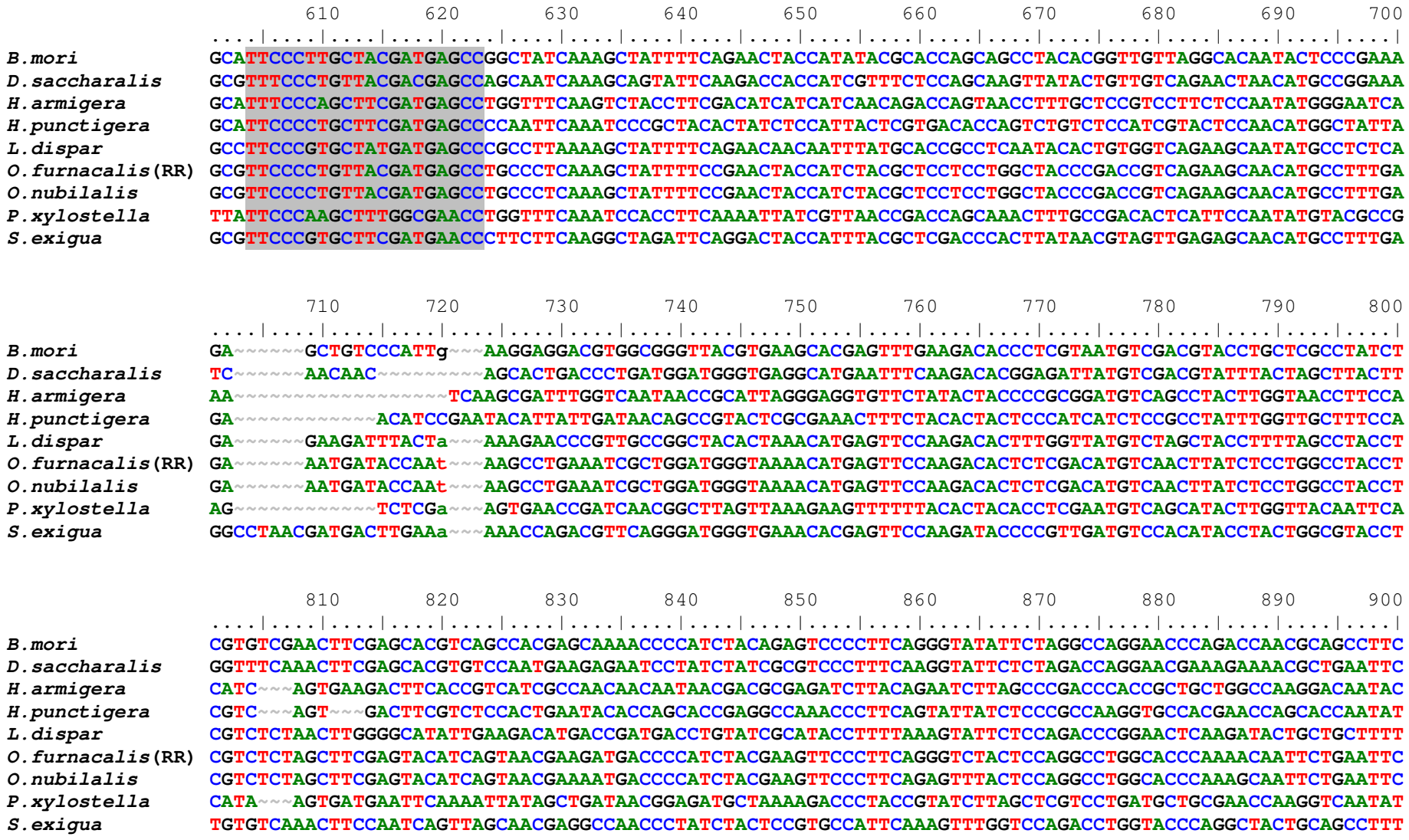
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O.nubilalis GCGTTCCCCTGTTACGATGAGCCTGCCCTCAAAGCTATTTTCCGAACTACCATCTACGCTCCTCCTGGCTACCCGACCGTCAGAAGCAACATGCCTTTGA
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H.armigera AA~~~~~~~~~~~~~~~~~~TCAAGCGATTTGGTCAATAACCGCATTAGGGAGGTGTTCTATACTACCCCGCGGATGTCAGCCTACTTGGTAACCTTCCA
H.punctigera GA~~~~~~~~~~~~~ACATCCGAATACATTATTGATAACAGCCGTACTCGCGAAACTTTCTACACTACTCCCATCATCTCCGCCTATTTGGTTGCTTTCCA
L.dispar GA~~~~~~GAAGATTTACTa~~~AAAGAACCCGTTGCCGGCTACACTAAACATGAGTTCCAAGACACTTTGGTTATGTCTAGCTACCTTTTAGCCTACCT
O.furnacalis(RR) GA~~~~~~AATGATACCAAt~~~AAGCCTGAAATCGCTGGATGGGTAAAACATGAGTTCCAAGACACTCTCGACATGTCAACTTATCTCCTGGCCTACCT
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S.exigua GGCCTAACGATGACTTGAAa~~~AAACCAGACGTTCAGGGATGGGTGAAACACGAGTTCCAAGATACCCCGTTGATGTCCACATACCTACTGGCGTACCT
810 820 830 840 850 860 870 880 890 900
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D.saccharalis GGTTTCAAACTTCGAGCACGTGTCCAATGAAGAGAATCCTATCTATCGCGTCCCTTTCAAGGTATTCTCTAGACCAGGAACGAAAGAAAACGCTGAATTC
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L.dispar CGTCTCTAACTTGGGGCATATTGAAGACATGACCGATGACCTGTATCGCATACCTTTTAAAGTATTCTCCAGACCCGGAACTCAAGATACTGCTGCTTTT
O.furnacalis(RR) CGTCTCTAGCTTCGAGTACATCAGTAACGAAGATGACCCCATCTACGAAGTTCCCTTCAGGGTCTACTCCAGGCCTGGCACCCAAAACAATTCTGAATTC
O.nubilalis CGTCTCTAGCTTCGAGTACATCAGTAACGAAAATGACCCCATCTACGAAGTTCCCTTCAGAGTTTACTCCAGGCCTGGCACCCAAAGCAATTCTGAATTC
P.xylostella CATA~~~~AGTGATGAATTCAAAATTATAGCTGATAACGGAGATGCTAAAAGACCCTACCGTATCTTAGCTCGTCCTGATGCTGCGAACCAAGGTCAATAT
S.exigua TGTGTCAAACTTCCAATCAGTTAGCAACGAGGCCAACCCTATCTACTCCGTGCCATTCAAAGTTTGGTCCAGACCTGGTACCCAGGCTACTGCAGCCTTT


910 920 930 940 950 960 970 980 990 1000
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D.saccharalis GCTATGGATTTTGGTCAAAAGAATATGGTCAAATTGGAAGAATATACGGAATTCGATTAt~~~~~~~~~~~~~~~~~~~~GTCTTCCCGAAACTAGACAAAG
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H.punctigera GCTGCTGAAATTGGTCTTAAGATCACTAACGAACTCGATGACTACTTTGGCATCCAGTACCATGAGATGGGACAAGGTGCCTTGATGAAGAATGACCACA
L.dispar GCTCTAGATTTTGGTCAAAAAAATATGCAAGCACTAGAAGACTATACTGAATTTCCATAt~~~~~~~~~~~~~~~~~~~~TTGCTACCAAAAATGGATAAAG
O.furnacalis(RR) GCTTTGGACTTTGGACAGAAGAACATGATCGCTTTGGAGAATTACATTGAACTGCCCTAt~~~~~~~~~~~~~~~~~~~~GCGTTCCCTAAGCTGGACAAAG
O.nubilalis GCTTTGGACTTTGGACAGAAGAACATGATCGCTTTGGAAAATTACATTGAACTGCCCTAt~~~~~~~~~~~~~~~~~~~~GCGTTCCCTAAGCTGGATAAAG
P.xylostella GCTTTGGAGGTTGGCCCTCCACTGACGAAATGGCTGGAGGAATATTTGGGTAAGCCATACTACGAAATGGCTGAGAAC~~~~~~~ATGAAGAATGATCAAA
S.exigua GCTCTGGAGTTTGGTCAACAGAACATGGTAGAACTAGAAAAATACACTGAGTTTAAATAc~~~~~~~~~~~~~~~~~~~~GATGTTCCAAAACTTGACAAAG
1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
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H.armigera TAGCTTCTCCTTATTGGGCTTCAGGAGCCACTGAAAACTGGGGATTGGTAACATACAGGGAACTGCGTCTGTTATACCAAGAAGGTGAGACCAATGCTTT
H.punctigera TCGCACTTCCTGACTTTCCCTCCGGTGCTATGGAAAACTGGGGAATGGTAAACTATAGAGAGGCCTACCTGTTGTACGACGCAAACAACACCAACTTGAA
L.dispar CTGCTGTACCTGATTTTGCTGCCGGCGCTATGGAAAACTGGGGATTGGTTATCTATAGAGAAGTAGCACTTCTGGTTACTGAAGGTGTAACCACAACGCA
O.furnacalis(RR) CTGCAGTTCCTGATTTTGCTGCCGGTGCTATGGAGAACTGGGGACTGGTGATTTACAGAGAAGTTGCCCTCCTTGTCACTGAAGGTGTCACGACCACCCA
O.nubilalis CTGCAGTTCCTGATTTTGCTGCCGGTGCTATGGAGAACTGGGGACTGGTGATTTACAGAGAAGTTGCCCTCCTTGTCACTGAAGGTGTCACGACCACCCA
P.xylostella TTGCTTCACCGTTTTGGGCGTCTGGTGCTACGGAGAACTGGGGATTGGTGACGTACAGAGAGCCCCGCCTCCTCTACGAAGAGGGGGAGACGAATGCCGT
S.exigua CTGCTGTTCCTGACTTTGCTGCTGGTGCTATGGAGAACTGGGGATTGGTCATTTACAGAGAAGTAGCTCTTCTGGTCACTGATGGAGTGACGACCACTGC
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
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D.saccharalis AACGAGACAAAACATCGGCAGAATAATATGCCACGAAAATATGCACATGTGGTTTGGTAATGAAGTAAGCCCCTACTCTTGGACTTATACATGGCTCAAC
H.armigera GGACAAGATGTACATCGGTACTATTACTGCTCACGAGCTCGCTCACAAGTGGTTCGGTAACCTCATCACCTGCAGATGGTGGGATAACGTCTGGATCAAT
H.punctigera CAACAAGATTTTCATCGCTACAATCATGGCTCACGAATTGGGACACAAATGGTTCGGTAACCTCGTCACCTGCTTTTGGTGGAGCAACCTTTGGCTAAAC
L.dispar GACTAAGCAGAATATAGGCAGAATTATCTGCCATGAAAACGTGCATCAATGGTTCGGTAATGAGGTTGGACCCCAATCTTGGACTTTCACCTGGCTGAAC
O.furnacalis(RR) AACCCTTCAGAACATTGGTCGCATCATCTGCCACGAGAACATGCACATGTGGTTCGGAAACGAGGTCGGGCCTTACTCTTGGACCTATACCTGGCTCAAC
O.nubilalis AACCCTTCAGAACATTGGTCGCATCATCTGCCACGAGAACATGCACATGTGGTTCGGAAACGAGGTCGGGCCTTACTCTTGGACCTATACCTGGCTCAAC
P.xylostella GGACAAGATGAGCATCGGCACCATCATGGCCCACGAGCTCGGACACAAGTGGTTCGGCAACCTGGTGACGGCGCGCTGGTGGGACAACGTGTGGATCAAC
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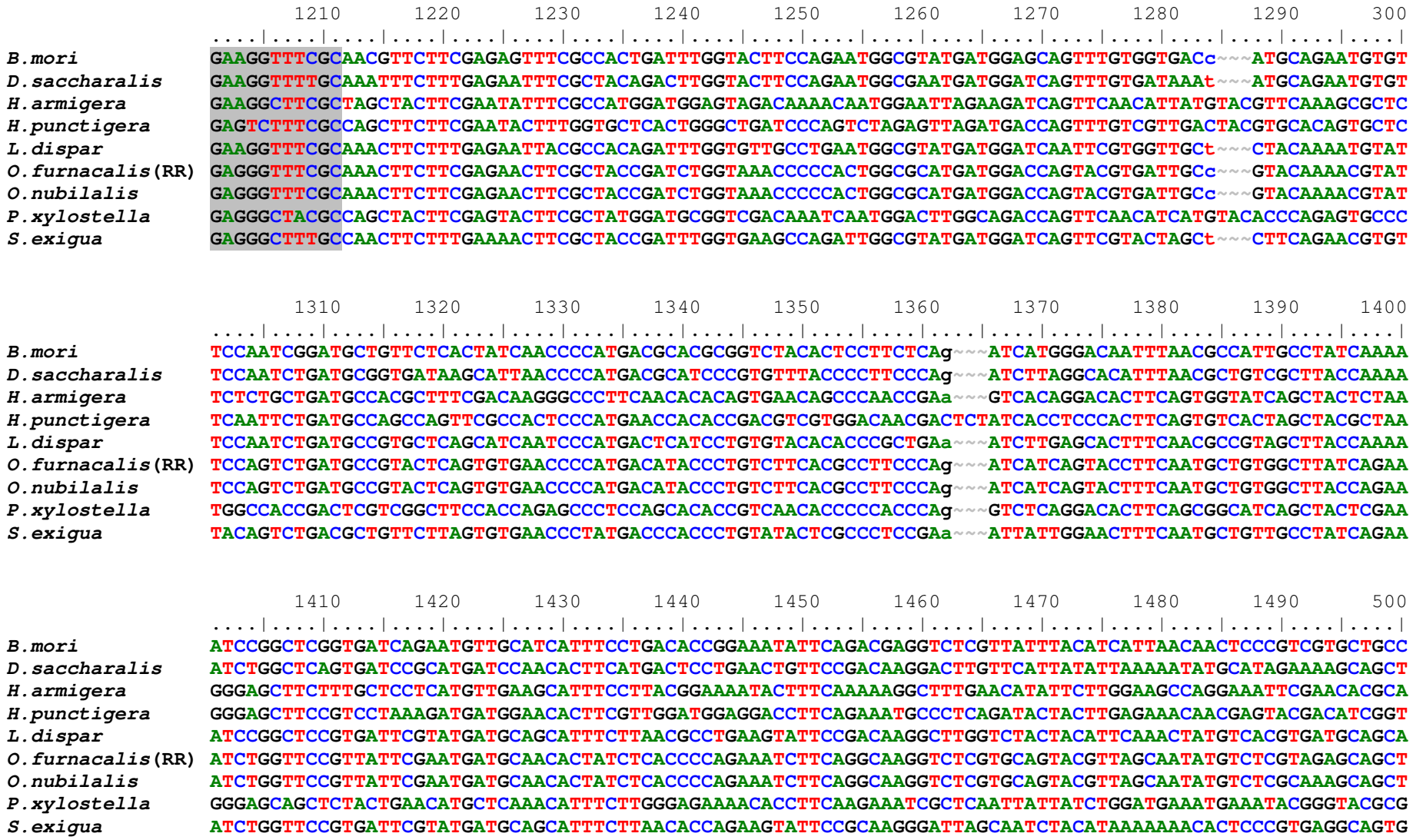
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H.armigera GAAGGCTTCGCTAGCTACTTCGAATATTTCGCCATGGATGGAGTAGACAAAACAATGGAATTAGAAGATCAGTTCAACATTATGTACGTTCAAAGCGCTC
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L.dispar GAAGGTTTCGCAAACTTCTTTGAGAATTACGCCACAGATTTGGTGTTGCCTGAATGGCGTATGATGGATCAATTCGTGGTTGCt~~~CTACAAAATGTAT
O.furnacalis(RR) GAGGGTTTCGCAAACTTCTTCGAGAACTTCGCTACCGATCTGGTAAACCCCCACTGGCGCATGATGGACCAGTACGTGATTGCc~~~GTACAAAACGTAT
O.nubilalis GAGGGTTTCGCAAACTTCTTCGAGAACTTCGCTACCGATCTGGTAAACCCCCACTGGCGCATGATGGACCAGTACGTGATTGCc~~~GTACAAAACGTAT
P.xylostella GAGGGCTACGCCAGCTACTTCGAGTACTTCGCTATGGATGCGGTCGACAAATCAATGGACTTGGCAGACCAGTTCAACATCATGTACACCCAGAGTGCCC
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D.saccharalis TCCAATCTGATGCGGTGATAAGCATTAACCCCATGACGCATCCCGTGTTTACCCCTTCCCAg~~~ATCTTAGGCACATTTAACGCTGTCGCTTACCAAAA
H.armigera TCTCTGCTGATGCCACGCTTTCGACAAGGGCCCTTCAACACACAGTGAACAGCCCAACCGAa~~~GTCACAGGACACTTCAGTGGTATCAGCTACTCTAA
H.punctigera TCAATTCTGATGCCAGCCAGTTCGCCACTCCCATGAACCACACCGACGTCGTGGACAACGACTCTATCACCTCCCACTTCAGTGTCACTAGCTACGCTAA
L.dispar TCCAATCTGATGCCGTGCTCAGCATCAATCCCATGACTCATCCTGTGTACACACCCGCTGAa~~~ATCTTGAGCACTTTCAACGCCGTAGCTTACCAAAA
O.furnacalis(RR) TCCAGTCTGATGCCGTACTCAGTGTGAACCCCATGACATACCCTGTCTTCACGCCTTCCCAg~~~ATCATCAGTACCTTCAATGCTGTGGCTTATCAGAA
O.nubilalis TCCAGTCTGATGCCGTACTCAGTGTGAACCCCATGACATACCCTGTCTTCACGCCTTCCCAg~~~ATCATCAGTACTTTCAATGCTGTGGCTTACCAGAA
P.xylostella TGGCCACCGACTCGTCGGCTTCCACCAGAGCCCTCCAGCACACCGTCAACACCCCCACCCAg~~~GTCTCAGGACACTTCAGCGGCATCAGCTACTCGAA
S.exigua TACAGTCTGACGCTGTTCTTAGTGTGAACCCTATGACCCACCCTGTATACTCGCCCTCCGAa~~~ATTATTGGAACTTTCAATGCTGTTGCCTATCAGAA
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D.saccharalis ATCTGGCTCAGTGATCCGCATGATCCAACACTTCATGACTCCTGAACTGTTCCGACAAGGACTTGTTCATTATATTAAAAATATGCATAGAAAAGCAGCT
H.armigera GGGAGCTTCTTTGCTCCTCATGTTGAAGCATTTCCTTACGGAAAATACTTTCAAAAAGGCTTTGAACATATTCTTGGAAGCCAGGAAATTCGAACACGCA
H.punctigera GGGAGCTTCCGTCCTAAAGATGATGGAACACTTCGTTGGATGGAGGACCTTCAGAAATGCCCTCAGATACTACTTGAGAAACAACGAGTACGACATCGGT
L.dispar ATCCGGCTCCGTGATTCGTATGATGCAGCATTTCTTAACGCCTGAAGTATTCCGACAAGGCTTGGTCTACTACATTCAAACTATGTCACGTGATGCAGCA
O.furnacalis(RR) ATCTGGTTCCGTTATTCGAATGATGCAACACTATCTCACCCCAGAAATCTTCAGGCAAGGTCTCGTGCAGTACGTTAGCAATATGTCTCGTAGAGCAGCT
O.nubilalis ATCTGGTTCCGTTATTCGAATGATGCAACACTATCTCACCCCAGAAATCTTCAGGCAAGGTCTCGTGCAGTACGTTAGCAATATGTCTCGCAAAGCAGCT
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S.exigua ATCTGGTTCCGTGATTCGTATGATGCAGCATTTCTTAACACCAGAAGTATTCCGCAAGGGATTAGCAATCTACATAAAAAAACACTCCCGTGAGGCAGTG


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H.armigera TTCCCAGCAGACCTCTTCAGTGCTTTTGCTACAGCAGTTCAGCAAGAT~~~~~~~~~gGTGTACCATCAAACACCTTCGATATTGCATCATTCATGAAAT
H.punctigera TTCCCCGTTGATATGTACACGGCATTCAAGCAAGCAGTTGCTGAAGATGTTAACTACCAACGTGATTTCCCCAATGTTGACGTTGGCGCAGTATTCGACA
L.dispar GCACCTATTCACCTGTATGCTGCATTACAGCGAGCATTAACTGAGTCT~~~aACCATTCCATCCCGTACGCC~~~~~~~~~TTGAACACAGTCATGGATC
O.furnacalis(RR) CAGCCCTCCGACCTCTTCGCTAACCTCCAGGAAGTGTTAGACGCCTCC~~~aACCACAGCATTCCGTGGCCG~~~~~~~~~ATAGCCACCATCATGGACC
O.nubilalis CAGCCCTCCGACCTCTTCGCTAACCTCCAGGAAGTGTTAGACGCCTCC~~~aACCACAGCATTCCGTGGCCG~~~~~~~~~ATAGCCACCATCATGGACC
P.xylostella AATCCGGACGATGTGTTCCGTGGTTTCGCAAGGGCCGTGCAAGAAGAT~~~gGCGCTCTCACTCAGTTCACGAATGTGAACATAACTGACTTCCTGTCCG
S.exigua GTTCCCTCTCAACTATACAGCGCTCTCCAACAAGCACTGGATGAGTCT~~~gATCATACTATTCCTTTCCAA~~~~~~~~~ATTATCGATGTGATGTCCC
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D.saccharalis GCTGGACTACTCAAGGTGGTTTCCCAGTCCTTAATGTTACAAGATCTTCTGCTGCTGCCAACTCCTTAGTATTCCAGCAGGAACGATATTTGACCGACCG
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H.punctigera CCTGGGTCCAGAACCGTGGATCTCCCGTCATCAACGTTTCTCGTAACAACAGCACCGGTGTC~~~ATCACTGTCAGACAGCAACGTTACGTGCTCTCTGG
L.dispar GATGGGTCAATCAGGGTGGCTTCCCAGTATTGACAGTCACCAGAAGCGCTGCAACTGCTGAATCTCTTGTTGTCGAACAGGTTCGATTCTTGACTGATAA
O.furnacalis(RR) GCTGGACAAACCAGGGAGGATTCCCGGTCCTCACTGTGTTCAGGTCTGCACCTACTGCCAACTCTTTGACCATCGCTCAGGAACGCTTCCTCACCGACCG
O.nubilalis GCTGGACCAACCAGGGAGGATTCCCGGTCCTCACTGTGTTCGGGTCCGCACCTACTGCCAACTCTTTGACCATCGCTCAGGAACGTTTCCTCACCGACCG
P.xylostella ACTGGGTCTACGAGCCAGGGTACCCCGTCATCAACGTCGACATCAATATGAACACGGGGAAT~~~ATTTACATAGAACAGGAGCGTTTCTTCACGACAAC
S.exigua GTTGGGCGTACCAGGGTGGTTTCCCAGTTTTGACAGTCACACGAAGCGCTGCCGCTGCCAATTCAATTACCGTTGCACAGGAACGTTACCTCACCGACAA
1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
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D.saccharalis TTCACTTTCATCTCCTGATCGt~~~TGGCACGTACCCATCAACGTGGTCTTGAATGACAATCCCGATTTCTCAGATACTAAGCCTGATGGCTGGGTATCA
H.armigera CACTGCCACGCCCACCGACCAAGTTTGGCCGCTGCCCCTTACTTACACAACTGAGAGCAACCCTGACTGGCAGAACTTGTTGCCTAGTAAA~~~~~~~~~~
H.punctigera CGCTGTAGCCCCAGCg~~~~~~CTTTGGCACATTCCTCTCACCTGGACTCAACATGGCTCCCTCAACTTTAACAGCACCAGGCCTAGCACC~~~~~~~~~~
L.dispar CACTTTAGTATCATCTGATCGc~~~TGGCATGTACCTATCAACTGGGTTCTATCTACAGACCCTGACTTCTCCGACACTCAACCAATGGAATGGATACCG
O.furnacalis(RR) CAGTCTGACCTCCACTGACCGt~~~TGGCACGTACCAGTCAACTGGGTGCTGTCAACCGACCCCAACTTCAACGACACCAGCCCACAAGGATGGATTCCA
O.nubilalis CAGTCTGACCTCCACTGACCGt~~~TGGCACGTACCAGTCAACTGGGTGCTGTCAACCGACCCCAACTTCAACGACACCAGCCCACAAGGATGGATTCCA
P.xylostella CGGT~~~~~~TCGTCCAATCAGCTGTGGCCGCTGCCCCTGACGTATACTTCGGCCAGCAGCCCCGACTGGAGCAGCACCAGGGCTAGCCAC~~~~~~~~~~
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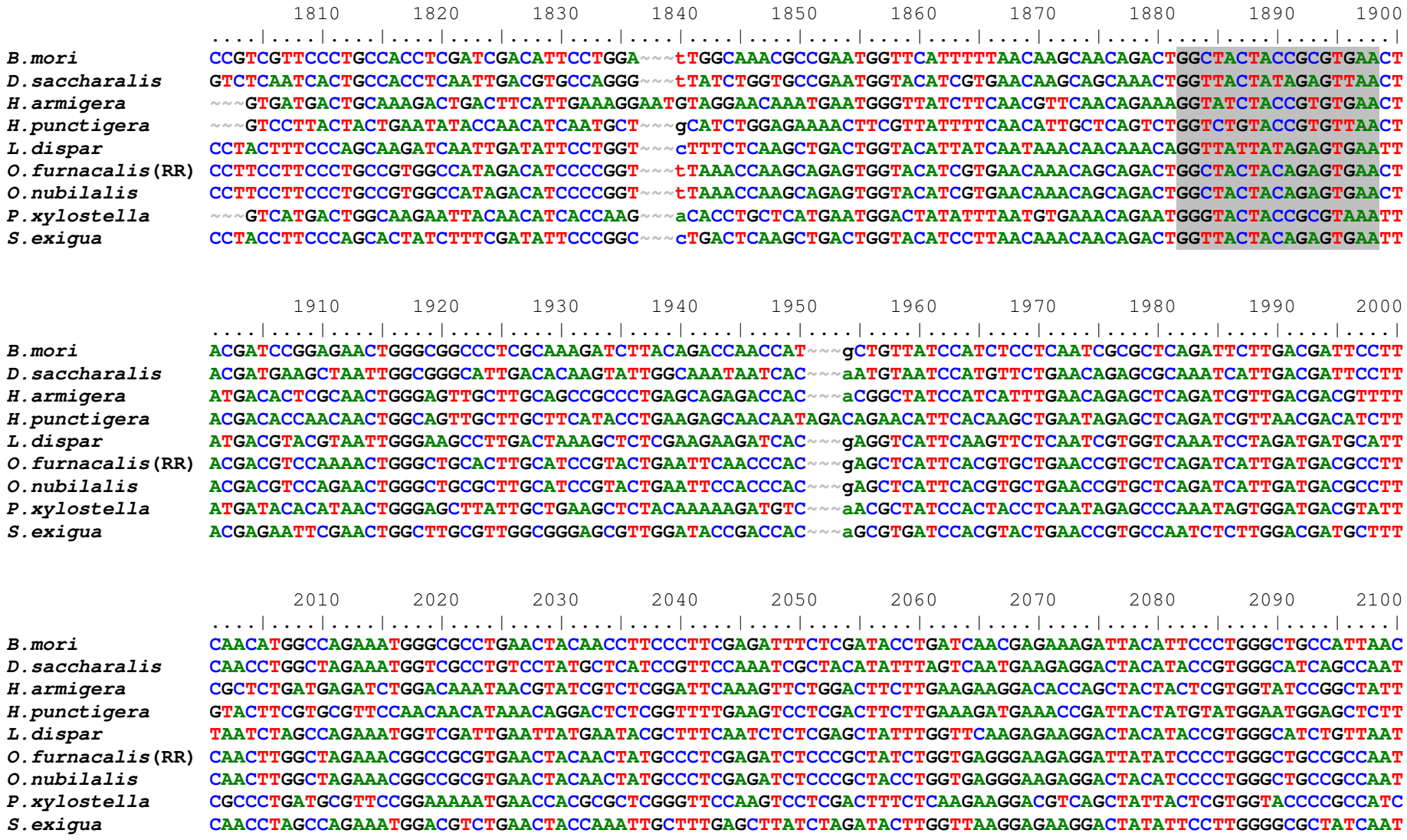
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D.saccharalis GTCTCAATCACTGCCACCTCAATTGACGTGCCAGGG~~~tTATCTGGTGCCGAATGGTACATCGTGAACAAGCAGCAAACTGGTTACTATAGAGTTAACT
H.armigera ~~~GTGATGACTGCAAAGACTGACTTCATTGAAAGGAATGTAGGAACAAATGAATGGGTTATCTTCAACGTTCAACAGAAAGGTATCTACCGTGTGAACT
H.punctigera ~~~GTCCTTACTACTGAATATACCAACATCAATGCT~~~gCATCTGGAGAAAACTTCGTTATTTTCAACATTGCTCAGTCTGGTCTGTACCGTGTTAACT
L.dispar CCTACTTTCCCAGCAAGATCAATTGATATTCCTGGT~~~cTTTCTCAAGCTGACTGGTACATTATCAATAAACAACAAACAGGTTATTATAGAGTGAATT
O.furnacalis(RR) CCTTCCTTCCCTGCCGTGGCCATAGACATCCCCGGT~~~tTAAACCAAGCAGAGTGGTACATCGTGAACAAACAGCAGACTGGCTACTACAGAGTGAACT
O.nubilalis CCTTCCTTCCCTGCCGTGGCCATAGACATCCCCGGT~~~tTAAACCAAGCAGAGTGGTACATCGTGAACAAACAGCAGACTGGCTACTACAGAGTGAACT
P.xylostella ~~~GTCATGACTGGCAAGAATTACAACATCACCAAG~~~aCACCTGCTCATGAATGGACTATATTTAATGTGAAACAGAATGGGTACTACCGCGTAAATT
S.exigua CCTACCTTCCCAGCACTATCTTTCGATATTCCCGGC~~~cTGACTCAAGCTGACTGGTACATCCTTAACAAACAACAGACTGGTTACTACAGAGTGAATT
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
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D.saccharalis ACGATGAAGCTAATTGGCGGGCATTGACACAAGTATTGGCAAATAATCAC~~~aATGTAATCCATGTTCTGAACAGAGCGCAAATCATTGACGATTCCTT
H.armigera ATGACACTCGCAACTGGGAGTTGCTTGCAGCCGCCCTGAGCAGAGACCAC~~~aCGGCTATCCATCATTTGAACAGAGCTCAGATCGTTGACGACGTTTT
H.punctigera ACGACACCAACAACTGGCAGTTGCTTGCTTCATACCTGAAGAGCAACAATAGACAGAACATTCACAAGCTGAATAGAGCTCAGATCGTTAACGACATCTT
L.dispar ATGACGTACGTAATTGGGAAGCCTTGACTAAAGCTCTCGAAGAAGATCAC~~~gAGGTCATTCAAGTTCTCAATCGTGGTCAAATCCTAGATGATGCATT
O.furnacalis(RR) ACGACGTCCAAAACTGGGCTGCACTTGCATCCGTACTGAATTCAACCCAC~~~gAGCTCATTCACGTGCTGAACCGTGCTCAGATCATTGATGACGCCTT
O.nubilalis ACGACGTCCAGAACTGGGCTGCGCTTGCATCCGTACTGAATTCCACCCAC~~~gAGCTCATTCACGTGCTGAACCGTGCTCAGATCATTGATGACGCCTT
P.xylostella ATGATACACATAACTGGGAGCTTATTGCTGAAGCTCTACAAAAAGATGTC~~~aACGCTATCCACTACCTCAATAGAGCCCAAATAGTGGATGACGTATT
S.exigua ACGAGAATTCGAACTGGCTTGCGTTGGCGGGAGCGTTGGATACCGACCAC~~~aGCGTGATCCACGTACTGAACCGTGCCAATCTCTTGGACGATGCTTT
2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
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D.saccharalis CAACCTGGCTAGAAATGGTCGCCTGTCCTATGCTCATCCGTTCCAAATCGCTACATATTTAGTCAATGAAGAGGACTACATACCGTGGGCATCAGCCAAT
H.armigera CGCTCTGATGAGATCTGGACAAATAACGTATCGTCTCGGATTCAAAGTTCTGGACTTCTTGAAGAAGGACACCAGCTACTACTCGTGGTATCCGGCTATT
H.punctigera GTACTTCGTGCGTTCCAACAACATAAACAGGACTCTCGGTTTTGAAGTCCTCGACTTCTTGAAAGATGAAACCGATTACTATGTATGGAATGGAGCTCTT
L.dispar TAATCTAGCCAGAAATGGTCGATTGAATTATGAATACGCTTTCAATCTCTCGAGCTATTTGGTTCAAGAGAAGGACTACATACCGTGGGCATCTGTTAAT
O.furnacalis(RR) CAACTTGGCTAGAAACGGCCGCGTGAACTACAACTATGCCCTCGAGATCTCCCGCTATCTGGTGAGGGAAGAGGATTATATCCCCTGGGCTGCCGCCAAT
O.nubilalis CAACTTGGCTAGAAACGGCCGCGTGAACTACAACTATGCCCTCGAGATCTCCCGCTACCTGGTGAGGGAAGAGGACTACATCCCCTGGGCTGCCGCCAAT
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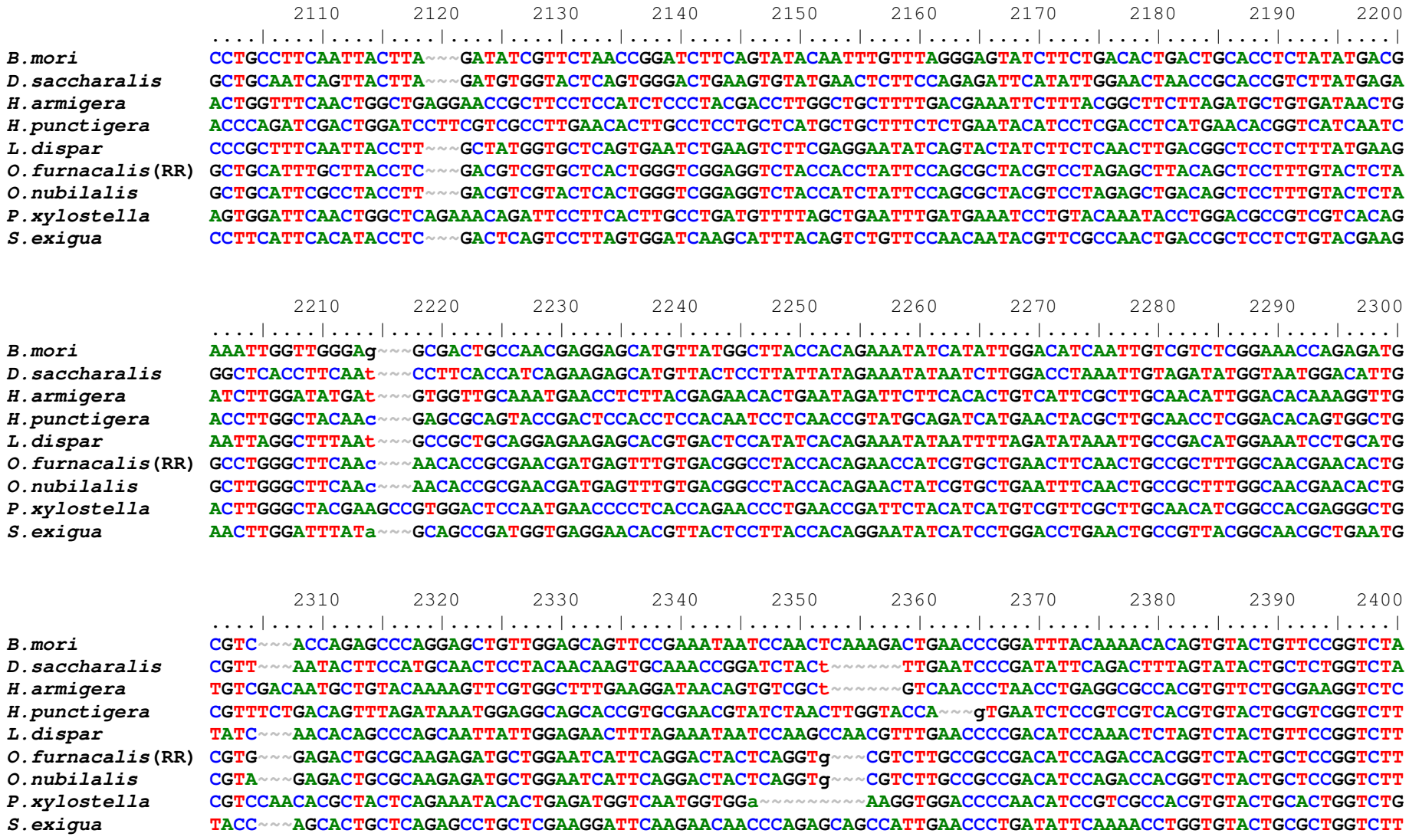
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H.armigera ACTGGTTTCAACTGGCTGAGGAACCGCTTCCTCCATCTCCCTACGACCTTGGCTGCTTTTGACGAAATTCTTTACGGCTTCTTAGATGCTGTGATAACTG
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L.dispar CCCGCTTTCAATTACCTT~~~GCTATGGTGCTCAGTGAATCTGAAGTCTTCGAGGAATATCAGTACTATCTTCTCAACTTGACGGCTCCTCTTTATGAAG
O.furnacalis(RR) GCTGCATTTGCTTACCTC~~~GACGTCGTGCTCACTGGGTCGGAGGTCTACCACCTATTCCAGCGCTACGTCCTAGAGCTTACAGCTCCTTTGTACTCTA
O.nubilalis GCTGCATTCGCCTACCTT~~~GACGTCGTACTCACTGGGTCGGAGGTCTACCATCTATTCCAGCGCTACGTCCTAGAGCTGACAGCTCCTTTGTACTCTA
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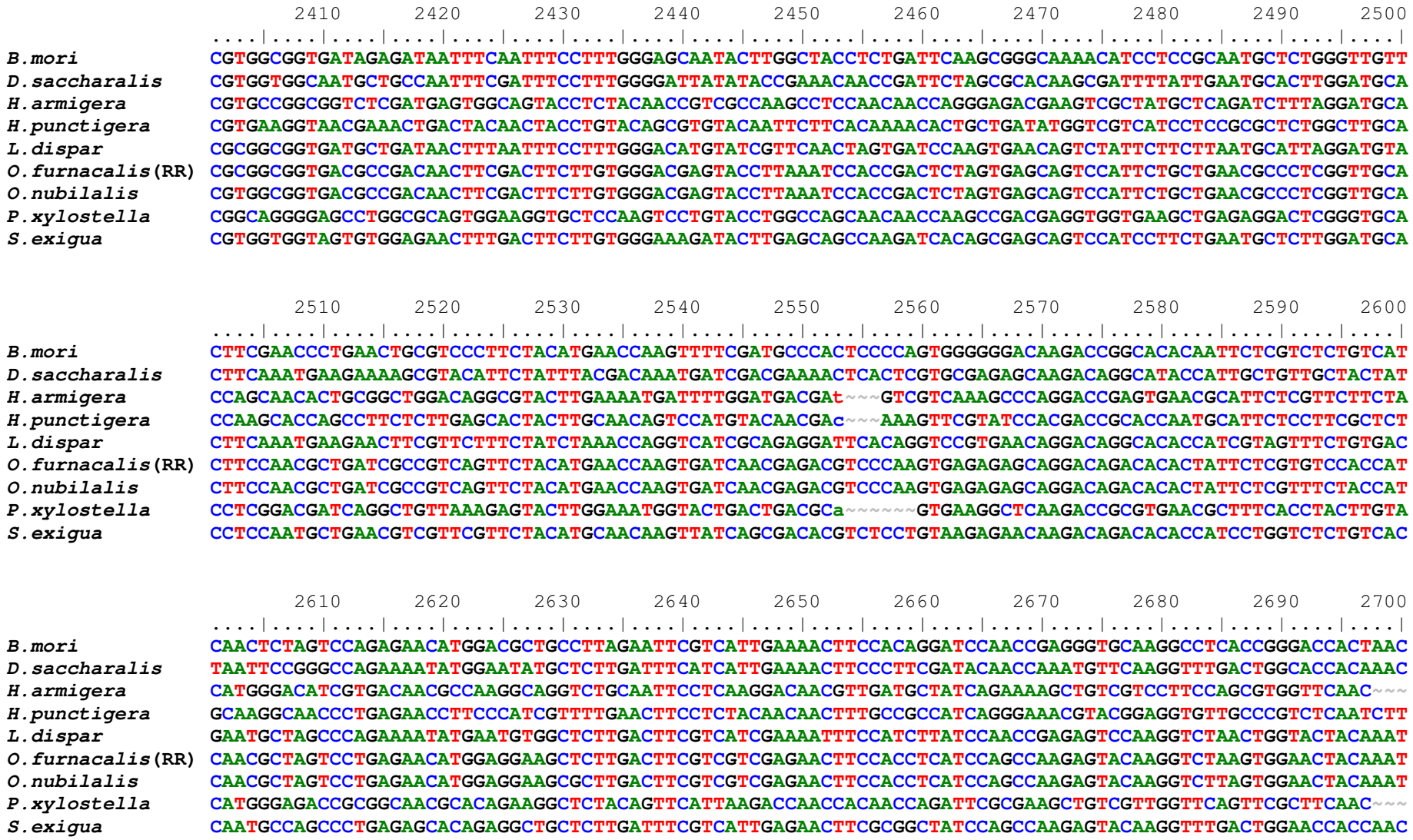
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H.punctigera CCAAGCACCAGCCTTCTCTTGAGCACTACTTGCAACAGTCCATGTACAACGAc~~~AAAGTTCGTATCCACGACCGCACCAATGCATTCTCCTTCGCTCT
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S.exigua CCTCCAATGCTGAACGTCGTTCGTTCTACATGCAACAAGTTATCAGCGACACGTCTCCTGTAAGAGAACAAGACAGACACACCATCCTGGTCTCTGTCAC
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H.armigera CATGGGACATCGTGACAACGCCAAGGCAGGTCTGCAATTCCTCAAGGACAACGTTGATGCTATCAGAAAAGCTGTCGTCCTTCCAGCGTGGTTCAAC~~~
H.punctigera GCAAGGCAACCCTGAGAACCTTCCCATCGTTTTGAACTTCCTCTACAACAACTTTGCCGCCATCAGGGAAACGTACGGAGGTGTTGCCCGTCTCAATCTT
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O.nubilalis CAACGCTAGTCCTGAGAACATGGAGGAAGCGCTTGACTTCGTCGTCGAGAACTTCCACCTCATCCAGCCAAGAGTACAAGGTCTTAGTGGAACTACAAAT
P.xylostella CATGGGAGACCGCGGCAACGCACAGAAGGCTCTACAGTTCATTAAGACCAACCACAACCAGATTCGCGAAGCTGTCGTTGGTTCAGTTCGCTTCAAC~~~
S.exigua CAATGCCAGCCCTGAGAGCACAGAGGCTGCTCTTGATTTCGTCATTGAGAACTTCGCGGCTATCCAGCCAAGAGTACAAGGTTTGACTGGAACCACCAAC

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D.saccharalis     ATTTTGAATGCTCTGGCCCGTAGATTGACAACTACATCTCATTTACAAAAGATTAATGCGTTTGCTACAAGTCATGCAAACATc~~~~~~~~~~~~tTCA
H.armigera        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~aaCGTTCTCACAACCACCGCCGGCTATTTGGACGAAGCTGGTCTGAGGGATATGGAAGAATGGC
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L.dispar          ATCCTTAACGCCTTTGCAAGGAGGTTAACGTCCCAAGCTCATTCTGATAAAGTGGACGAGCTTTACAGCCGTCATGAGTCAATa~~~~~~~~~~~~tTCA
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O.nubilalis       ATTCTGAATGCTCTCTCCAGAAGGCTCACAACGCAAGCTCATTCTGACAAGGTTGACCAGCTGTTCAACCGACACCAGTTCATc~~~~~~~~~~~~tTCA
P.xylostella      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~acCGTGTTGGCGAACTTGGCCGCTTACACTGATGAAGAGGGACTACAGGATATGGAATCATGGC
S.exigua          ATCCTGAACGCATTCGCCAGAAGACTAACAACTCAAGAACATTCCAGCAAGATTGATGAGCTTAGCAGTCGCCACCAATCTATt~~~~~~~~~~~~tTAA

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H.armigera        TGTTGGCTAACCAAAACGCTGTCCCCGAATTCGCt~~~GTGGGCATCAGCGCCATAACCTCAGCTAGAAATAACATGCAGTGGGGTTCAGACAATGCTGC
H.punctigera      TGGAATCCAATCAATTGGAGCTGGCTGGTTCTGTCAACGCTGGAAATAATGTCGTCAGCACCGCCTTGGCTAATCTCGATTGGGGCAACGACGCAGCTGT
L.dispar          CAGCAGGTGAATTG~~~~~~~~~~~~gCTTCAATt~~~GCCGGCATCAGAGAAAATATCGCAGCTTCA~~~~~~aTTACGTGGAGCAATGAAAACGCAAA
O.furnacalis(RR)  CCGCCGGAGAACTG~~~~~~~~~~~~gCCTCCATt~~~GGGGCCATCAGGGAGAACATCGCAGCCTCT~~~~~~cTCACGTGGAGTGCGGAATACTTGGA
O.nubilalis       CCGCCGGAGAACTG~~~~~~~~~~~~gCCTCCATt~~~GGGGCCATCAGGGAAAACATCGCAGCCTCT~~~~~~cTCACGTGGAGTGCAGAATACTTGGA
P.xylostella      TAGAGGAGAACAAAGATTCGATTCCCGAGTATTCt~~~GTCGGCGTGAGCGCGGTGGCATCAGCTCGGGCCAACATGGCGTGGGGCACCCAGAACGCTGA
S.exigua          CCGCTGGAGAGCTA~~~~~~~~~~~~gCATCCATc~~~GCTGGAATCAGAGAAAATATTGCTGCCTCA~~~~~~aTTACATGGAATACTGATAACGCTGC

                        2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
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H.armigera        TACCATTATAGCTGCAGCCAATGATGAAGATCCCCCAGAAGATGGT~~~~~~~~~~~~GGTTCAGGAGAAGAAGTAGACCCG~~~~~~~~~~~~~~~~~~
H.punctigera      TGAAATTGTCAACTTCCTCAACGCTAGAAGCGGa~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
L.dispar          TACGGTAGAAGACTGGCTGTTTGAAAACTACGGa~~~~~~~~~~~~~~~~~~~~~~~~~~~AGCGGT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
O.furnacalis(RR)  CACTGTGGAATCCTGGCTTGTTGAGAACTATACt~~~~~~~~~~~~~~~~~~~~~GACAGT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
O.nubilalis       CACTGTGGAATCCTGGCTTGTTGAGAACTATACt~~~~~~~~~~~~~~~~~~~~~GACAGT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
P.xylostella      CTCCATATTGAAAGCTGCCCGA~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
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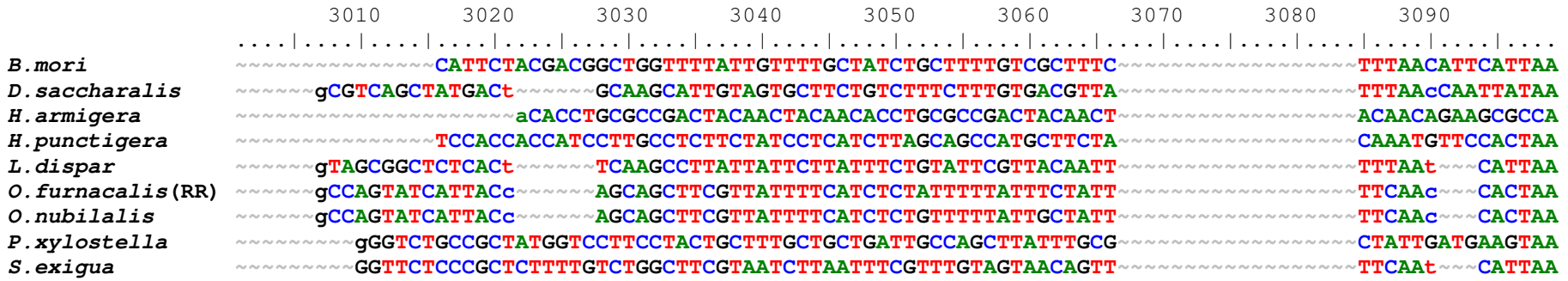
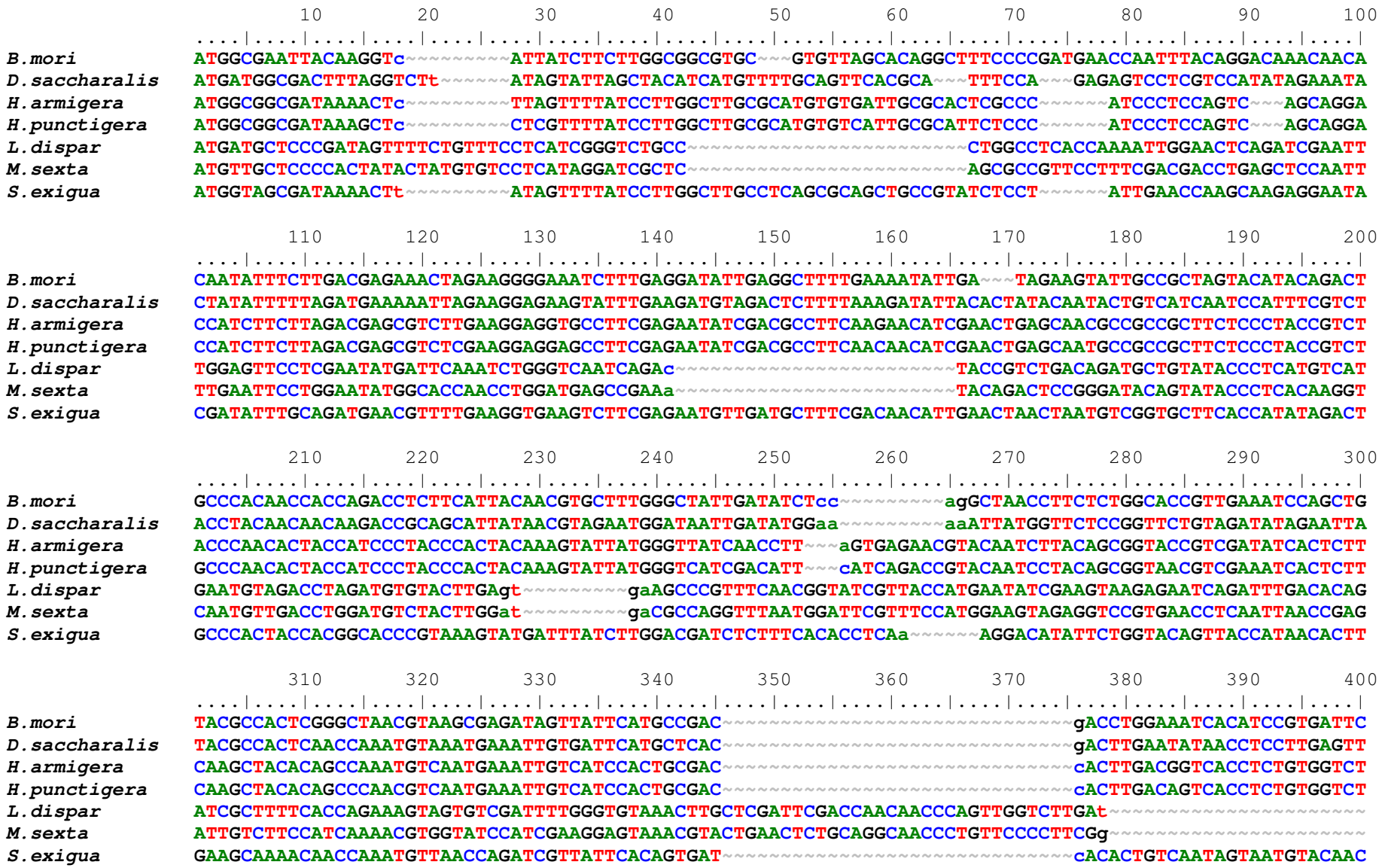
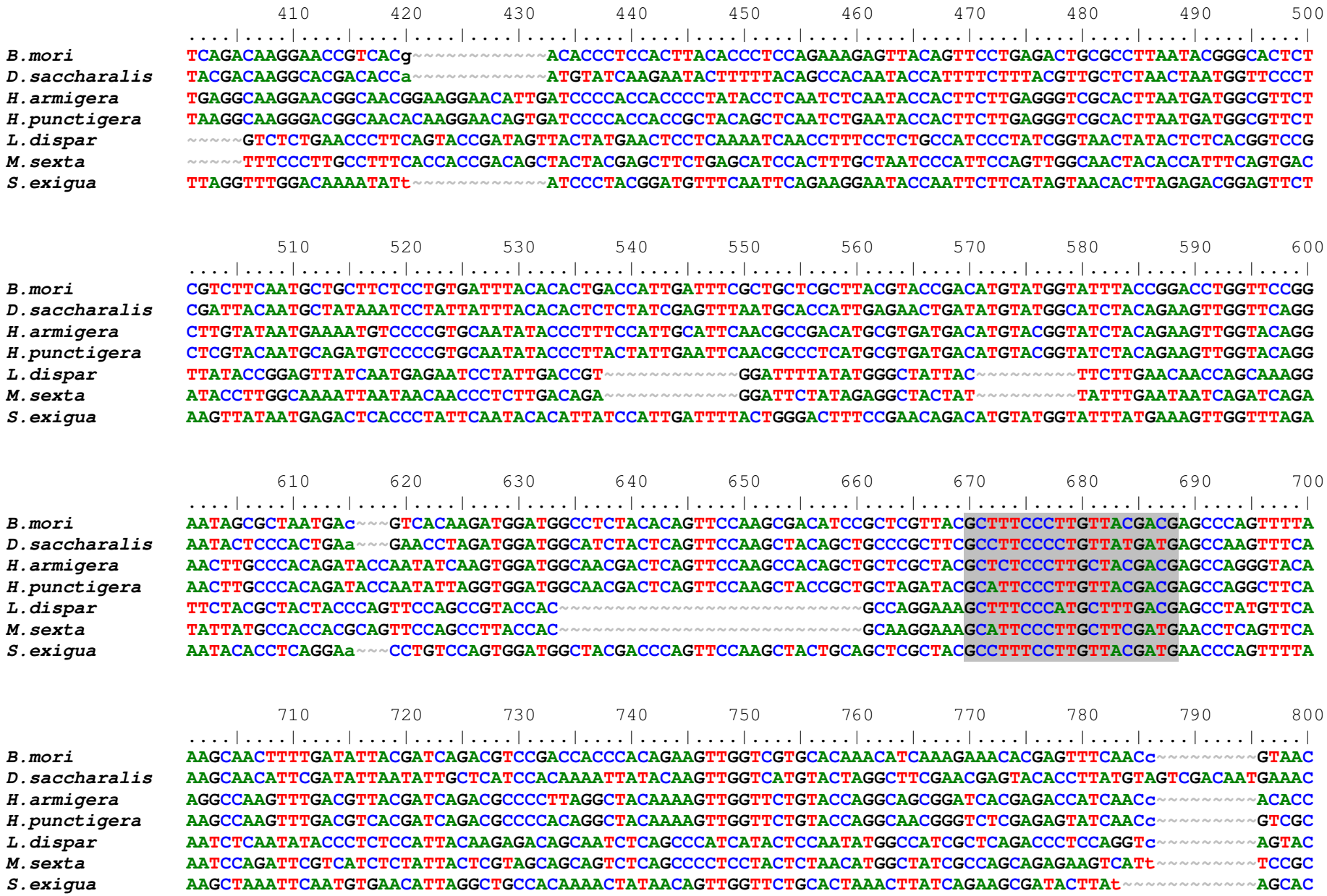


Figure B.2: DNA sequence alignment *of aminopeptidase N2* (*APN2*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (112983995),*Diatraea saccharalis* (302403440), *Helicoverpa armigera* (33641858), *Helicoverpa punctigera* (7158841), *Lymantria dispar* (4868146), *Ostrinia furnacalis* (RR) (194220239), *Ostrinia nubilalis* (215261003), *Plutella xylostella* (48526295) and *Spodoptera exigua* (37788337).

B.3. *Aminopeptidase N3* (*APN3*)

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D.saccharalis TACGACAAGGCACGACACCa~~~~~~~~~~~~~~ATGTATCAAGAATACTTTTTACAGCCACAATACCATTTTCTTTACGTTGCTCTAACTAATGGTTCCCT
H.armigera TGAGGCAAGGAACGGCAACGGAAGGAACATTGATCCCCACCACCCCTATACCTCAATCTCAATACCACTTCTTGAGGGTCGCACTTAATGATGGCGTTCT
H.punctigera TAAGGCAAGGGACGGCAACACAAGGAACAGTGATCCCCACCACCGCTACAGCTCAATCTGAATACCACTTCTTGAGGGTCGCACTTAATGATGGCGTTCT
L.dispar ~~~~~GTCTCTGAACCCTTCAGTACCGATAGTTACTATGAACTCCTCAAAATCAACCTTTCCTCTGCCATCCCTATCGGTAACTATACTCTCACGGTCCG
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S.exigua TTAGGTTTGGACAAAATATt~~~~~~~~~~~~~~ATCCCTACGGATGTTTCAATTCAGAAGGAATACCAATTCTTCATAGTAACACTTAGAGACGGAGTTCT
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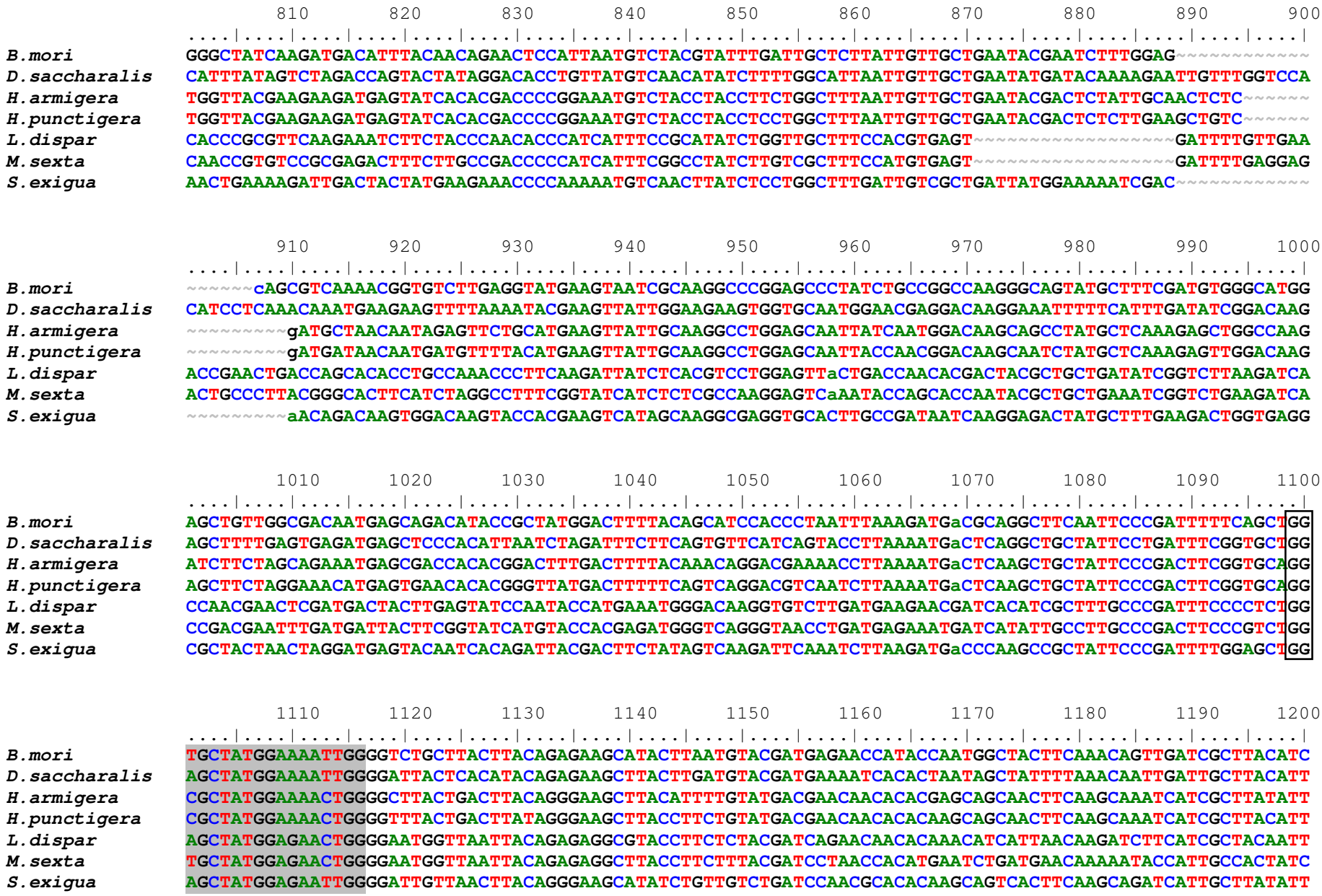
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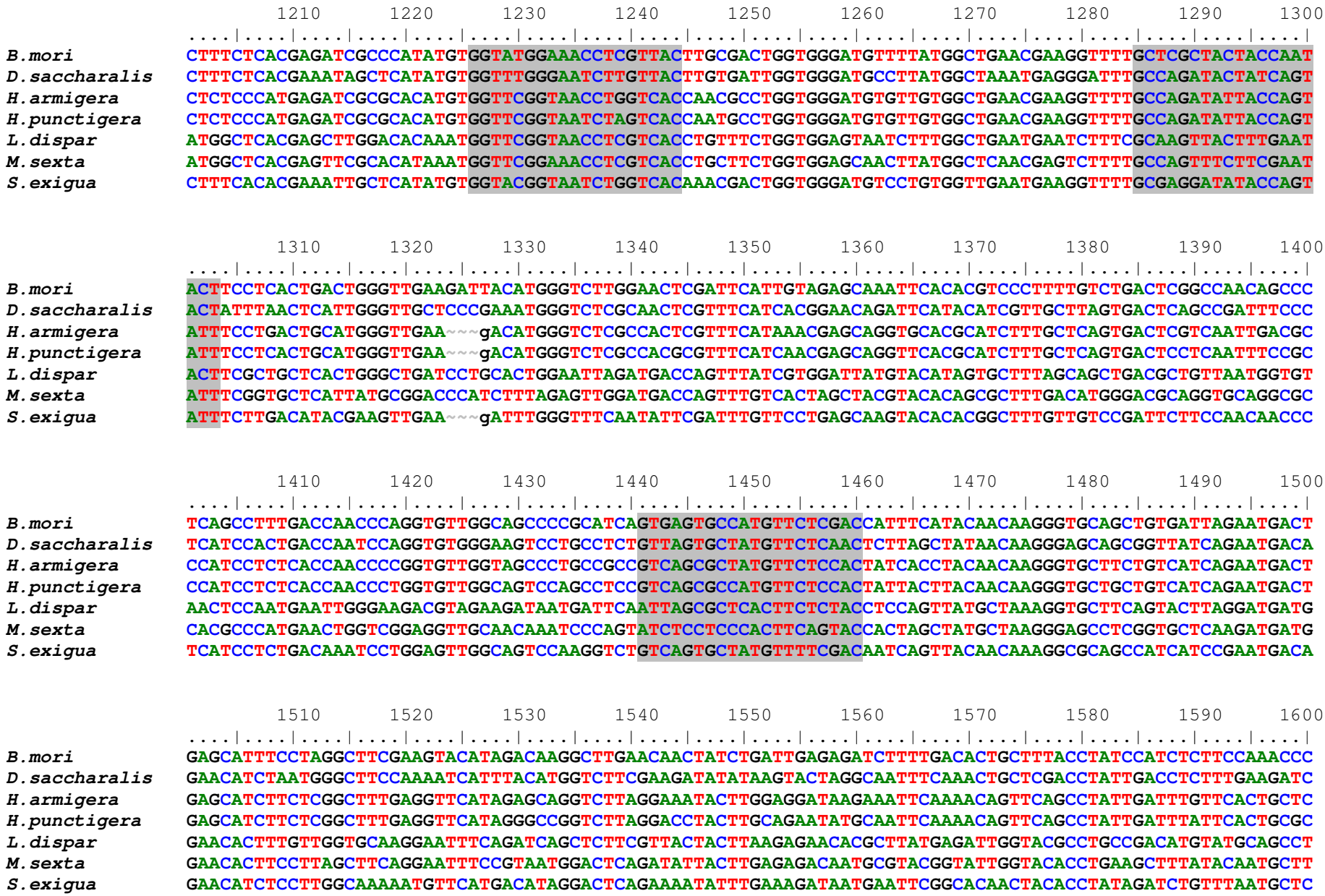
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D.saccharalis CTTTCTCACGAAATAGCTCATATGTGGTTTGGGAATCTTGTTACTTGTGATTGGTGGGATGCCTTATGGCTAAATGAGGGATTTGCCAGATACTATCAGT
H.armigera CTCTCCCATGAGATCGCGCACATGTGGTTCGGTAACCTGGTCACCAACGCCTGGTGGGATGTGTTGTGGCTGAACGAAGGTTTTGCCAGATATTACCAGT
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1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
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M.sexta CACGCCCATGAACTGGTCGGAGGTTGCAACAAATCCCAGTATCTCCTCCCACTTCAGTACCACTAGCTATGCTAAGGGAGCCTCGGTGCTCAAGATGATG
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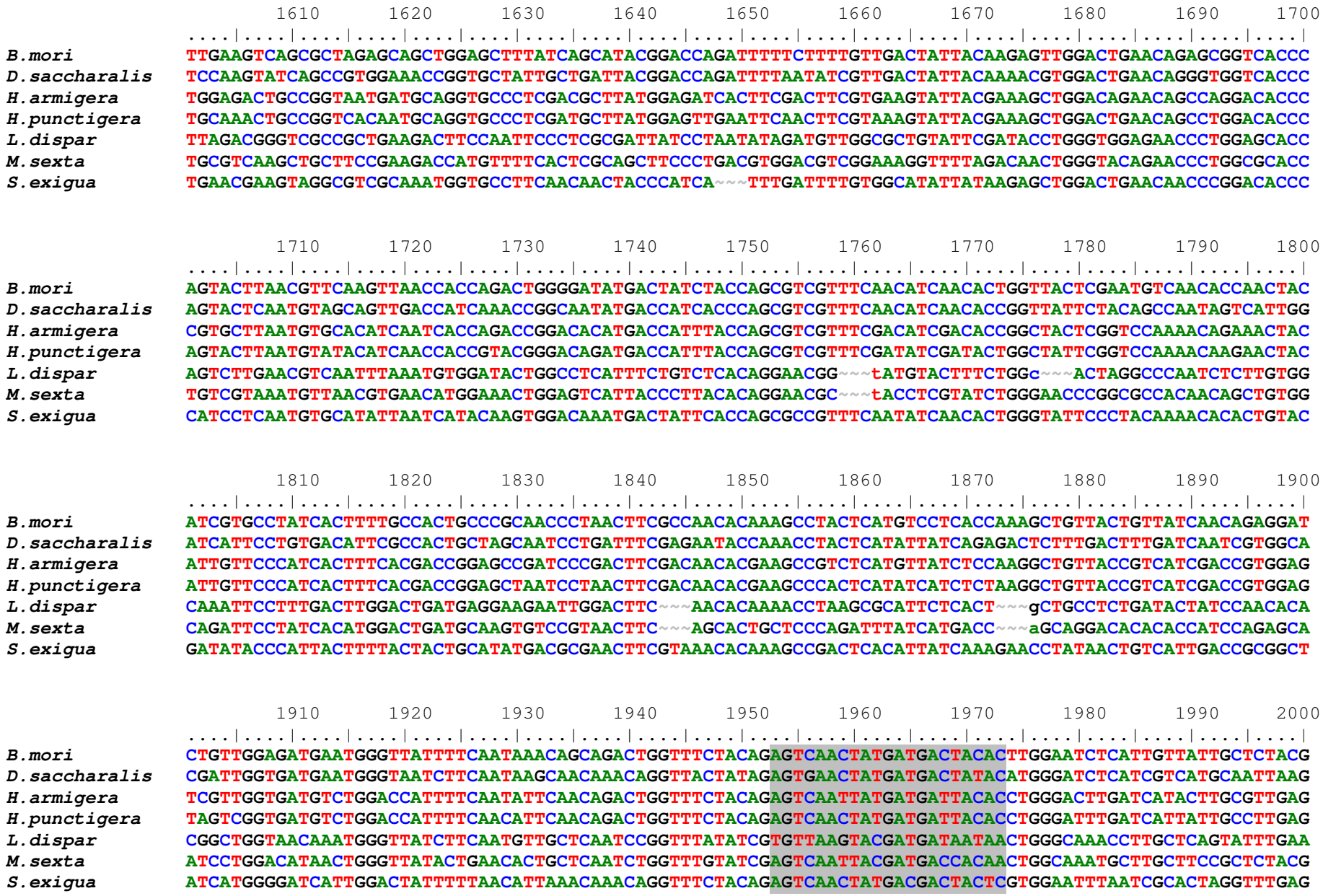
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H.punctigera TGCAAACTGCCGGTCACAATGCAGGTGCCCTCGATGCTTATGGAGTTGAATTCAACTTCGTAAAGTATTACGAAAGCTGGACTGAACAGCCTGGACACCC
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M.sexta TGCGTCAAGCTGCTTCCGAAGACCATGTTTTCACTCGCAGCTTCCCTGACGTGGACGTCGGAAAGGTTTTAGACAACTGGGTACAGAACCCTGGCGCACC
S.exigua TGAACGAAGTAGGCGTCGCAAATGGTGCCTTCAACAACTACCCATCA~~~TTTGATTTTGTGGCATATTATAAGAGCTGGACTGAACAACCCGGACACCC
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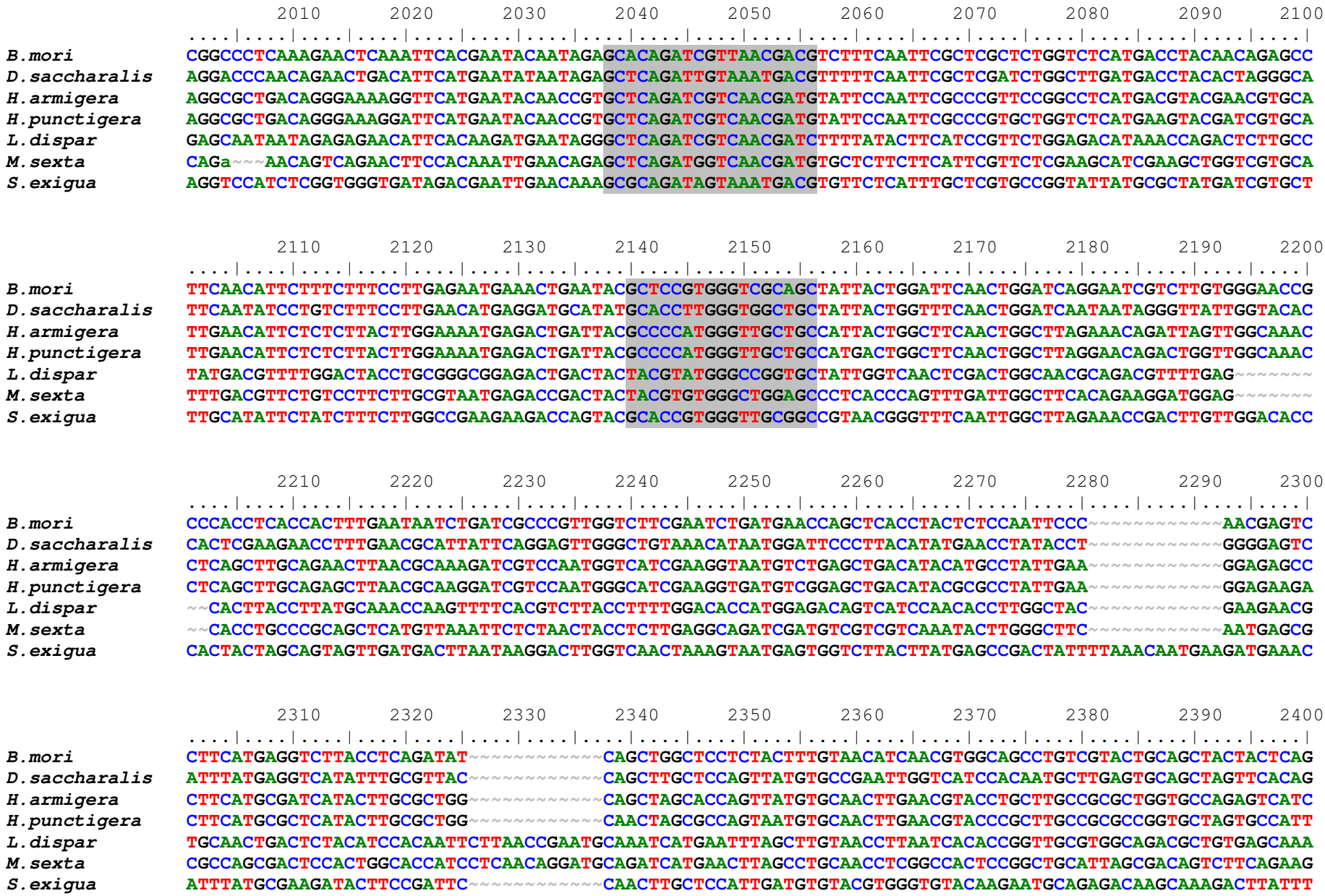
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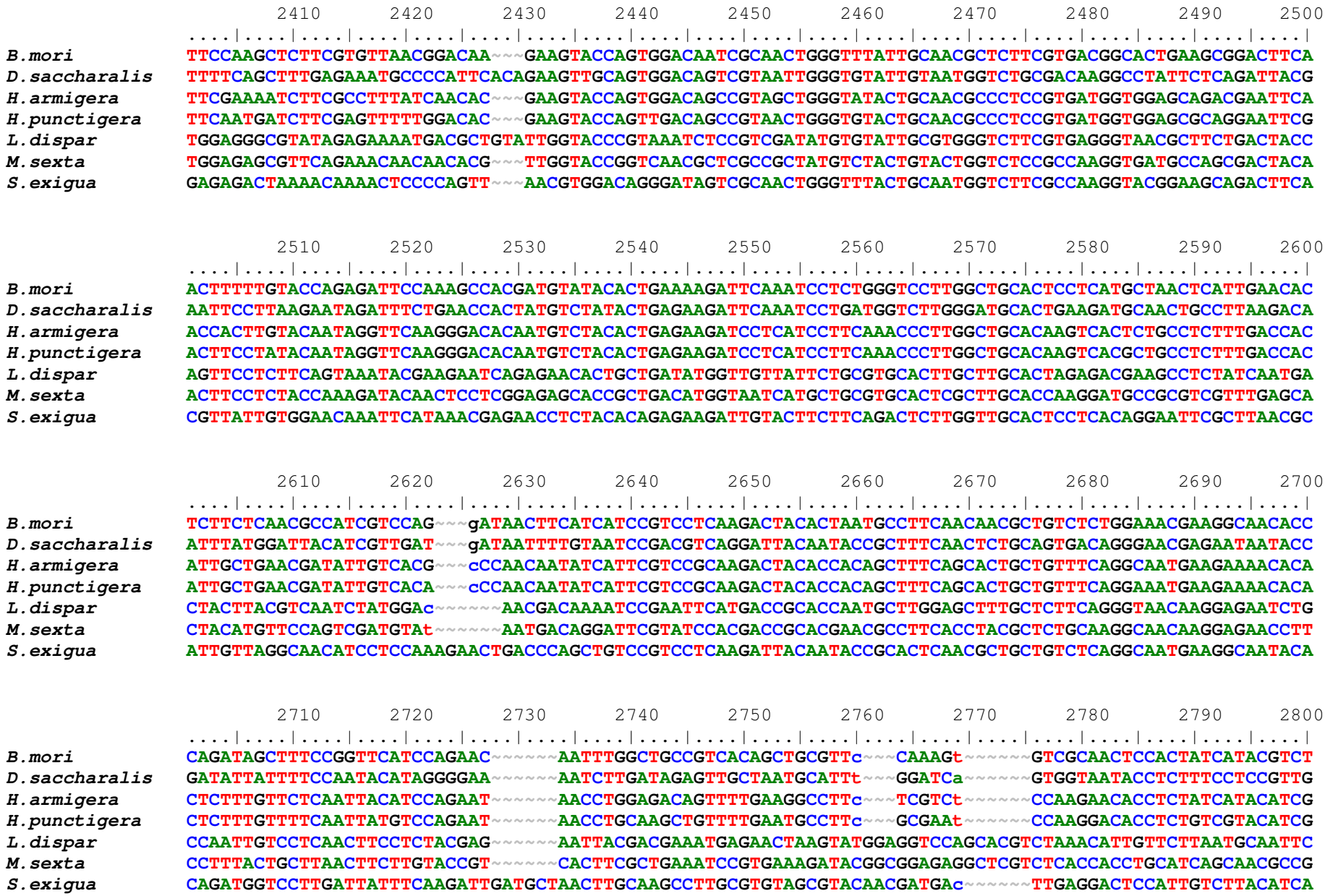
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D.saccharalis TTTTCAGCTTTGAGAAATGCCCCATTCACAGAAGTTGCAGTGGACAGTCGTAATTGGGTGTATTGTAATGGTCTGCGACAAGGCCTATTCTCAGATTACG
H.armigera TTCGAAAATCTTCGCCTTTATCAACAC~~~GAAGTACCAGTGGACAGCCGTAGCTGGGTATACTGCAACGCCCTCCGTGATGGTGGAGCAGACGAATTCA
H.punctigera TTCAATGATCTTCGAGTTTTTGGACAC~~~GAAGTACCAGTTGACAGCCGTAACTGGGTGTACTGCAACGCCCTCCGTGATGGTGGAGCGCAGGAATTCG
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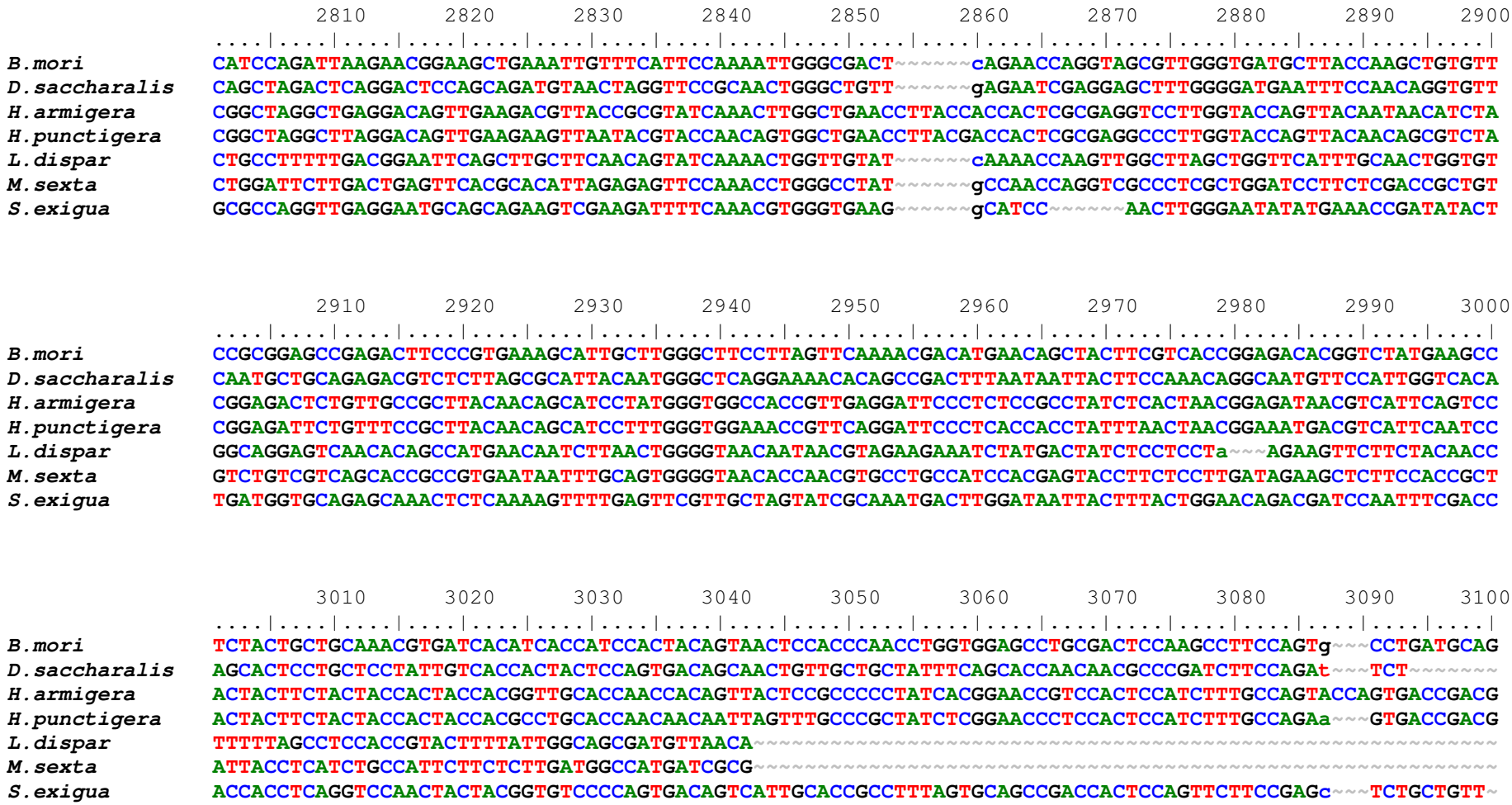
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L.dispar CTGCCTTTTTTGACGGAATTCAGCTTGCTTCAACAGTATCAAAACTGGTTGTAT~~~~~~cAAAACCAAGTTGGCTTAGCTGGTTCATTTGCAACTGGTGT
M.sexta CTGGATTCTTGACTGAGTTCACGCACATTAGAGAGTTCCAAACCTGGGCCTAT~~~~~~gCCAACCAGGTCGCCCTCGCTGGATCCTTCTCGACCGCTGT
S.exigua GCGCCAGGTTGAGGAATGCAGCAGAAGTCGAAGATTTTCAAACGTGGGTGAAG~~~~~~gCATCC~~~~~~AACTTGGGAATATATGAAACCGATATACT
2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
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3010 3020 3030 3040 3050 3060 3070 3080 3090 3100
B.mori TCTACTGCTGCAAACGTGATCACATCACCATCCACTACAGTAACTCCACCCAACCTGGTGGAGCCTGCGACTCCAAGCCTTCCAGTg~~~CCTGATGCAG
D.saccharalis AGCACTCCTGCTCCTATTGTCACCACTACTCCAGTGACAGCAACTGTTGCTGCTATTTCAGCACCAACAACGCCCGATCTTCCAGAt~~~TCT~~~~~~~~
H.armigera ACTACTTCTACTACCACTACCACGGTTGCACCAACCACAGTTACTCCGCCCCCTATCACGGAACCGTCCACTCCATCTTTGCCAGTACCAGTGACCGACG
H.punctigera ACTACTTCTACTACCACTACCACGCCTGCACCAACAACAATTAGTTTGCCCGCTATCTCGGAACCCTCCACTCCATCTTTGCCAGAa~~~GTGACCGACG
L.dispar TTTTTAGCCTCCACCGTACTTTTATTGGCAGCGATGTTAACA~~~~~~
M.sexta ATTACCTCATCTGCCATTCTTCTCTTGATGGCCATGATCGCG~~~~~~
S.exigua ACCACCTCAGGTCCAACTACTACGGTGTCCCCAGTGACAGTCATTGCACCGCCTTTAGTGCAGCCGACCACTCCAGTTCTTCCGAGc~~~TCTGCTGTT~

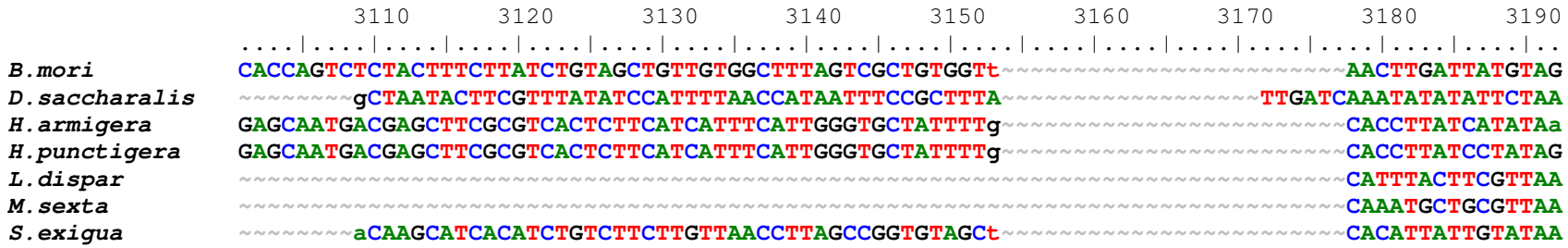

Figure B.3: DNA sequence alignment of *aminopeptidase N3* (*APN3*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (19070648),*Diatraea saccharalis* (302403442), *Helicoverpa armigera* (30961824), *Helicoverpa punctigera* (7158843), *Lymantria dispar* (16588785), *Manduca sexta* (20279108) and *Spodoptera exigua* (60739178).

B.4. *Aminopeptidase N4* (*APN4*)

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                            10        20        30        40        50        60        70        80        90       100
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         ATGGGTGCCAACATGGTGCTTCCCACCGTATTCTGCATCCTCCTGGGATCCATa~~~~~~~~~~~~~~~~~~GCGGCCATTCCTCAAGAGGACTTCAGGT
L.dispar           ATGGCAATTTGGTATCATCTCCTTATAGGAGCACTTTTCATACAAGGCTAT~~~~~~tTGGCTTATAGCCCTATCCCTGAAGAGAATTCCGCGGAAGAAT
M.sexta            ATGGCAGGCCTAAGACGACAAATCTTCGCTTTGGCATGCGTTCTATCCAACGTCGCGTCCTTCGACCCGCCGGTGATGCGGACAGCGTCCACCATCTTCG
O.furnacalis(R)    ATGGGGTCAATCATGTTACTCCCAATCCTGTTCTTCCTCGCGGGGGTa~~~~~~~~~~~~~~~~~~~~~~~~GCTGCTTACCCCAACGAGGATATGAGAT
O.nubilalis        ATGGGGTCAATCATGTTACTCCCAATCCTGTTCTTCCTCGCGGGGGTa~~~~~~~~~~~~~~~~~~~~~~~~GCTGCTTACCCCAATGAGGACATGAGAT
P.xylostella       ATGCGTTTACTTATCTGCCTGCCTCTGTTGGGCTTAGTc~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~T
S.exigua           ATGGGTACCAAAATGTTGGTTCCCGCTCTGTTTTGCGTTCTTATGGGATTTGCg~~~~~~~~~~~~~~~~~~GTAGCTATTCCCCCAGAAGATTTCCGGT

                           110       120       130       140       150       160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         CCAACTTGGAGTGGTCTGACTACAGCACCAACTTAGAC~~~~~~~~~~~~~~~~~~~~~~~~~~~gAGCCGGCGTACCGTCTGCGTGATGTGGTCTATCC
L.dispar           GGATTCAATACAACGAATTACTGAGG~~~~~~~~~~~~~~~~~~~~~~~~~~~gATCCCAGTTATCGCTTGCCTACTACTACTCGTCCCAGTCATTACAC
M.sexta            GTGATGAAAAACTCAAAGGCGAGATTTTCGAAGACATCGACGAACAAGAAGTCGCGATGTCATCAGCTGTGACGCGTAATTCTGCCTATCGTCTACCTAC
O.furnacalis(R)    ATGACTTGGAGTTCTTCGGGTACTCCACAAACTTGGAC~~~~~~~~~~~~~~~~~~~~~~~~~~~gATCCTAAGTACCGTCTCACAGACTCTGTGCAGCC
O.nubilalis        CTGACTTGGAGTTCTTCGGGTACTCCACAAACTTGGAC~~~~~~~~~~~~~~~~~~~~~~~~~~~gATCCTAAATACCGTCTCACAGACTCTGTGCAGCC
P.xylostella       GCGGGAACCCCGTGCAACTGACAGACAACAGCATCGCGCTACAAAACACATATGAGAACTACGTTCTGCCAGGGGAGTCATTCCCGACCTTCTACGATGT
S.exigua           CAAACTTGGAGTTCTTTGACTATTCCTCAAATGTAGCT~~~~~~~~~~~~~~~~~~~~~~~~~~~gAACCAGCTTACCGGCTCGTCAACAATGTGTATCC

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                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         TACTGATGTCAACCTGGATCTT~~~gATGTCTACCTa~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AACCACCTGAACTTCTCTGGACTCGTACAGATT
L.dispar           AGTAACTTTA~~~gCTCCTATTTTAGATACTGTGCCTGCTAACCTTCCTAATGCACTACCATTCACGTTTGATGGTGAAGTTAATATTATCATTAGAGCC
M.sexta            CACCACAAGACCTTCGCGGTACAACGTTCATTGG~~~aCCATAGATATg~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TCCAGAAGGACATACACCGGT
O.furnacalis(R)    CAGGGATGTGTATGTGCATTTG~~~gATGTGCATGTc~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GTGGAGGCTAGATTCGACGGCTTTGTACAGCTC
O.nubilalis        CAGAGACGTGTATGTGCATTTG~~~gATGTGCATGTc~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GTGGAGGCTAGATTCGACGGTTTTATTCAGCTC
P.xylostella       CCAACTA~~~tTTTTTGACCCt~~~~~~~~~~~~~~~~~~~~~~~~~~~GAATACGAAGCGTCCTTCAATGGCACAGTAGCAATAAGAGTCGTGCCCAGA
S.exigua           TACTGATGTGAAAGTAAACCTAGACAATATTAACCTt~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GAAGAAGCTCGATTCACAGGAAGTGTGGAGATG

                           310       320       330       340       350       360       370       380       390       400
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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L.dispar           ACAGAGCCTAATATAAATGAAATTGTTCTGCATTGCAATGACTTGACCATTAATACACTATCGGTATAT~~~~~~GCTACAACGGCTCCTAATACCAATA
M.sexta            AATGTGGCAATCCAGCTCTTCGCTACACAGTCTGGCGTCAACGAAATTGTCATCCATTCTGACCACGTGACAATCCAATCCGTGGTCCTGCAGCAAGGAT
O.furnacalis(R)    GACATTGAAATCGAAGAATCGGGCATGACTCAGATCGTGCTGCACCAAAAAGTGGTATCGATCCAGACTGTCAATGTCCTT~~~~~~GATTCCGCTGGCA
O.nubilalis        GACGTTGAAATCGAAGAATCGGGCATGACTCAGATCGTGCTGCATCAAAAAGTGGTGTCGATCCAGACTGTCAATGTCCTT~~~~~~GATTCCGCTGGCA
P.xylostella       ~~~aTTGCTACACAAGAGATTGTCCTGCACGCTATGGAAATGGAGATACTGTCTATCCGTGCTTACTCAGACTTGCCTAGTGATGATAATTTGAATGAAA
S.exigua           ATCGTTATTGTGAGAGAAAACGATTTACATCAAATTTCCATGCACCAGAACAATCTCATTATCACCAGGATCAGTGTTGTT~~~AACAACACAAATGGCG
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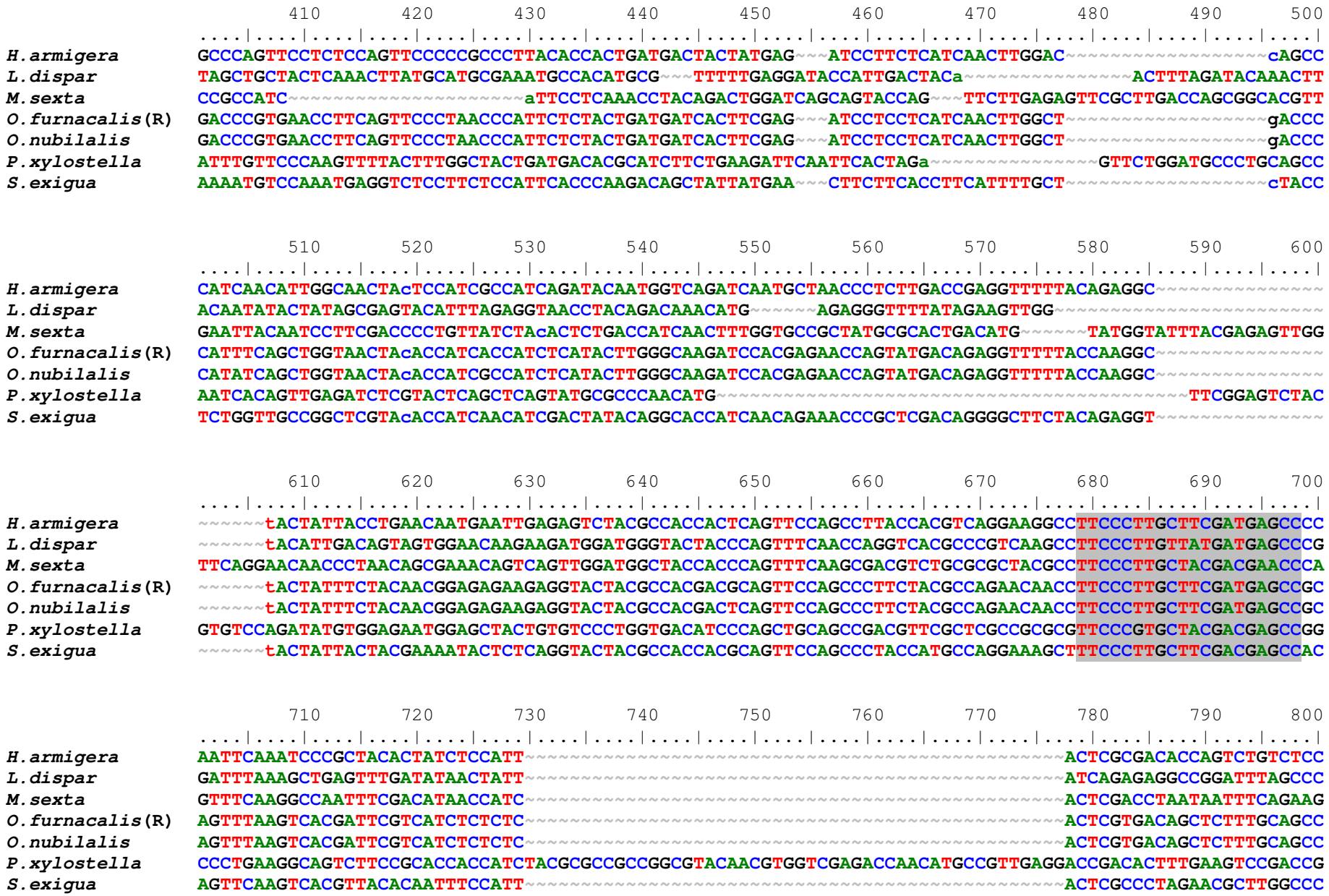
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L.dispar TAGCTGCTACTCAAACTTATGCATGCGAAATGCCACATGCG~~~TTTTTGAGGATACCATTGACTACa~~~~~~~~~~~~~~~~ACTTTAGATACAAACTT
M.sexta CCGCCATC~~~~~~~~~~~~~~~~~~~~~~~~aTTCCTCAAACCTACAGACTGGATCAGCAGTACCAG~~~TTCTTGAGAGTTCGCTTGACCAGCGGCACGTT
O.furnacalis(R) GACCCGTGAACCTTCAGTTCCCTAACCCATTCTCTACTGATGATCACTTCGAG~~~ATCCTCCTCATCAACTTGGCT~~~~~~~~~~~~~~~~~~~~gACCC
O.nubilalis GACCCGTGAACCTTCAGTTCCCTAACCCATTCTCTACTGATGATCACTTCGAG~~~ATCCTCCTCATCAACTTGGCT~~~~~~~~~~~~~~~~~~~~gACCC
P.xylostella ATTTGTTCCCAAGTTTTACTTTGGCTACTGATGACACGCATCTTCTGAAGATTCAATTCACTAGa~~~~~~~~~~~~~~~~GTTCTGGATGCCCTGCAGCC
S.exigua AAAATGTCCAAATGAGGTCTCCTTCTCCATTCACCCAAGACAGCTATTATGAA~~~CTTCTTCACCTTCATTTTGCT~~~~~~~~~~~~~~~~~~~~cTACC
510 520 530 540 550 560 570 580 590 600
H.armigera CATCAACATTGGCAACTAcTCCATCGCCATCAGATACAATGGTCAGATCAATGCTAACCCTCTTGACCGAGGTTTTTACAGAGGC~~~~~~~~~~~~~~~~
L.dispar ACAATATACTATAGCGAGTACATTTAGAGGTAACCTACAGACAAACATG~~~~~~~AGAGGGTTTTATAGAAGTTGG~~~~~~~~~~~~~~~~~~~~~~~~
M.sexta GAATTACAATCCTTCGACCCCTGTTATCTAcACTCTGACCATCAACTTTGGTGCCGCTATGCGCACTGACATG~~~~~~~TATGGTATTTACGAGAGTTGG
O.furnacalis(R) CATTTCAGCTGGTAACTAcACCATCACCATCTCATACTTGGGCAAGATCCACGAGAACCAGTATGACAGAGGTTTTTACCAAGGC~~~~~~~~~~~~~~~~
O.nubilalis CATATCAGCTGGTAACTAcACCATCGCCATCTCATACTTGGGCAAGATCCACGAGAACCAGTATGACAGAGGTTTTTACCAAGGC~~~~~~~~~~~~~~~~
P.xylostella AATCACAGTTGAGATCTCGTACTCAGCTCAGTATGCGCCCAACATG~~~~~~TTCGGAGTCTAC
S.exigua TCTGGTTGCCGGCTCGTAcACCATCAACATCGACTATACAGGCACCATCAACAGAAACCCGCTCGACAGGGGCTTCTACAGAGGT~~~~~~~~~~~~~~~~
610 620 630 640 650 660 670 680 690 700
H.armigera ~~~~~~tACTATTACCTGAACAATGAATTGAGAGTCTACGCCACCACTCAGTTCCAGCCTTACCACGTCAGGAAGGCCTTCCCTTGCTTCGATGAGCCCC
L.dispar ~~~~~~tACATTGACAGTAGTGGAACAAGAAGATGGATGGGTACTACCCAGTTTCAACCAGGTCACGCCCGTCAAGCCTTCCCTTGTTATGATGAGCCCG
M.sexta TTCAGGAACAACCCTAACAGCGAAACAGTCAGTTGGATGGCTACCACCCAGTTTCAAGCGACGTCTGCGCGCTACGCCTTCCCTTGCTACGACGAACCCA
O.furnacalis(R) ~~~~~~tACTATTTCTACAACGGAGAGAAGAGGTACTACGCCACGACGCAGTTCCAGCCCTTCTACGCCAGAACAACCTTCCCTTGCTTCGATGAGCCGC
O.nubilalis ~~~~~~tACTATTTCTACAACGGAGAGAAGAGGTACTACGCCACGACTCAGTTCCAGCCCTTCTACGCCAGAACAACCTTCCCTTGCTTCGATGAGCCGC
P.xylostella GTGTCCAGATATGTGGAGAATGGAGCTACTGTGTCCCTGGTGACATCCCAGCTGCAGCCGACGTTCGCTCGCCGCGCGTTCCCGTGCTACGACGAGCCGG
S.exigua ~~~~~~tACTATTACTACGAAAATACTCTCAGGTACTACGCCACCACGCAGTTCCAGCCCTACCATGCCAGGAAAGCTTTCCCTTGCTTCGACGAGCCAC
710 720 730 740 750 760 770 780 790 800
H.armigera AATTCAAATCCCGCTACACTATCTCCATT~~~~~~ACTCGCGACACCAGTCTGTCTCC
L.dispar GATTTAAAGCTGAGTTTGATATAACTATT~~~~~~ATCAGAGAGGCCGGATTTAGCCC
M.sexta GTTTCAAGGCCAATTTCGACATAACCATC~~~~~~ACTCGACCTAATAATTTCAGAAG
O.furnacalis(R) AGTTTAAGTCACGATTCGTCATCTCTCTC~~~~~~ACTCGTGACAGCTCTTTGCAGCC
O.nubilalis AGTTTAAGTCACGATTCGTCATCTCTCTC~~~~~~ACTCGTGACAGCTCTTTGCAGCC
P.xylostella CCCTGAAGGCAGTCTTCCGCACCACCATCTACGCGCCGCCGGCGTACAACGTGGTCGAGACCAACATGCCGTTGAGGACCGACACTTTGAAGTCCGACCG
S.exigua AGTTCAAGTCACGTTACACAATTTCCATT~~~~~~ACTCGCCCTAGAACGCTTGGCCC

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                            810       820       830       840       850       860       870       880       890       900
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L.dispar            A~~~ACTATATCAAATATGGCGATAAGATCAACTTCAATTCTTACAGGTGGAAGAATTTCAGAGACCTTTTATACTACACCTGTGACATCCACTTACTTA
M.sexta             CTGGTCCTGTACCAGGATTAAGGAGACCAGAGCCTCTAGTGTCTTGAACTTCCAGGAT~~~GACATCTACCACACGACGCCCTGCATGTCAACTTACCTC
O.furnacalis(R)     ATCCTAC~~~tCTAACATGCCCATTGGTGAAACTGTTGAGACTTCTCCCGGCCGTATTCGTGAGACCTTCCTCCCCACTTCTATCGTTTCTGCATATCTC
O.nubilalis         CTCCTAC~~~tCTAATATGCCCATTGGTGAAACTGTTGAGACTTCTCCCGGCCGTATTCGTGAGACCTTCCTCCCCACTCCTATCGTTTCTGTATATCTC
P.xylostella        GCCAGGCTTCGCCAAGCACGAG~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TTCCAGGACACCCTGGTGATGTCCAGCTACCTG
S.exigua            ATCCTAC~~~tCCAACATGGCCATCAGTTCTACCGAAGATCTTGGc~~~AATAGTATTCGAGAAACATTCTACCCAACTCCTATAATTTCGGCATACCTA

                            910       920       930       940       950       960       970       980       990      1000
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          GTCGCTTTCCACGTC~~~~~~agTGACTTCGTCTCCACTGAATACACCAGCACCGATGCCAAACCCTTCAGTATTATCTCCCGCCAAGGT~~~~~~gCCA
L.dispar            CTTGCCTTCATTGTATCt~~~~~~CATTATGCAACAGTGGCTAGCAATGAAAGCGTAGAAAGGCCTTTCTATATTTATGCTAGAGATAAT~~~~~~gCTG
M.sexta             ATCGCTCTGATCGTTGCg~~~~~~GAGTACGATTCGCTAGAATTAAGACAAAACAATGTCGTCATGTATGAGGTTATTGCCCGACCTGGCGCACTTTCTG
O.furnacalis(R)     GTCGCCTTCACAGTCAGt~~~~~~GACTTCGTTGCCACCAACTTAACCACCACGTCCACCAGACCCTTCCAAATCGTCTCTCGCCCAGGA~~~~~~gTTA
O.nubilalis         GTCGCCTTCACAGTCAGt~~~~~~GATTTCGTTGCCACCAACTTAACCACCACGTCCACCAGACCCTTCCAAATCGTCTCTCGCCCAGGA~~~~~~gTTA
P.xylostella        CTGGCCTACCTGGTCTCCAAGTTCGACTACATCTCCAACGAAAACAACCCCACCTACGACAAATCCATGAAAGTGTTCTCGAGACCAGGG~~~~~~aCCC
S.exigua            GTGGCTTTCCATGTGAGc~~~~~~GATTTTGTGCCCACAACCGTTACAACCACCAATCGTAGACCATTCAGCATTATCTCTCGCCAAGGA~~~~~~gTGA

                           1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          CGAACCAGCACCAATATGCTGCTGAAATCGGTCTTAAGATCACCAACGAACTCGATGACTACTTTGGCATCCAGTACCATGAGATGGGACAAGGTGCTTT
L.dispar            GAACAACTGGTGAATTTGCTCTTGATATCGGTGAAAGACTTCTTATTGCTATGGAAGACTTTACAGGATACCCATACTACTCGGTAGCTTATAATATGAT
M.sexta             CTGGTCAGGGTCAATACGCTTTCGACGTCGGTCAAGAGCTTCTCGCTGAGATGAGTAAGCACACAGCCATGGACTTCTATACCATGGATCCCAACCTTAA
O.furnacalis(R)     CCTCACAGCACGTGTACGCCGCTGGTATTGGTTTGGACATCACCAACGAACTGGATGATTACTTGGGAATTGAGTATTATGAAATGGGACAAGGAGTGCC
O.nubilalis         CCTCACAGCACGTGTACGCCGCTGGTATCGGTTTGGACATCACCAACGAACTGGATGATTACTTGGGAATTGAGTATTATGAAATGGGACAAGGAGTGCC
P.xylostella        AGAACACGGCAGAGTTCGCCTTGGACTTTGGCCAGAAGAACATGGTAGAACTGGAGAAGTATACTGAGTTCCCGTAC~~~~~~~~~~~~~~~~~~GCCTT
S.exigua            CGGAACAGCACTCGTATGCTGCAGAAATCGGTGTGGAAATAACCAACCAGTTAGATGACTACCTTGGTATTGAGTACCATGAAATGGGTCAGGGTCAAAT

                           1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          GATGAAGAATGACCACATAGCTCTTCCTGACTTCCCCTCCGGTGCTATGGAGAACTGGGGAATGGTTAACTACAGGGAAGCCTACCTCTTATACGACGAA
L.dispar            TATGCAa~~~~~~CAAGCAGCTATTCCAGACTTTTCGGCGGGTGCTATGGAGAACTGGGGTCTTTTGACCTATAGAGAGGCTCTGATGTTGTATGATCCG
M.sexta             GATGACt~~~~~~CAAGCCTCCATCCCTGACTTCTCAGCTGGTGCTATGGAAAATTGGGGTCTTCTTACGTACAGAGAAGCGTACCTGATGTACGATGCG
O.furnacalis(R)     AATGAAGAACGATCACCTTGCTATTCCTGATTTTCCTTCTGGTGCTATGGAGAACAGGGGAATGGTAAACTACAGGGAAGCGTACTTGTTGTGCGATGAG
O.nubilalis         AATGAAGAACGATCACCTTGCTATTCCTGATTTTCCTTCTGGTGCTATGGAGAACTGGGGGATGGTAAACTACAGGGAAGCGTACTTGTTGTACGATGAG
P.xylostella        CCCAAAGATCGACAAAGTGGCGGTGCCAGACTTTGCCGCTGGTGCCATGGAGAACTGGGGACTGGTTATTTACAGAGAAATAGCGCTGCTAGTCCAAGAA
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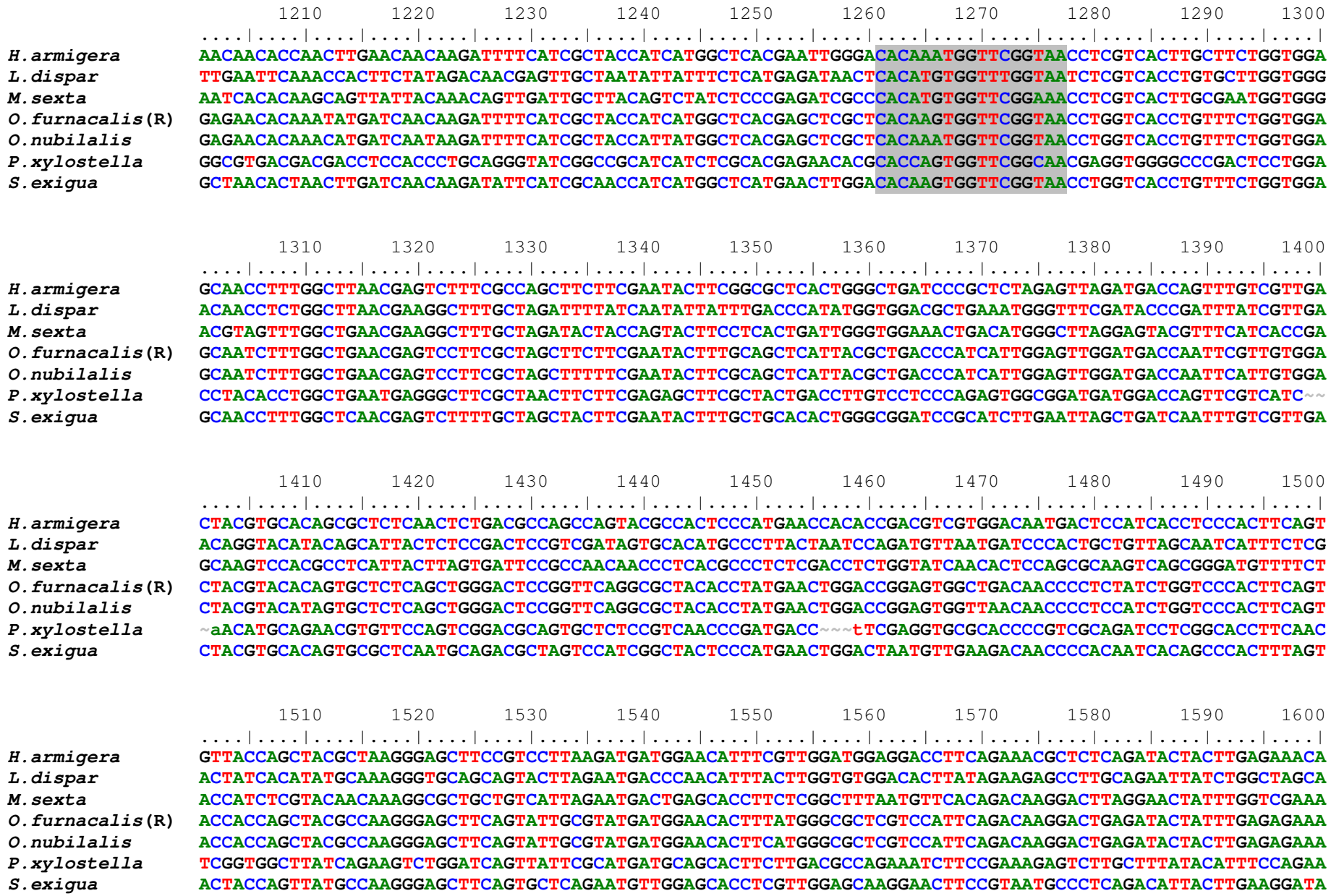
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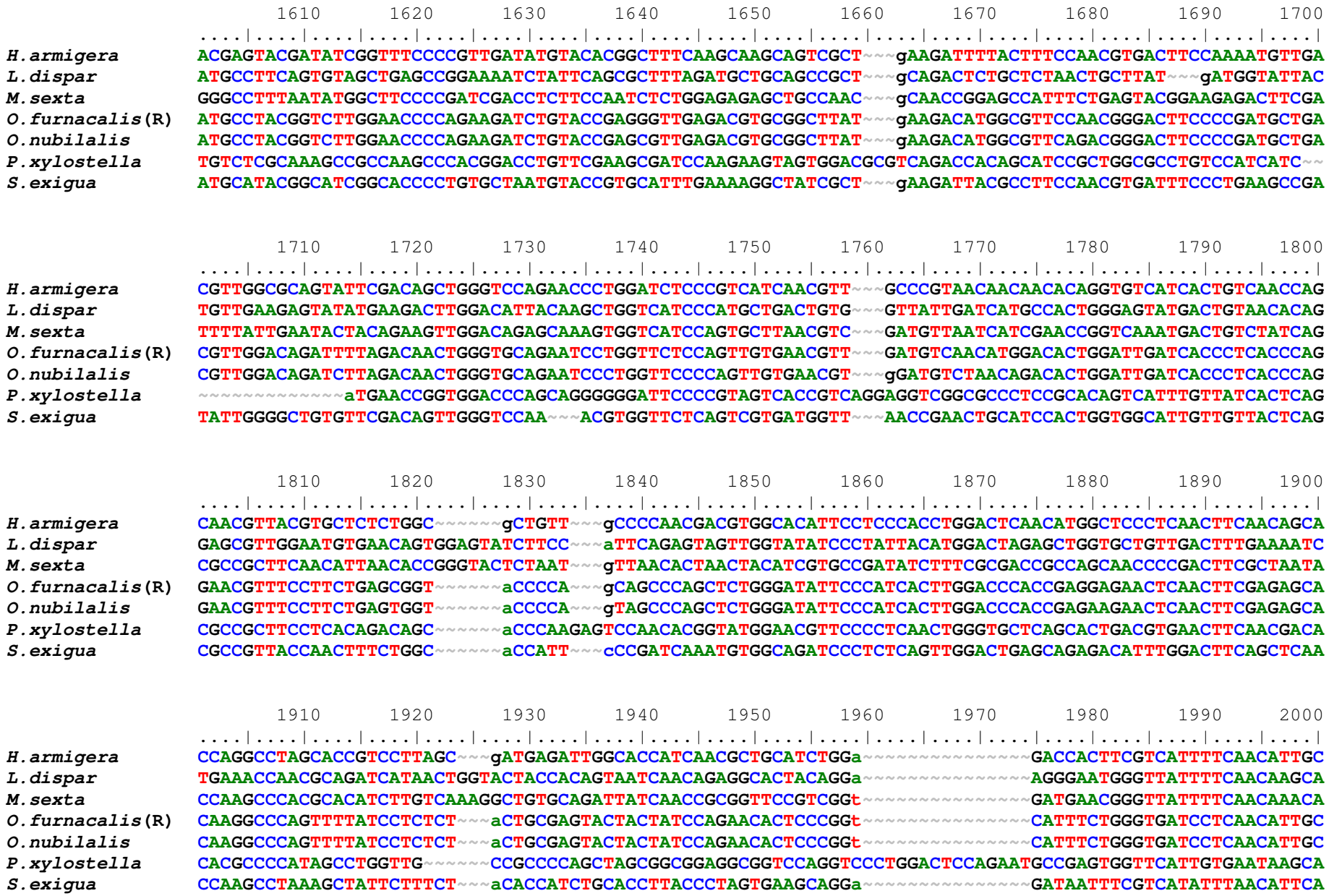
L.dispar TTGAATTCAAACCACTTCTATAGACAACGAGTTGCTAATATTATTTCTCATGAGATAACTCACATGTGGTTTGGTAATCTCGTCACCTGTGCTTGGTGGG
M.sexta AATCACACAAGCAGTTATTACAAACAGTTGATTGCTTACAGTCTATCTCCCGAGATCGCCCACATGTGGTTCGGAAACCTCGTCACTTGCGAATGGTGGG
O.furnacalis(R) GAGAACACAAATATGATCAACAAGATTTTCATCGCTACCATCATGGCTCACGAGCTCGCTCACAAGTGGTTCGGTAACCTGGTCACCTGTTTCTGGTGGA
O.nubilalis GAGAACACAAACATGATCAATAAGATTTTCATCGCTACCATTATGGCTCACGAGCTCGCTCACAAATGGTTCGGTAACCTGGTCACCTGTTTCTGGTGGA
P.xylostella GGCGTGACGACGACCTCCACCCTGCAGGGTATCGGCCGCATCATCTCGCACGAGAACACGCACCAGTGGTTCGGCAACGAGGTGGGGCCCGACTCCTGGA
S.exigua GCTAACACTAACTTGATCAACAAGATATTCATCGCAACCATCATGGCTCATGAACTTGGACACAAGTGGTTCGGTAACCTGGTCACCTGTTTCTGGTGGA
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
H.armigera GCAACCTTTGGCTTAACGAGTCTTTCGCCAGCTTCTTCGAATACTTCGGCGCTCACTGGGCTGATCCCGCTCTAGAGTTAGATGACCAGTTTGTCGTTGA
L.dispar ACAACCTCTGGCTTAACGAAGGCTTTGCTAGATTTTATCAATATTATTTGACCCATATGGTGGACGCTGAAATGGGTTTCGATACCCGATTTATCGTTGA
M.sexta ACGTAGTTTGGCTGAACGAAGGCTTTGCTAGATACTACCAGTACTTCCTCACTGATTGGGTGGAAACTGACATGGGCTTAGGAGTACGTTTCATCACCGA
O.furnacalis(R) GCAATCTTTGGCTGAACGAGTCCTTCGCTAGCTTCTTCGAATACTTTGCAGCTCATTACGCTGACCCATCATTGGAGTTGGATGACCAATTCGTTGTGGA
O.nubilalis GCAATCTTTGGCTGAACGAGTCCTTCGCTAGCTTTTTCGAATACTTCGCAGCTCATTACGCTGACCCATCATTGGAGTTGGATGACCAATTCATTGTGGA
P.xylostella CCTACACCTGGCTGAATGAGGGCTTCGCTAACTTCTTCGAGAGCTTCGCTACTGACCTTGTCCTCCCAGAGTGGCGGATGATGGACCAGTTCGTCATC~~
S.exigua GCAACCTTTGGCTCAACGAGTCTTTTGCTAGCTACTTCGAATACTTTGCTGCACACTGGGCGGATCCGCATCTTGAATTAGCTGATCAATTTGTCGTTGA
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
H.armigera CTACGTGCACAGCGCTCTCAACTCTGACGCCAGCCAGTACGCCACTCCCATGAACCACACCGACGTCGTGGACAATGACTCCATCACCTCCCACTTCAGT
L.dispar ACAGGTACATACAGCATTACTCTCCGACTCCGTCGATAGTGCACATGCCCTTACTAATCCAGATGTTAATGATCCCACTGCTGTTAGCAATCATTTCTCG
M.sexta GCAAGTCCACGCCTCATTACTTAGTGATTCCGCCAACAACCCTCACGCCCTCTCGACCTCTGGTATCAACACTCCAGCGCAAGTCAGCGGGATGTTTTCT
O.furnacalis(R) CTACGTACACAGTGCTCTCAGCTGGGACTCCGGTTCAGGCGCTACACCTATGAACTGGACCGGAGTGGCTGACAACCCCTCTATCTGGTCCCACTTCAGT
O.nubilalis CTACGTACATAGTGCTCTCAGCTGGGACTCCGGTTCAGGCGCTACACCTATGAACTGGACCGGAGTGGTTAACAACCCCTCCATCTGGTCCCACTTCAGT
P.xylostella ~aACATGCAGAACGTGTTCCAGTCGGACGCAGTGCTCTCCGTCAACCCGATGACC~~~tTCGAGGTGCGCACCCCGTCGCAGATCCTCGGCACCTTCAAC
S.exigua CTACGTGCACAGTGCGCTCAATGCAGACGCTAGTCCATCGGCTACTCCCATGAACTGGACTAATGTTGAAGACAACCCCACAATCACAGCCCACTTTAGT
1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
H.armigera GTTACCAGCTACGCTAAGGGAGCTTCCGTCCTTAAGATGATGGAACATTTCGTTGGATGGAGGACCTTCAGAAACGCTCTCAGATACTACTTGAGAAACA
L.dispar ACTATCACATATGCAAAGGGTGCAGCAGTACTTAGAATGACCCAACATTTACTTGGTGTGGACACTTATAGAAGAGCCTTGCAGAATTATCTGGCTAGCA
M.sexta ACCATCTCGTACAACAAAGGCGCTGCTGTCATTAGAATGACTGAGCACCTTCTCGGCTTTAATGTTCACAGACAAGGACTTAGGAACTATTTGGTCGAAA
O.furnacalis(R) ACCACCAGCTACGCCAAGGGAGCTTCAGTATTGCGTATGATGGAACACTTTATGGGCGCTCGTCCATTCAGACAAGGACTGAGATACTATTTGAGAGAAA
O.nubilalis ACCACCAGCTACGCCAAGGGAGCTTCAGTATTGCGTATGATGGAACACTTCATGGGCGCTCGTCCATTCAGACAAGGACTGAGATACTACTTGAGAGAAA
P.xylostella TCGGTGGCTTATCAGAAGTCTGGATCAGTTATTCGCATGATGCAGCACTTCTTGACGCCAGAAATCTTCCGAAAGAGTCTTGCTTTATACATTTCCAGAA
S.exigua ACTACCAGTTATGCCAAGGGAGCTTCAGTGCTCAGAATGTTGGAGCACCTCGTTGGAGCAAGGAACTTCCGTAATGCCCTCAGACATTACTTGAAGGATA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L.dispar ATGCCTTCAGTGTAGCTGAGCCGGAAAATCTATTCAGCGCTTTAGATGCTGCAGCCGCT~~~gCAGACTCTGCTCTAACTGCTTAT~~~gATGGTATTAC
M.sexta GGGCCTTTAATATGGCTTCCCCGATCGACCTCTTCCAATCTCTGGAGAGAGCTGCCAAC~~~gCAACCGGAGCCATTTCTGAGTACGGAAGAGACTTCGA
O.furnacalis(R) ATGCCTACGGTCTTGGAACCCCAGAAGATCTGTACCGAGGGTTGAGACGTGCGGCTTAT~~~gAAGACATGGCGTTCCAACGGGACTTCCCCGATGCTGA
O.nubilalis ATGCCTACGGTCTTGGAACCCCAGAAGATCTGTACCGAGCGTTGAGACGTGCGGCTTAT~~~gAAGACATGGCGTTCAGACGGGACTTCCCCGATGCTGA
P.xylostella TGTCTCGCAAAGCCGCCAAGCCCACGGACCTGTTCGAAGCGATCCAAGAAGTAGTGGACGCGTCAGACCACAGCATCCGCTGGCGCCTGTCCATCATC~~
S.exigua ATGCATACGGCATCGGCACCCCTGTGCTAATGTACCGTGCATTTGAAAAGGCTATCGCT~~~gAAGATTACGCCTTCCAACGTGATTTCCCTGAAGCCGA
1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
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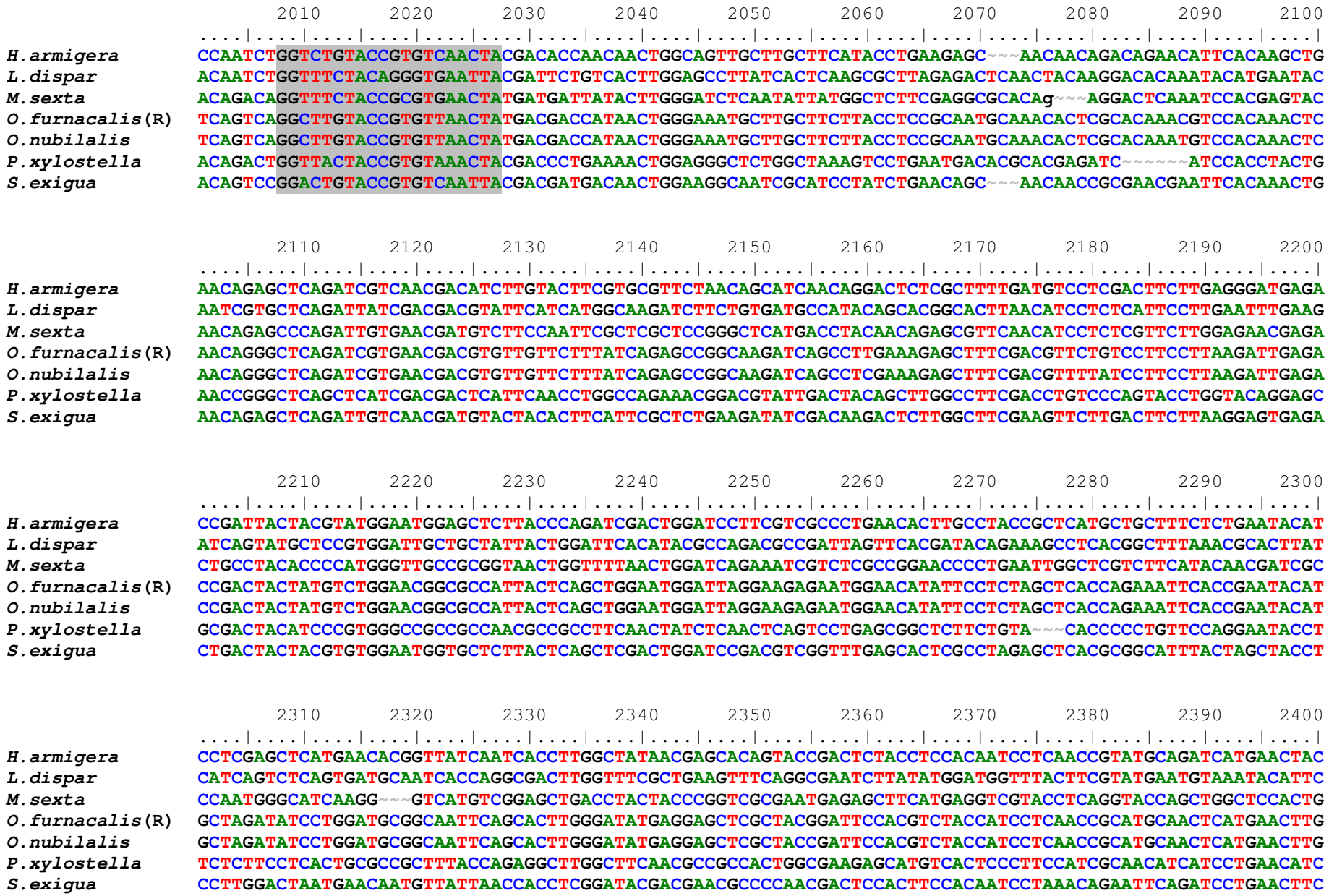
L.dispar TGTTGAAGAGTATATGAAGACTTGGACATTACAAGCTGGTCATCCCATGCTGACTGTG~~~GTTATTGATCATGCCACTGGGAGTATGACTGTAACACAG
M.sexta TTTTATTGAATACTACAGAAGTTGGACAGAGCAAAGTGGTCATCCAGTGCTTAACGTC~~~GATGTTAATCATCGAACCGGTCAAATGACTGTCTATCAG
O.furnacalis(R) CGTTGGACAGATTTTAGACAACTGGGTGCAGAATCCTGGTTCTCCAGTTGTGAACGTT~~~GATGTCAACATGGACACTGGATTGATCACCCTCACCCAG
O.nubilalis CGTTGGACAGATCTTAGACAACTGGGTGCAGAATCCCTGGTTCCCCAGTTGTGAACGT~~~gGATGTCTAACAGACACTGGATTGATCACCCTCACCCAG
P.xylostella ~~~~~~~~~~~~~~aTGAACCGGTGGACCCAGCAGGGGGGATTCCCCGTAGTCACCGTCAGGAGGTCGGCGCCCTCCGCACAGTCATTTGTTATCACTCAG
S.exigua TATTGGGGCTGTGTTCGACAGTTGGGTCCAA~~~ACGTGGTTCTCAGTCGTGATGGTT~~~AACCGAACTGCATCCACTGGTGGCATTGTTGTTACTCAG
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
H.armigera CAACGTTACGTGCTCTCTGGC~~~~~~gCTGTT~~~gCCCCAACGACGTGGCACATTCCTCCCACCTGGACTCAACATGGCTCCCTCAACTTCAACAGCA
L.dispar GAGCGTTGGAATGTGAACAGTGGAGTATCTTCC~~~aTTCAGAGTAGTTGGTATATCCCTATTACATGGACTAGAGCTGGTGCTGTTGACTTTGAAAATC
M.sexta CGCCGCTTCAACATTAACACCGGGTACTCTAAT~~~gTTAACACTAACTACATCGTGCCGATATCTTTCGCGACCGCCAGCAACCCCGACTTCGCTAATA
O.furnacalis(R) GAACGTTTCCTTCTGAGCGGT~~~~~~aCCCCA~~~gCAGCCCAGCTCTGGGATATTCCCATCACTTGGACCCACCGAGGAGAACTCAACTTCGAGAGCA
O.nubilalis GAACGTTTCCTTCTGAGTGGT~~~~~~aCCCCA~~~gTAGCCCAGCTCTGGGATATTCCCATCACTTGGACCCACCGAGAAGAACTCAACTTCGAGAGCA
P.xylostella CGCCGCTTCCTCACAGACAGC~~~~~~aCCCAAGAGTCCAACACGGTATGGAACGTTCCCCTCAACTGGGTGCTCAGCACTGACGTGAACTTCAACGACA
S.exigua CGCCGTTACCAACTTTCTGGC~~~~~~aCCATT~~~cCCGATCAAATGTGGCAGATCCCTCTCAGTTGGACTGAGCAGAGACATTTGGACTTCAGCTCAA
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
H.armigera CCAGGCCTAGCACCGTCCTTAGC~~~gATGAGATTGGCACCATCAACGCTGCATCTGGa~~~~~~~~~~~~~~~~GACCACTTCGTCATTTTCAACATTGC
L.dispar TGAAACCAACGCAGATCATAACTGGTACTACCACAGTAATCAACAGAGGCACTACAGGa~~~~~~~~~~~~~~~~AGGGAATGGGTTATTTTCAACAAGCA
M.sexta CCAAGCCCACGCACATCTTGTCAAAGGCTGTGCAGATTATCAACCGCGGTTCCGTCGGt~~~~~~~~~~~~~~~~GATGAACGGGTTATTTTCAACAAACA
O.furnacalis(R) CAAGGCCCAGTTTTATCCTCTCT~~~aCTGCGAGTACTACTATCCAGAACACTCCCGGt~~~~~~~~~~~~~~~~CATTTCTGGGTGATCCTCAACATTGC
O.nubilalis CAAGGCCCAGTTTTATCCTCTCT~~~aCTGCGAGTACTACTATCCAGAACACTCCCGGt~~~~~~~~~~~~~~~~CATTTCTGGGTGATCCTCAACATTGC
P.xylostella CACGCCCCATAGCCTGGTTG~~~~~~CCGCCCCAGCTAGCGGCGGAGGCGGTCCAGGTCCCTGGACTCCAGAATGCCGAGTGGTTCATTGTGAATAAGCA
S.exigua CCAAGCCTAAAGCTATTCTTTCT~~~aCACCATCTGCACCTTACCCTAGTGAAGCAGGa~~~~~~~~~~~~~~~~GATAATTTCGTCATATTTAACATTCA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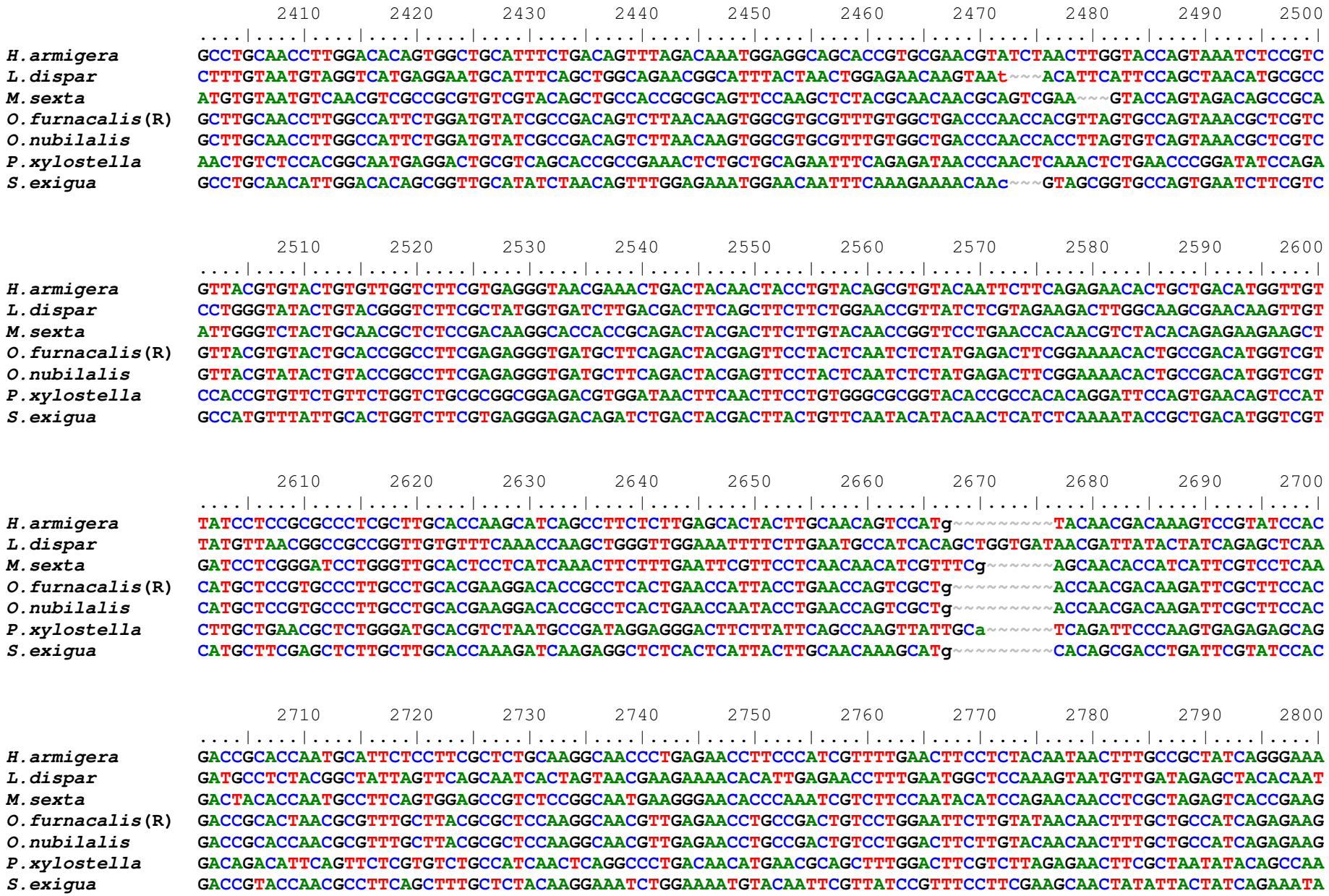
L.dispar ACAATCTGGTTTCTACAGGGTGAATTACGATTCTGTCACTTGGAGCCTTATCACTCAAGCGCTTAGAGACTCAACTACAAGGACACAAATACATGAATAC
M.sexta ACAGACAGGTTTCTACCGCGTGAACTATGATGATTATACTTGGGATCTCAATATTATGGCTCTTCGAGGCGCACAg~~~AGGACTCAAATCCACGAGTAC
O.furnacalis(R) TCAGTCAGGCTTGTACCGTGTTAACTATGACGACCATAACTGGGAAATGCTTGCTTCTTACCTCCGCAATGCAAACACTCGCACAAACGTCCACAAACTC
O.nubilalis TCAGTCAGGCTTGTACCGTGTTAACTATGACGACCATAACTGGGAAATGCTTGCTTCTTACCTCCGCAATGCAAACACTCGCACAAATGTCCACAAACTC
P.xylostella ACAGACTGGTTACTACCGTGTAAACTACGACCCTGAAAACTGGAGGGCTCTGGCTAAAGTCCTGAATGACACGCACGAGATC~~~~~~ATCCACCTACTG
S.exigua ACAGTCCGGACTGTACCGTGTCAATTACGACGATGACAACTGGAAGGCAATCGCATCCTATCTGAACAGC~~~AACAACCGCGAACGAATTCACAAACTG
2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
H.armigera AACAGAGCTCAGATCGTCAACGACATCTTGTACTTCGTGCGTTCTAACAGCATCAACAGGACTCTCGCTTTTGATGTCCTCGACTTCTTGAGGGATGAGA
L.dispar AATCGTGCTCAGATTATCGACGACGTATTCATCATGGCAAGATCTTCTGTGATGCCATACAGCACGGCACTTAACATCCTCTCATTCCTTGAATTTGAAG
M.sexta AACAGAGCCCAGATTGTGAACGATGTCTTCCAATTCGCTCGCTCCGGGCTCATGACCTACAACAGAGCGTTCAACATCCTCTCGTTCTTGGAGAACGAGA
O.furnacalis(R) AACAGGGCTCAGATCGTGAACGACGTGTTGTTCTTTATCAGAGCCGGCAAGATCAGCCTTGAAAGAGCTTTCGACGTTCTGTCCTTCCTTAAGATTGAGA
O.nubilalis AACAGGGCTCAGATCGTGAACGACGTGTTGTTCTTTATCAGAGCCGGCAAGATCAGCCTCGAAAGAGCTTTCGACGTTTTATCCTTCCTTAAGATTGAGA
P.xylostella AACCGGGCTCAGCTCATCGACGACTCATTCAACCTGGCCAGAAACGGACGTATTGACTACAGCTTGGCCTTCGACCTGTCCCAGTACCTGGTACAGGAGC
S.exigua AACAGAGCTCAGATTGTCAACGATGTACTACACTTCATTCGCTCTGAAGATATCGACAAGACTCTTGGCTTCGAAGTTCTTGACTTCTTAAGGAGTGAGA
2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
H.armigera CCGATTACTACGTATGGAATGGAGCTCTTACCCAGATCGACTGGATCCTTCGTCGCCCTGAACACTTGCCTACCGCTCATGCTGCTTTCTCTGAATACAT
L.dispar ATCAGTATGCTCCGTGGATTGCTGCTATTACTGGATTCACATACGCCAGACGCCGATTAGTTCACGATACAGAAAGCCTCACGGCTTTAAACGCACTTAT
M.sexta CTGCCTACACCCCATGGGTTGCCGCGGTAACTGGTTTTAACTGGATCAGAAATCGTCTCGCCGGAACCCCTGAATTGGCTCGTCTTCATACAACGATCGC
O.furnacalis(R) CCGACTACTATGTCTGGAACGGCGCCATTACTCAGCTGGAATGGATTAGGAAGAGAATGGAACATATTCCTCTAGCTCACCAGAAATTCACCGAATACAT
O.nubilalis CCGACTACTATGTCTGGAACGGCGCCATTACTCAGCTGGAATGGATTAGGAAGAGAATGGAACATATTCCTCTAGCTCACCAGAAATTCACCGAATACAT
P.xylostella GCGACTACATCCCGTGGGCCGCCGCCAACGCCGCCTTCAACTATCTCAACTCAGTCCTGAGCGGCTCTTCTGTA~~~CACCCCCTGTTCCAGGAATACCT
S.exigua CTGACTACTACGTGTGGAATGGTGCTCTTACTCAGCTCGACTGGATCCGACGTCGGTTTGAGCACTCGCCTAGAGCTCACGCGGCATTTACTAGCTACCT
2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
H.armigera CCTCGAGCTCATGAACACGGTTATCAATCACCTTGGCTATAACGAGCACAGTACCGACTCTACCTCCACAATCCTCAACCGTATGCAGATCATGAACTAC
L.dispar CATCAGTCTCAGTGATGCAATCACCAGGCGACTTGGTTTCGCTGAAGTTTCAGGCGAATCTTATATGGATGGTTTACTTCGTATGAATGTAAATACATTC
M.sexta CCAATGGGCATCAAGG~~~GTCATGTCGGAGCTGACCTACTACCCGGTCGCGAATGAGAGCTTCATGAGGTCGTACCTCAGGTACCAGCTGGCTCCACTG
O.furnacalis(R) GCTAGATATCCTGGATGCGGCAATTCAGCACTTGGGATATGAGGAGCTCGCTACGGATTCCACGTCTACCATCCTCAACCGCATGCAACTCATGAACTTG
O.nubilalis GCTAGATATCCTGGATGCGGCAATTCAGCACTTGGGATATGAGGAGCTCGCTACCGATTCCACGTCTACCATCCTCAACCGCATGCAACTCATGAACTTG
P.xylostella TCTCTTCCTCACTGCGCCGCTTTACCAGAGGCTTGGCTTCAACGCCGCCACTGGCGAAGAGCATGTCACTCCCTTCCATCGCAACATCATCCTGAACATC
S.exigua CCTTGGACTAATGAACAATGTTATTAACCACCTCGGATACGACGAACGCCCCAACGACTCCACTTCCACAATCCTAAACAGAATTCAGATCCTGAACTTC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L.dispar CTTTGTAATGTAGGTCATGAGGAATGCATTTCAGCTGGCAGAACGGCATTTACTAACTGGAGAACAAGTAAt~~~ACATTCATTCCAGCTAACATGCGCC
M.sexta ATGTGTAATGTCAACGTCGCCGCGTGTCGTACAGCTGCCACCGCGCAGTTCCAAGCTCTACGCAACAACGCAGTCGAA~~~GTACCAGTAGACAGCCGCA
O.furnacalis(R) GCTTGCAACCTTGGCCATTCTGGATGTATCGCCGACAGTCTTAACAAGTGGCGTGCGTTTGTGGCTGACCCAACCACGTTAGTGCCAGTAAACGCTCGTC
O.nubilalis GCTTGCAACCTTGGCCATTCTGGATGTATCGCCGACAGTCTTAACAAGTGGCGTGCGTTTGTGGCTGACCCAACCACCTTAGTGTCAGTAAACGCTCGTC
P.xylostella AACTGTCTCCACGGCAATGAGGACTGCGTCAGCACCGCCGAAACTCTGCTGCAGAATTTCAGAGATAACCCAACTCAAACTCTGAACCCGGATATCCAGA
S.exigua GCCTGCAACATTGGACACAGCGGTTGCATATCTAACAGTTTGGAGAAATGGAACAATTTCAAAGAAAACAAc~~~GTAGCGGTGCCAGTGAATCTTCGTC
2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
H.armigera GTTACGTGTACTGTGTTGGTCTTCGTGAGGGTAACGAAACTGACTACAACTACCTGTACAGCGTGTACAATTCTTCAGAGAACACTGCTGACATGGTTGT
L.dispar CCTGGGTATACTGTACGGGTCTTCGCTATGGTGATCTTGACGACTTCAGCTTCTTCTGGAACCGTTATCTCGTAGAAGACTTGGCAAGCGAACAAGTTGT
M.sexta ATTGGGTCTACTGCAACGCTCTCCGACAAGGCACCACCGCAGACTACGACTTCTTGTACAACCGGTTCCTGAACCACAACGTCTACACAGAGAAGAAGCT
O.furnacalis(R) GTTACGTGTACTGCACCGGCCTTCGAGAGGGTGATGCTTCAGACTACGAGTTCCTACTCAATCTCTATGAGACTTCGGAAAACACTGCCGACATGGTCGT
O.nubilalis GTTACGTATACTGTACCGGCCTTCGAGAGGGTGATGCTTCAGACTACGAGTTCCTACTCAATCTCTATGAGACTTCGGAAAACACTGCCGACATGGTCGT
P.xylostella CCACCGTGTTCTGTTCTGGTCTGCGCGGCGGAGACGTGGATAACTTCAACTTCCTGTGGGCGCGGTACACCGCCACACAGGATTCCAGTGAACAGTCCAT
S.exigua GCCATGTTTATTGCACTGGTCTTCGTGAGGGAGACAGATCTGACTACGACTTACTGTTCAATACATACAACTCATCTCAAAATACCGCTGACATGGTCGT
2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
H.armigera TATCCTCCGCGCCCTCGCTTGCACCAAGCATCAGCCTTCTCTTGAGCACTACTTGCAACAGTCCATg~~~~~~~~~~TACAACGACAAAGTCCGTATCCAC
L.dispar TATGTTAACGGCCGCCGGTTGTGTTTCAAACCAAGCTGGGTTGGAAATTTTCTTGAATGCCATCACAGCTGGTGATAACGATTATACTATCAGAGCTCAA
M.sexta GATCCTCGGGATCCTGGGTTGCACTCCTCATCAAACTTCTTTGAATTCGTTCCTCAACAACATCGTTTCg~~~~~~AGCAACACCATCATTCGTCCTCAA
O.furnacalis(R) CATGCTCCGTGCCCTTGCCTGCACGAAGGACACCGCCTCACTGAACCATTACCTGAACCAGTCGCTg~~~~~~~~~~ACCAACGACAAGATTCGCTTCCAC
O.nubilalis CATGCTCCGTGCCCTTGCCTGCACGAAGGACACCGCCTCACTGAACCAATACCTGAACCAGTCGCTg~~~~~~~~~~ACCAACGACAAGATTCGCTTCCAC
P.xylostella CTTGCTGAACGCTCTGGGATGCACGTCTAATGCCGATAGGAGGGACTTCTTATTCAGCCAAGTTATTGCa~~~~~~TCAGATTCCCAAGTGAGAGAGCAG
S.exigua CATGCTTCGAGCTCTTGCTTGCACCAAAGATCAAGAGGCTCTCACTCATTACTTGCAACAAAGCATg~~~~~~~~~~CACAGCGACCTGATTCGTATCCAC
2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
H.armigera GACCGCACCAATGCATTCTCCTTCGCTCTGCAAGGCAACCCTGAGAACCTTCCCATCGTTTTGAACTTCCTCTACAATAACTTTGCCGCTATCAGGGAAA
L.dispar GATGCCTCTACGGCTATTAGTTCAGCAATCACTAGTAACGAAGAAAACACATTGAGAACCTTTGAATGGCTCCAAAGTAATGTTGATAGAGCTACACAAT
M.sexta GACTACACCAATGCCTTCAGTGGAGCCGTCTCCGGCAATGAAGGGAACACCCAAATCGTCTTCCAATACATCCAGAACAACCTCGCTAGAGTCACCGAAG
O.furnacalis(R) GACCGCACTAACGCGTTTGCTTACGCGCTCCAAGGCAACGTTGAGAACCTGCCGACTGTCCTGGAATTCTTGTATAACAACTTTGCTGCCATCAGAGAAG
O.nubilalis GACCGCACCAACGCGTTTGCTTACGCGCTCCAAGGCAACGTTGAGAACCTGCCGACTGTCCTGGACTTCTTGTACAACAACTTTGCTGCCATCAGAGAAG
P.xylostella GACAGACATTCAGTTCTCGTGTCTGCCATCAACTCAGGCCCTGACAACATGAACGCAGCTTTGGACTTCGTCTTAGAGAACTTCGCTAATATACAGCCAA
S.exigua GACCGTACCAACGCCTTCAGCTTTGCTCTACAAGGAAATCTGGAAAATGTACAATTCGTTATCCGTTTCCTTCGAAGCAACTATATTACTATCAGAAATA

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                          2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          CGTACGGAGGTGTTGCCCGTCTCAATCTTTGCATCAACGCTATTGCGGCATTCTTGACTGACTACCAG~~~~~~~~~aCCATCACTCAGTTCCAAACT~~
L.dispar            CGGGCTAc~~~~~~~~~~~~CTAGCTACTATAATAAGCTCCATTACAACTCGTCTGCTGAATGAGGAG~~~~~~~~~cAGATCGCTGTGGTATCCACT~~
M.sexta             CTTTTGGc~~~~~~ACTCCGAATACTCCCCTCTCGTACGTTTCTTCAAGATTGCGGACCGAGGCTGaa~~~~~~~~~ATAAATGCCTTCCAAGCg~~~~~
O.furnacalis(R)     CGCACGGAAACCAGGACCGTCTCATCGTCAATATCAACAACGTAGCTGGATTCTTGACCAACTACGAG~~~~~~~~~cATATTGTTGAATTCCAAACC~~
O.nubilalis         CGCACGGAAACCAGGACCGTCTCATCGTCAACATCAACAACGTAGCTGGATTCTTGACCAACTACGAG~~~~~~~~~cATATTGTTGAATTCCAAACC~~
P.xylostella        ACGTACAAGGCTTAACCGGCACTACGAACATCCTCAATGCCTTTGCAAGAACACTCACTACTCAGGAGCATGCCAACAAGATCGATGAATTCTCAAACAA
S.exigua            CATACGGTGGTGAAGCTCGTTTGAATATCTGCATCAACGCCGTTGCCGCCTTCTTGAACACGTTTGAG~~~~~~~~~gCAATAACTGAATTCCAGCAA~~

                          2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          ~~~~~~~TGGGTGTACTCCAACCAATTGGAGCTGGTTGGCTCTGTCGGCGTTGGAAATAACGTCGTCACCGCCGCCTTAGACAATCTc~~~~~~~~~~~~
L.dispar            ~~~~~~~TGGCTTGAAGCAAACCAACTTATACTTGGCACTGCCTACAATACCGGTATCAATGGCATCGCTACTGCCAGATCGAATATGCAATGGTACAAC
M.sexta             ~~~~~~~TGGGCCAACCAGACTCAGACGCAGCTCGGCAACAGTTACCAAGCCGTGTACAATGGAGCCGAATCATCTCGCCAGAGTATCGCTTGGGCCGCA
O.furnacalis(R)     ~~~~~~~TGGGCGTACGCTAACCAGTTGGCGCTCGGCAGCGCTTTCCAGAACGCTGTCAACGTCGTCAACAGTGCCATGAACAACCTc~~~~~~~~~~~~
O.nubilalis         ~~~~~~~TGGGCGTACGCTAACCAGTTGGCGCTCGGCAGCGCTTTCCAGAACGCTGTCAACGTCGTCAACAGTGCCATGAACAACCTc~~~~~~~~~~~~
P.xylostella        ATACGCCAACGTGTTTACGGCGGGGGAGATGGCTTCAGTGGCGGCCATCAAA~~~GAGAACATCGCCGCTTCCATCACGTGGAACTCTCAAAACGCTGCT
S.exigua            ~~~~~~~TGGGTGTACGATTCACAAATCGGCTTAGGAGGGTCCTTTAACGTCGCTGTGGGTGTAGTCAACAGTGCTATGGCCAATCTt~~~~~~~~~~~~

                          3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ACTTGGGGCA
L.dispar            CAGAGAATACCAGAGTTCCAGAGCTACTTCGATACGGGTTATACTGAAGAATCGTTTGAAGATGATACAACTACAACCACGACAACAACTACTACTACAA
M.sexta             ACTGTCCAATCTGACATGAACACTTACTTCACTAATGGCAACGAGGCAATCCAGCAGTCAACTACTGCTCCTACCACCACCACTACAACTACTACTGTAG
O.furnacalis(R)     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AACTGGGGAA
O.nubilalis         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AACTGGGGAA
P.xylostella        ACGGTCGAGGCCTGGCTGCGAAAGAACTTCGGT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~aCTGATGGAG
S.exigua            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GAATGGGGCG

                          3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera          ACGGCGCAGCTGTTGAAATTGTCAACTTCCTCAACTCTAGAAGCGGTTCCAgc~~~~~~~~~~~~ACCATCCTTGCCTCTTCAATCCTCATCTTAGCAGC
L.dispar            CAACAGAGGCTCCa~~~~~~~~~ACAACTGAATCTACAACGGGAACAACTCCA~~~TCATCAGCGACTGCTGCCACTTTAAGCTTTTTGACATTATTCAT
M.sexta             CACCACCAAGCATTTCGGAGCCAGTGACCCCAGTTCTACCAGAACCTGTCCCAGATTCGGCAGCGACAAGCTTCCTTTCTGCGATGGTGATACTATTCGC
O.furnacalis(R)     ACAGCGTTGCTGCTGAGGTCTACGACATACTCCTGGCGAGGAACTCCGCTGct~~~~~~~~~~~~GCCATTGTTTCTCCCCTCGCTCTCATCCTGGCTGC
O.nubilalis         ACAGCGTTGCTGCTGAGGTCTACGACATACTCCTGGCGAGGAACTCCGCTGct~~~~~~~~~~~~GCCATTGTTTCTCCCATCGCTCTCATCCTGGCTGC
P.xylostella        CA~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~aGCACTGTCAGTGCCTCAATTACCATTATTATCTCCGCTAT
S.exigua            CCAACACTTCACTTGAAATCTTCAATTTCGTTTCAACTAGAGGAGCTTCTTcc~~~~~~~~~~~~GCCATCTTCGCTTCGTCTTTCCTCATCCTAGCAGC
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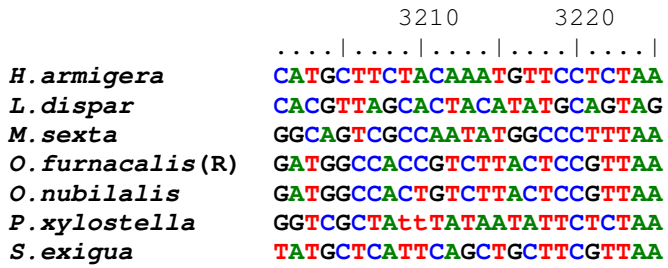
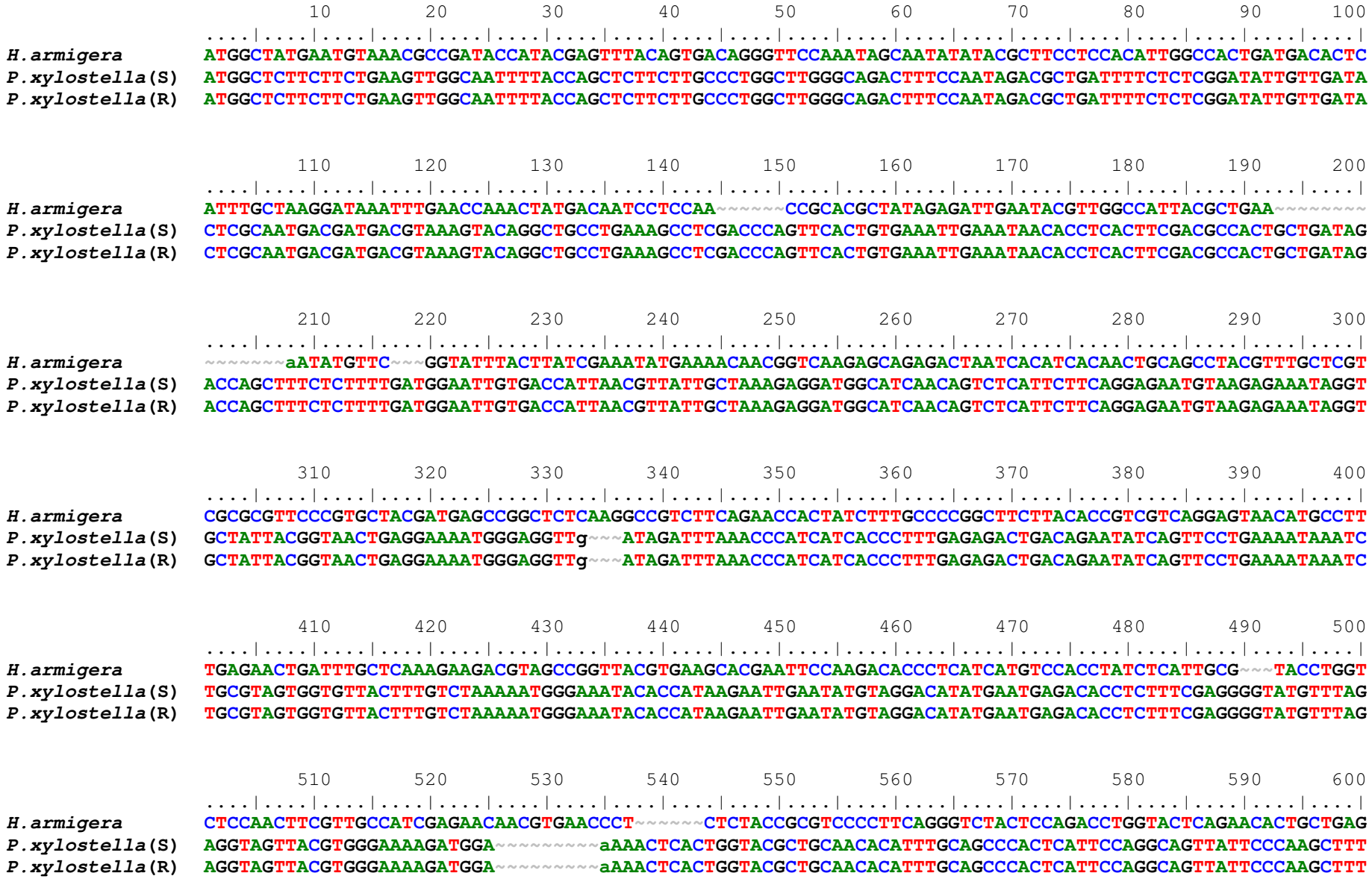


Figure B.4: DNA sequence alignment of *aminopeptidase N4* (*APN4*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Helicoverpa armigera* (27818924), *Lymantria dispar* (16588788), *Manduca sexta* (20260703) *Ostrinia furnacalis* (R) (207091423), *Ostrinia nubilalis* (258547213), *Plutella xylostella* (45685594) and *Spodoptera exigua* (37788343).

B.5. *Aminopeptidase N5* (*APN5*)



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                       610       620       630       640       650       660       670       680       690       700
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         TTCGCCCTGACCTTTGGACAGCAGAACATGGCAGCTTTGGAGAGATAC~~~ACTGAGTTCAACTAt~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
P.xylostella(S)    GACGAACCTGGTTTCAAATCCACCTTCAAAATTATCGTTAACCGACCAGCAAACTTTGCCGACACTCATTCCAATATG~~~~~~tACGCCGAGTCTCGAA
P.xylostella(R)    GACGAACCTGGTTTCAAATCCACCTTCAAAATTATCGTTAACCGACCAGCAAACTTTGCCGACACTCATTCCAATATG~~~~~~tACGCCGAGTCTCGAA

                       710       720       730       740       750       760       770       780       790       800
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
P.xylostella(S)    GTGAACCGATCAACGGCTTAGTTAAAGAAGTTTTTTACACTACACCTCGAATGTCAGCATACTTGGTTACAATTCACATAAGTGATGAATTCACAATTAT
P.xylostella(R)    GTGAACCGATCAACGGCTTAGTTAAAGAAGTTTTTTACACTACACCTCGAATGTCAGCATACTTGGTTACAATTCACATAAGTGATGAATTCACAATTAT

                       810       820       830       840       850       860       870       880       890       900
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
P.xylostella(S)    AGCTGATAACGGAGATGCTAAAAGACCCTACCGTATCTTa~~~~~~~~~~~~GCTCGTCCTGATGCTGCGAACCAAGGTCAATATGCTTTGGAGGTTGGC
P.xylostella(R)    AGCTGATAACGGAGATGCTAAAAGACCCTACCGTATCTTa~~~~~~~~~~~~GCTCGTCCTGATGCTGCGAACCAAGGTCAATATGCTTTGGAGGTTGGC

                       910       920       930       940       950       960       970       980       990      1000
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GAGTTCCCGAAGATGGATAAGGTGGCCGTACCTGATTTCGCTG
P.xylostella(S)    CCTCCACTGACGAAATGGCTGGAGGAATATTTGGGTAAGCCATACTATGAAATGGCTGAGAACATGAAGAATGACCAAATTGCTTCACCGTTTTGGGCGT
P.xylostella(R)    CCTCCACTGACGAAATGGCTGGAGGAATATTTGGGTAAGCCATACTATGAAATGGCTGAGAGCATGAAGAATGACCAAATTGCTTCACCGTTTTGGGCGT

                      1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         CTGGTGCTATGGAGAACTGGGGTCTGGTTATCTACAGAGAAGTGGCACTCCTAGTAACGGACGGAGTGACAACAACAGCCGTCAGACAGAATGTAGGCAG
P.xylostella(S)    CTGGTGCTACGGAGAACTGGGGATTGGTGACGTACAGAGAGCTCCGCCTCCTCTACGAAGAGGGGGAGACGAATGTCGTGGACAAGATGAGCATCGGCAC
P.xylostella(R)    CTGGTGCTACGGAGAACTGGGGATTGGTGACGTACAGAGAGCTCCGCCTCCTCTACGAAGAGGGGGAGACGAATGTCGTGGACAAGATGAGCATCGGCAC

                      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera         GATCATCTGTCACGAGAACGTTCATCAGTGGTTCGGTAACGAAGTGGGGCCTCTGTCCTGGACCTACACGTGGCTCAACGAAGGTTTTGCTAACTTCTTC
P.xylostella(S)    CATCACGGCCCACGAGCTCGGACACAAGTGGTTCGGCAACCTGGTGACGGCGCGCTGGTGGGACAACGTGTGGATCAACGAGGGCTACGCCAGCTACTTC
P.xylostella(R)    CATCACGGCCCACGAGCTCGGACACAAGTGGTTCGGCAACCTGGTGACGGCGCGCTGGTGGGACAACGTGTGGATCAACGAGGGCTACGCCAGCTACTTC
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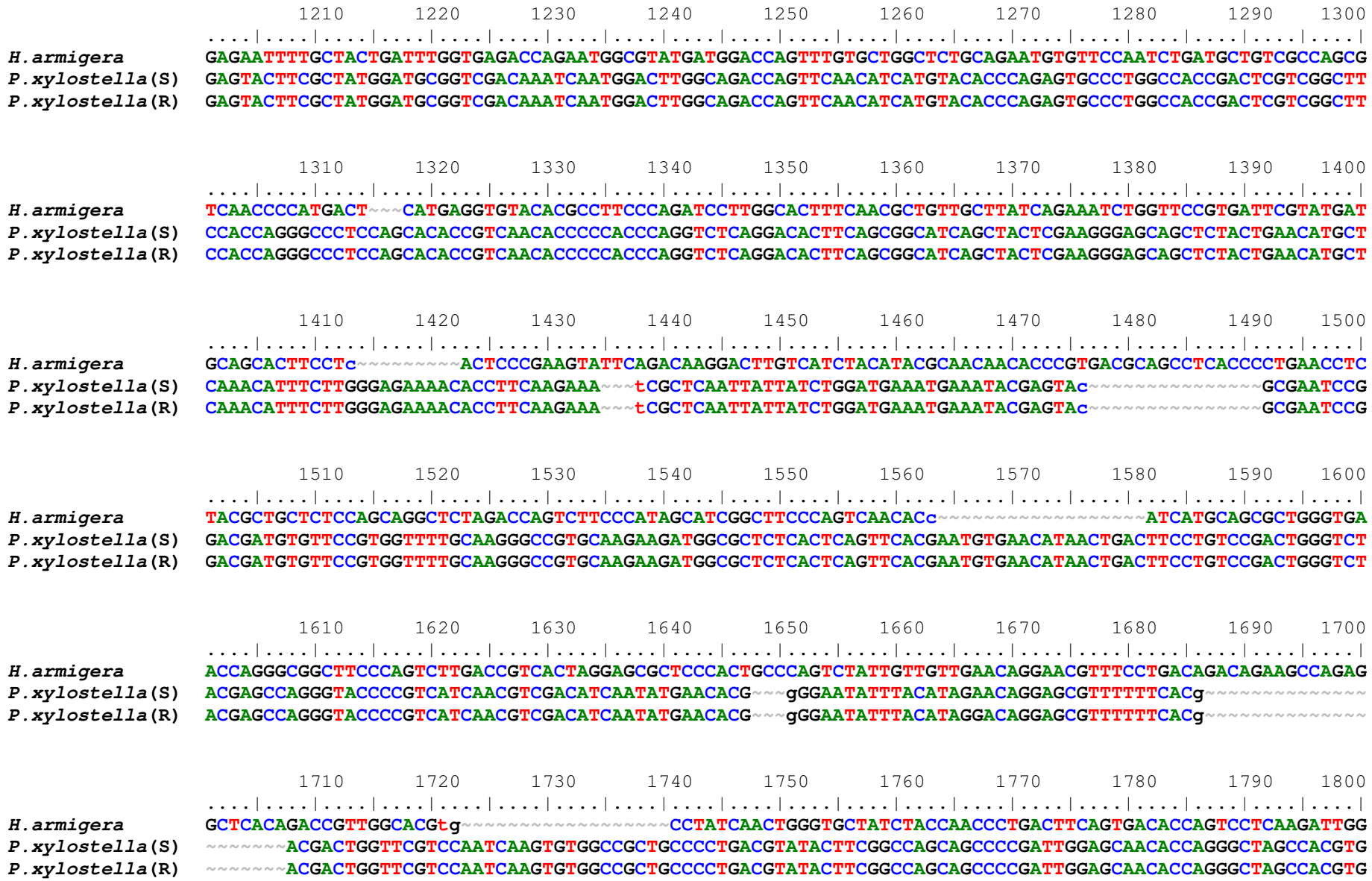
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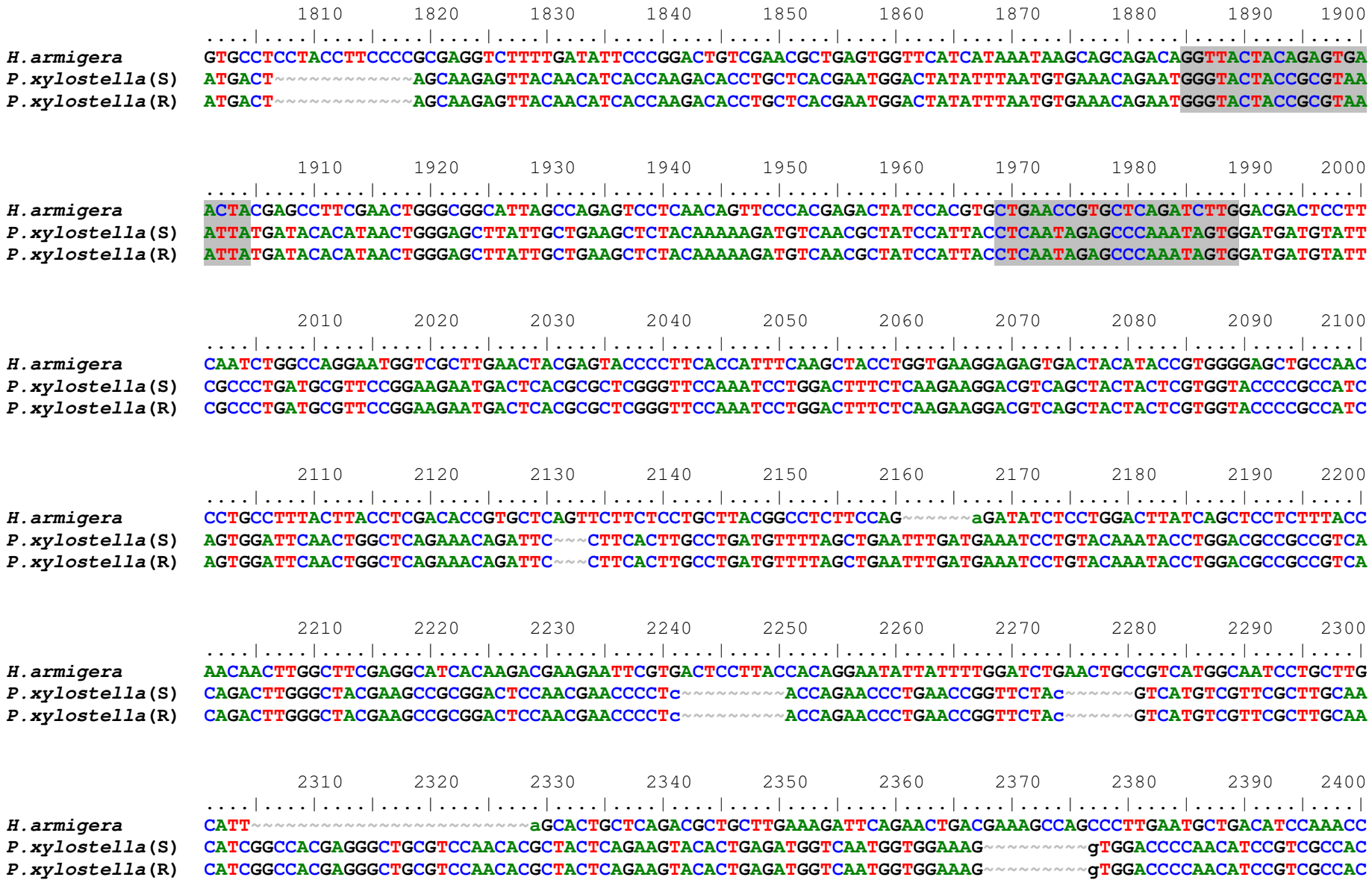
H.armigera GAGAATTTTGCTACTGATTTGGTGAGACCAGAATGGCGTATGATGGACCAGTTTGTGCTGGCTCTGCAGAATGTGTTCCAATCTGATGCTGTCGCCAGCG
P.xylostella(S) GAGTACTTCGCTATGGATGCGGTCGACAAATCAATGGACTTGGCAGACCAGTTCAACATCATGTACACCCAGAGTGCCCTGGCCACCGACTCGTCGGCTT
P.xylostella(R) GAGTACTTCGCTATGGATGCGGTCGACAAATCAATGGACTTGGCAGACCAGTTCAACATCATGTACACCCAGAGTGCCCTGGCCACCGACTCGTCGGCTT
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
H.armigera TCAACCCCATGACT~~~CATGAGGTGTACACGCCTTCCCAGATCCTTGGCACTTTCAACGCTGTTGCTTATCAGAAATCTGGTTCCGTGATTCGTATGAT
P.xylostella(S) CCACCAGGGCCCTCCAGCACACCGTCAACACCCCCACCCAGGTCTCAGGACACTTCAGCGGCATCAGCTACTCGAAGGGAGCAGCTCTACTGAACATGCT
P.xylostella(R) CCACCAGGGCCCTCCAGCACACCGTCAACACCCCCACCCAGGTCTCAGGACACTTCAGCGGCATCAGCTACTCGAAGGGAGCAGCTCTACTGAACATGCT
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
H.armigera GCAGCACTTCCTc~~~~~~~~~~ACTCCCGAAGTATTCAGACAAGGACTTGTCATCTACATACGCAACAACACCCGTGACGCAGCCTCACCCCTGAACCTC
P.xylostella(S) CAAACATTTCTTGGGAGAAAACACCTTCAAGAAA~~~tCGCTCAATTATTATCTGGATGAAATGAAATACGAGTAc~~~~~~~~~~~~~~~~GCGAATCCG
P.xylostella(R) CAAACATTTCTTGGGAGAAAACACCTTCAAGAAA~~~tCGCTCAATTATTATCTGGATGAAATGAAATACGAGTAc~~~~~~~~~~~~~~~~GCGAATCCG
1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
H.armigera TACGCTGCTCTCCAGCAGGCTCTAGACCAGTCTTCCCATAGCATCGGCTTCCCAGTCAACACc~~~~~~~~~~~~~~~~~~ATCATGCAGCGCTGGGTGA
P.xylostella(S) GACGATGTGTTCCGTGGTTTTGCAAGGGCCGTGCAAGAAGATGGCGCTCTCACTCAGTTCACGAATGTGAACATAACTGACTTCCTGTCCGACTGGGTCT
P.xylostella(R) GACGATGTGTTCCGTGGTTTTGCAAGGGCCGTGCAAGAAGATGGCGCTCTCACTCAGTTCACGAATGTGAACATAACTGACTTCCTGTCCGACTGGGTCT
1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
H.armigera ACCAGGGCGGCTTCCCAGTCTTGACCGTCACTAGGAGCGCTCCCACTGCCCAGTCTATTGTTGTTGAACAGGAACGTTTCCTGACAGACAGAAGCCAGAG
P.xylostella(S) ACGAGCCAGGGTACCCCGTCATCAACGTCGACATCAATATGAACACG~~~gGGAATATTTACATAGAACAGGAGCGTTTTTTCACg~~~~~~~~~~~~~~
P.xylostella(R) ACGAGCCAGGGTACCCCGTCATCAACGTCGACATCAATATGAACACG~~~gGGAATATTTACATAGGACAGGAGCGTTTTTTCACg~~~~~~~~~~~~~~
1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
H.armigera GCTCACAGACCGTTGGCACGtg~~~~~~~~~~~~~~~~~~~~CCTATCAACTGGGTGCTATCTACCAACCCTGACTTCAGTGACACCAGTCCTCAAGATTGG
P.xylostella(S) ~~~~~~~~ACGACTGGTTCGTCCAATCAAGTGTGGCCGCTGCCCCTGACGTATACTTCGGCCAGCAGCCCCGATTGGAGCAACACCAGGGCTAGCCACGTG
P.xylostella(R) ~~~~~~~~ACGACTGGTTCGTCCAATCAAGTGTGGCCGCTGCCCCTGACGTATACTTCGGCCAGCAGCCCCGATTGGAGCAACACCAGGGCTAGCCACGTG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P.xylostella(R) ATGACT~~~~~~~~~~~~~AGCAAGAGTTACAACATCACCAAGACACCTGCTCACGAATGGACTATATTTAATGTGAAACAGAATGGGTACTACCGCGTAA
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
H.armigera ACTACGAGCCTTCGAACTGGGCGGCATTAGCCAGAGTCCTCAACAGTTCCCACGAGACTATCCACGTGCTGAACCGTGCTCAGATCTTGGACGACTCCTT
P.xylostella(S) ATTATGATACACATAACTGGGAGCTTATTGCTGAAGCTCTACAAAAAGATGTCAACGCTATCCATTACCTCAATAGAGCCCAAATAGTGGATGATGTATT
P.xylostella(R) ATTATGATACACATAACTGGGAGCTTATTGCTGAAGCTCTACAAAAAGATGTCAACGCTATCCATTACCTCAATAGAGCCCAAATAGTGGATGATGTATT
2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
H.armigera CAATCTGGCCAGGAATGGTCGCTTGAACTACGAGTACCCCTTCACCATTTCAAGCTACCTGGTGAAGGAGAGTGACTACATACCGTGGGGAGCTGCCAAC
P.xylostella(S) CGCCCTGATGCGTTCCGGAAGAATGACTCACGCGCTCGGGTTCCAAATCCTGGACTTTCTCAAGAAGGACGTCAGCTACTACTCGTGGTACCCCGCCATC
P.xylostella(R) CGCCCTGATGCGTTCCGGAAGAATGACTCACGCGCTCGGGTTCCAAATCCTGGACTTTCTCAAGAAGGACGTCAGCTACTACTCGTGGTACCCCGCCATC
2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
H.armigera CCTGCCTTTACTTACCTCGACACCGTGCTCAGTTCTTCTCCTGCTTACGGCCTCTTCCAG~~~~~~aGATATCTCCTGGACTTATCAGCTCCTCTTTACC
P.xylostella(S) AGTGGATTCAACTGGCTCAGAAACAGATTC~~~CTTCACTTGCCTGATGTTTTAGCTGAATTTGATGAAATCCTGTACAAATACCTGGACGCCGCCGTCA
P.xylostella(R) AGTGGATTCAACTGGCTCAGAAACAGATTC~~~CTTCACTTGCCTGATGTTTTAGCTGAATTTGATGAAATCCTGTACAAATACCTGGACGCCGCCGTCA
2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
H.armigera AACAACTTGGCTTCGAGGCATCACAAGACGAAGAATTCGTGACTCCTTACCACAGGAATATTATTTTGGATCTGAACTGCCGTCATGGCAATCCTGCTTG
P.xylostella(S) CAGACTTGGGCTACGAAGCCGCGGACTCCAACGAACCCCTc~~~~~~~~~~ACCAGAACCCTGAACCGGTTCTAc~~~~~~GTCATGTCGTTCGCTTGCAA
P.xylostella(R) CAGACTTGGGCTACGAAGCCGCGGACTCCAACGAACCCCTc~~~~~~~~~~ACCAGAACCCTGAACCGGTTCTAc~~~~~~GTCATGTCGTTCGCTTGCAA
2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
H.armigera CATT~~~~~~~~~~~~~~~~~~~~~~~~aGCACTGCTCAGACGCTGCTTGAAAGATTCAGAACTGACGAAAGCCAGCCCTTGAATGCTGACATCCAAACC
P.xylostella(S) CATCGGCCACGAGGGCTGCGTCCAACACGCTACTCAGAAGTACACTGAGATGGTCAATGGTGGAAAG~~~~~~~~~~gTGGACCCCAACACATCCGTCGCCAC
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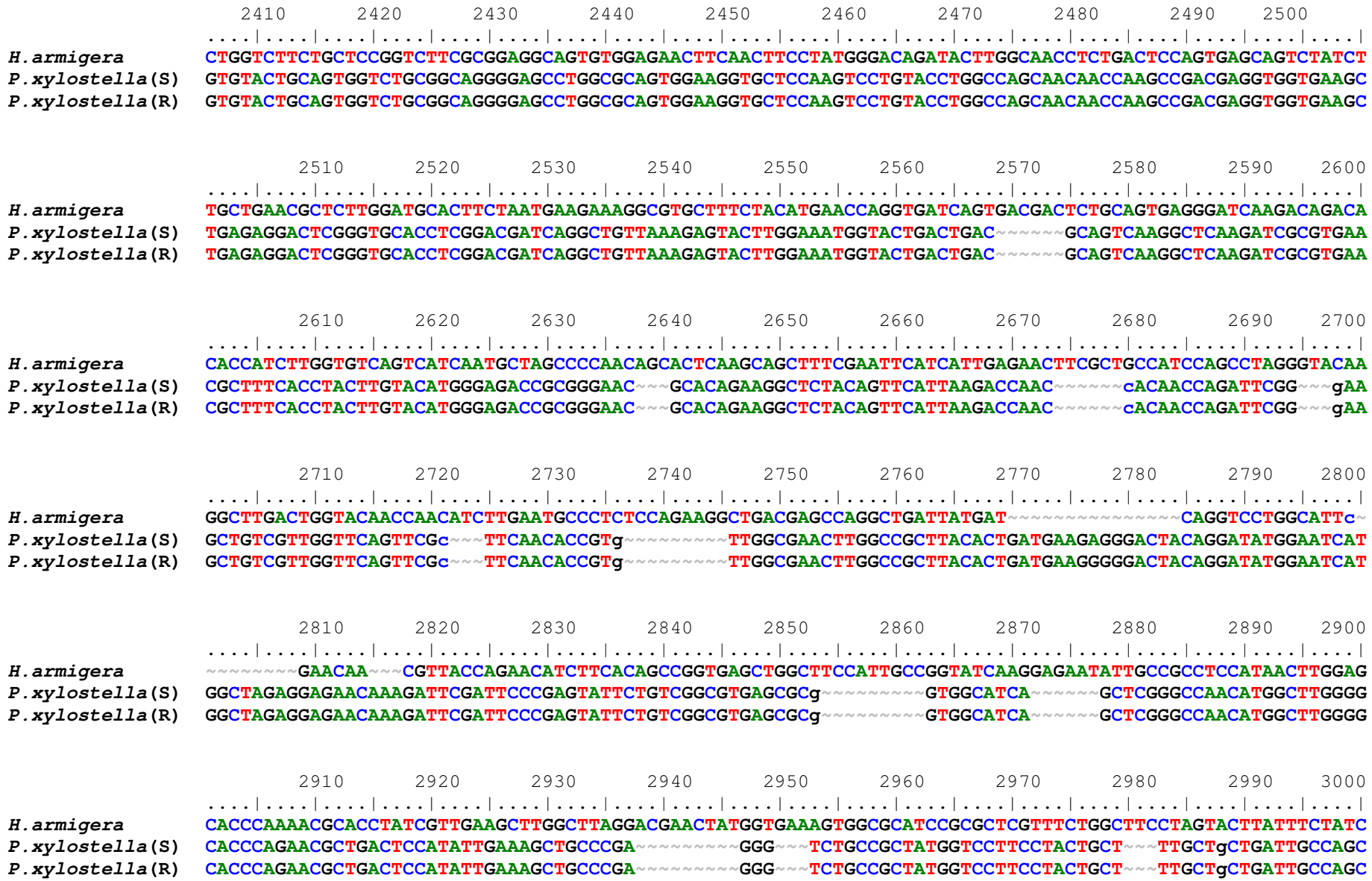
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H.armigera CTGGTCTTCTGCTCCGGTCTTCGCGGAGGCAGTGTGGAGAACTTCAACTTCCTATGGGACAGATACTTGGCAACCTCTGACTCCAGTGAGCAGTCTATCT
P.xylostella(S) GTGTACTGCAGTGGTCTGCGGCAGGGGAGCCTGGCGCAGTGGAAGGTGCTCCAAGTCCTGTACCTGGCCAGCAACAACCAAGCCGACGAGGTGGTGAAGC
P.xylostella(R) GTGTACTGCAGTGGTCTGCGGCAGGGGAGCCTGGCGCAGTGGAAGGTGCTCCAAGTCCTGTACCTGGCCAGCAACAACCAAGCCGACGAGGTGGTGAAGC
2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
H.armigera TGCTGAACGCTCTTGGATGCACTTCTAATGAAGAAAGGCGTGCTTTCTACATGAACCAGGTGATCAGTGACGACTCTGCAGTGAGGGATCAAGACAGACA
P.xylostella(S) TGAGAGGACTCGGGTGCACCTCGGACGATCAGGCTGTTAAAGAGTACTTGGAAATGGTACTGACTGAC~~~~~~GCAGTCAAGGCTCAAGATCGCGTGAA
P.xylostella(R) TGAGAGGACTCGGGTGCACCTCGGACGATCAGGCTGTTAAAGAGTACTTGGAAATGGTACTGACTGAC~~~~~~GCAGTCAAGGCTCAAGATCGCGTGAA
2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
H.armigera CACCATCTTGGTGTCAGTCATCAATGCTAGCCCCAACAGCACTCAAGCAGCTTTCGAATTCATCATTGAGAACTTCGCTGCCATCCAGCCTAGGGTACAA
P.xylostella(S) CGCTTTCACCTACTTGTACATGGGAGACCGCGGGAAC~~~GCACAGAAGGCTCTACAGTTCATTAAGACCAAC~~~~~~cACAACCAGATTCGG~~~gAA
P.xylostella(R) CGCTTTCACCTACTTGTACATGGGAGACCGCGGGAAC~~~GCACAGAAGGCTCTACAGTTCATTAAGACCAAC~~~~~~cACAACCAGATTCGG~~~gAA
2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
H.armigera GGCTTGACTGGTACAACCAACATCTTGAATGCCCTCTCCAGAAGGCTGACGAGCCAGGCTGATTATGAT~~~~~~~~~~~~~~~CAGGTCCTGGCATTc~
P.xylostella(S) GCTGTCGTTGGTTCAGTTCGc~~~TTCAACACCGTg~~~~~~~~~TTGGCGAACTTGGCCGCTTACACTGATGAAGAGGGACTACAGGATATGGAATCAT
P.xylostella(R) GCTGTCGTTGGTTCAGTTCGc~~~TTCAACACCGTg~~~~~~~~~TTGGCGAACTTGGCCGCTTACACTGATGAAGGGGGACTACAGGATATGGAATCAT
2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
H.armigera ~~~~~~~~GAACAA~~~CGTTACCAGAACATCTTCACAGCCGGTGAGCTGGCTTCCATTGCCGGTATCAAGGAGAATATTGCCGCCTCCATAACTTGGAG
P.xylostella(S) GGCTAGAGGAGAACAAAGATTCGATTCCCGAGTATTCTGTCGGCGTGAGCGCg~~~~~~~~~GTGGCATCA~~~~~~GCTCGGGCCAACATGGCTTGGGG
P.xylostella(R) GGCTAGAGGAGAACAAAGATTCGATTCCCGAGTATTCTGTCGGCGTGAGCGCg~~~~~~~~~GTGGCATCA~~~~~~GCTCGGGCCAACATGGCTTGGGG
2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
H.armigera CACCCAAAACGCACCTATCGTTGAAGCTTGGCTTAGGACGAACTATGGTGAAAGTGGCGCATCCGCGCTCGTTTCTGGCTTCCTAGTACTTATTTCTATC
P.xylostella(S) CACCCAGAACGCTGACTCCATATTGAAAGCTGCCCGA~~~~~~~~~GGG~~~TCTGCCGCTATGGTCCTTCCTACTGCT~~~TTGCTgCTGATTGCCAGC
P.xylostella(R) CACCCAGAACGCTGACTCCATATTGAAAGCTGCCCGA~~~~~~~~~GGG~~~TCTGCCGCTATGGTCCTTCCTACTGCT~~~TTGCTgCTGATTGCCAGC

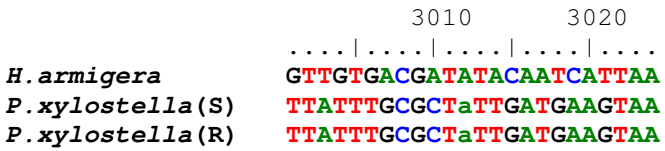
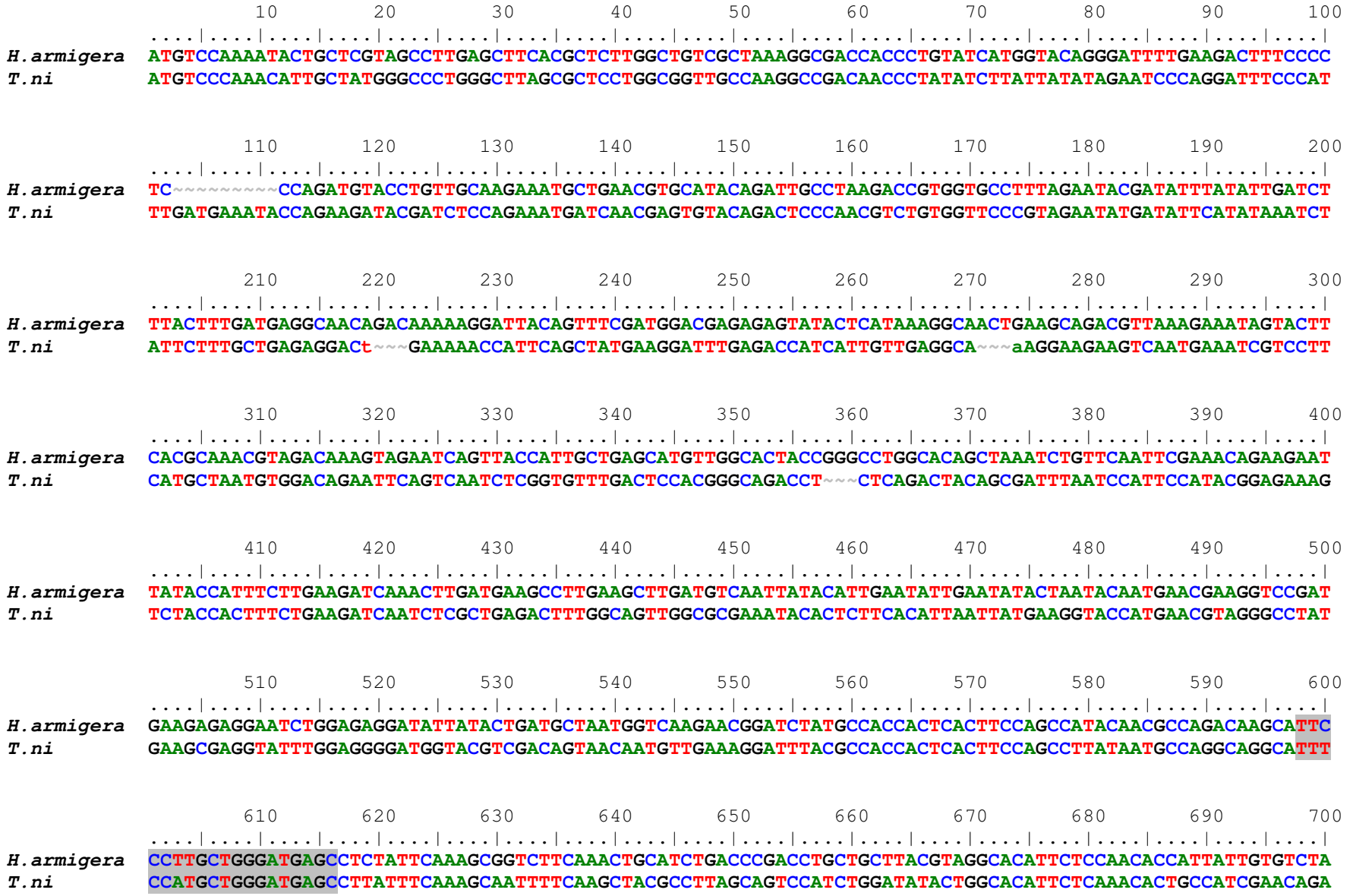
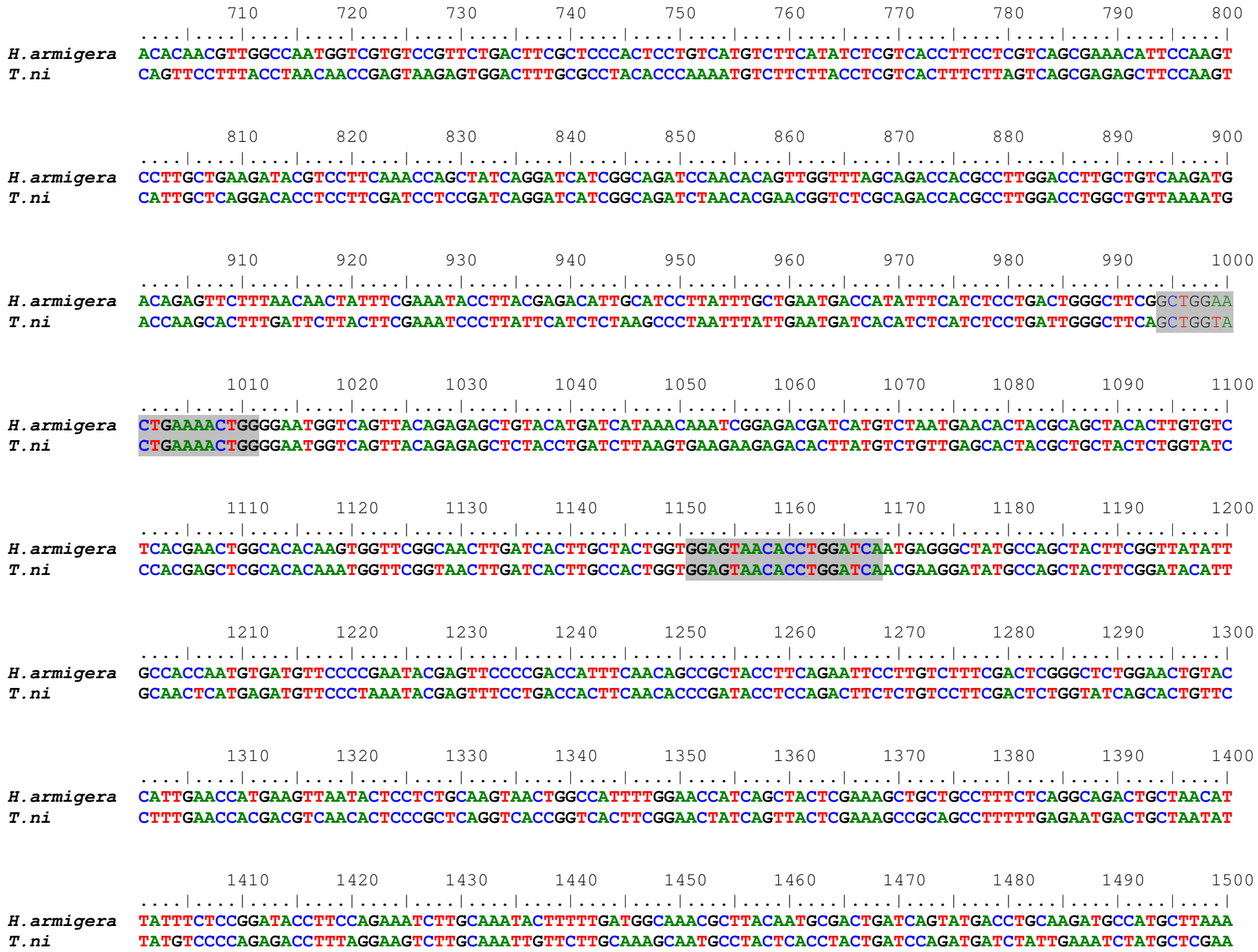


Figure B.5: DNA sequence alignment of *aminopeptidase N5* (*APN5*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Helicoverpa armigera* (126009702), *Plutella xylostella* (S) (281313031) and *Plutella xylostella* (R) (281313029).

B.6. *Aminopeptidase N6* (*APN6*)

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T.ni CAGTTCCTTTACCTAACAACCGAGTAAGAGTGGACTTTGCGCCTACACCCAAAATGTCTTCTTACCTCGTCACTTTCTTAGTCAGCGAGAGCTTCCAAGT
810 820 830 840 850 860 870 880 890 900
H.armigera CCTTGCTGAAGATACGTCCTTCAAACCAGCTATCAGGATCATCGGCAGATCCAACACAGTTGGTTTAGCAGACCACGCCTTGGACCTTGCTGTCAAGATG
T.ni CATTGCTCAGGACACCTCCTTCGATCCTCCGATCAGGATCATCGGCAGATCTAACACGAACGGTCTCGCAGACCACGCCTTGGACCTGGCTGTTAAAATG
910 920 930 940 950 960 970 980 990 1000
H.armigera ACAGAGTTCTTTAACAACTATTTCGAAATACCTTACGAGACATTGCATCCTTATTTGCTGAATGACCATATTTCATCTCCTGACTGGGCTTCGGCTGGAA
T.ni ACCAAGCACTTTGATTCTTACTTCGAAATCCCTTATTCATCTCTAAGCCCTAATTTATTGAATGATCACATCTCATCTCCTGATTGGGCTTCAGCTGGTA
1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
H.armigera CTGAAAACTGGGGAATGGTCAGTTACAGAGAGCTGTACATGATCATAAACAAATCGGAGACGATCATGTCTAATGAACACTACGCAGCTACACTTGTGTC
T.ni CTGAAAACTGGGGAATGGTCAGTTACAGAGAGCTCTACCTGATCTTAAGTGAAGAAGAGACACTTATGTCTGTTGAGCACTACGCTGCTACTCTGGTATC
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
H.armigera TCACGAACTGGCACACAAGTGGTTCGGCAACTTGATCACTTGCTACTGGTGGAGTAACACCTGGATCAATGAGGGCTATGCCAGCTACTTCGGTTATATT
T.ni CCACGAGCTCGCACACAAATGGTTCGGTAACTTGATCACTTGCCACTGGTGGAGTAACACCTGGATCAACGAAGGATATGCCAGCTACTTCGGATACATT
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
H.armigera GCCACCAATGTGATGTTCCCCGAATACGAGTTCCCCGACCATTTCAACAGCCGCTACCTTCAGAATTCCTTGTCTTTCGACTCGGGCTCTGGAACTGTAC
T.ni GCAACTCATGAGATGTTCCCTAAATACGAGTTTCCTGACCACTTCAACACCCGATACCTCCAGACTTCTCTGTCCTTCGACTCTGGTATCAGCACTGTTC
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
H.armigera CATTGAACCATGAAGTTAATACTCCTCTGCAAGTAACTGGCCATTTTGGAACCATCAGCTACTCGAAAGCTGCTGCCTTTCTCAGGCAGACTGCTAACAT
T.ni CTTTGAACCACGACGTCAACACTCCCGCTCAGGTCACCGGTCACTTCGGAACTATCAGTTACTCGAAAGCCGCAGCCTTTTTGAGAATGACTGCTAATAT
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
H.armigera TATTTCTCCGGATACCTTCCAGAAATCTTGCAAATACTTTTTGATGGCAAACGCTTACAATGCGACTGATCAGTATGACCTGCAAGATGCCATGCTTAAA
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                1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      GCGATTGAAGAAGACGGAAGCCTGGCTGATTACCCCAATTTCAGTTTTACGGAATACTATAGAATCTGGGTGAATGAGCCTGGATACCCTATTTTACAAG
T.ni            GCCATTGAAGAAGACAACTCTTTAGCTGACTACGGAAGCTTCAGCTTTGCTGACTACTACAACATCTGGGTTAACGAGCCCGGATACCCAATTCTAAATG

                1610      1620      1630      1640      1650      1660      1670      1680      1690      1700
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      TGAATGTTAACCATGCTACAGGAGTAATCACGCTCACCCAGGAACGCTTCTTCATAAGCGGAACAGCCAATTCCGCTGGAACAGTATACCCCATACCAAT
T.ni            TTACTGTTAACCACACTACCGGAGTGATCTCCTTGTCTCAGGAACGATTTTTCCTAAGTTCGTCTGCTGCACCTACTGGTCAAATCTATCCAATTCCTAT

                1710      1720      1730      1740      1750      1760      1770      1780      1790      1800
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      CACATACTCATCAAAGAGTAACAGAAACTTCGACAATTTGAAGCCTGAAAAGATGATGAGTCTTCCTAGTGACACTATTACGAAGAACGCTGCCGAAGAG
T.ni            TACTTTCTCAACAAAAACAAACCCCAGCTTTTCAATCCTGAAGCCTTCTCATATAATGACGGGAGCGACTCTCACCATAAACAAAGCGGCTGTCGAAGAA

                1810      1820      1830      1840      1850      1860      1870      1880      1890      1900
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      TGGGTCATATTCAATAATAAACAGCATGGTCACTACAGAGTAAACTACGATGAGAAGACATGGGGATTGATCGCCGAAGCTCTGCTTAATGAACCAGACA
T.ni            TGGGTGATATTTAATAATATGCAGCACGGCCACTATAGAGTTAACTATGATTCGAAAACCTGGTCTCTGATTGCGGAAGCTTTGTTAGAGGAACCCTCAC

                1910      1920      1930      1940      1950      1960      1970      1980      1990      2000
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      CTATTCACTATTTGAATAGAGCTCAGGTAGTTGATGACGTGTTTGCTCTTATGAGGTCTCAGAGGATGACCTTGAACTTTGGTTTCGATATCCTGAGGTT
T.ni            CAATCCATATTTTGAACAGGGCTCAGATCGTGGACGATGTCTTTGCCCTAATGAGATCGAACAGGATGACACACAATGAAGGCTTCAAAATTTTGAAGTT

                2010      2020      2030      2040      2050      2060      2070      2080      2090      2100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      CTTGGCCAACGAGACCAACTTCCACGTTTGGGAGCCAGCAATCTCTGGCTACACTTGGTACAGAAACAGATTGAGGCACATTCCTGACAAACAGGCTCAG
T.ni            CTTAGCCAAGGAGACCAGTATACACATTTGGAGCCCTGCTATAAGTGGGTTTACCTGGCTAAGGAACAGGCTGCGACACCTACCGGCAAAACAAGCTGAA

                2110      2120      2130      2140      2150      2160      2170      2180      2190      2200
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      TTTGACACATACATCTTAGGTCTGATGGAGCACGCGATCACTACTCTAGGCTTCGAGCCCGCTGCCAATGAGACTCCCACTGTGACGATGGCGAGACAGA
T.ni            TTTGATGCATTTCTTCTCAGTCAAATGGAACATGCAATCAACGAATTAGGTTATGAGCCAAAGCCCAATGAGACGCCTACAATTACGATGGCCCGTCAAG

                2210      2220      2230      2240      2250      2260      2270      2280      2290      2300
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
H.armigera      ACATCCTACACTTTGCCTGTATGCTCGGCCATGTTAGGTGTAATCAGGAATCTTGGGACCGATTCGTTAACTTGAGAGATAATGGTGTGCCGATCAATTC
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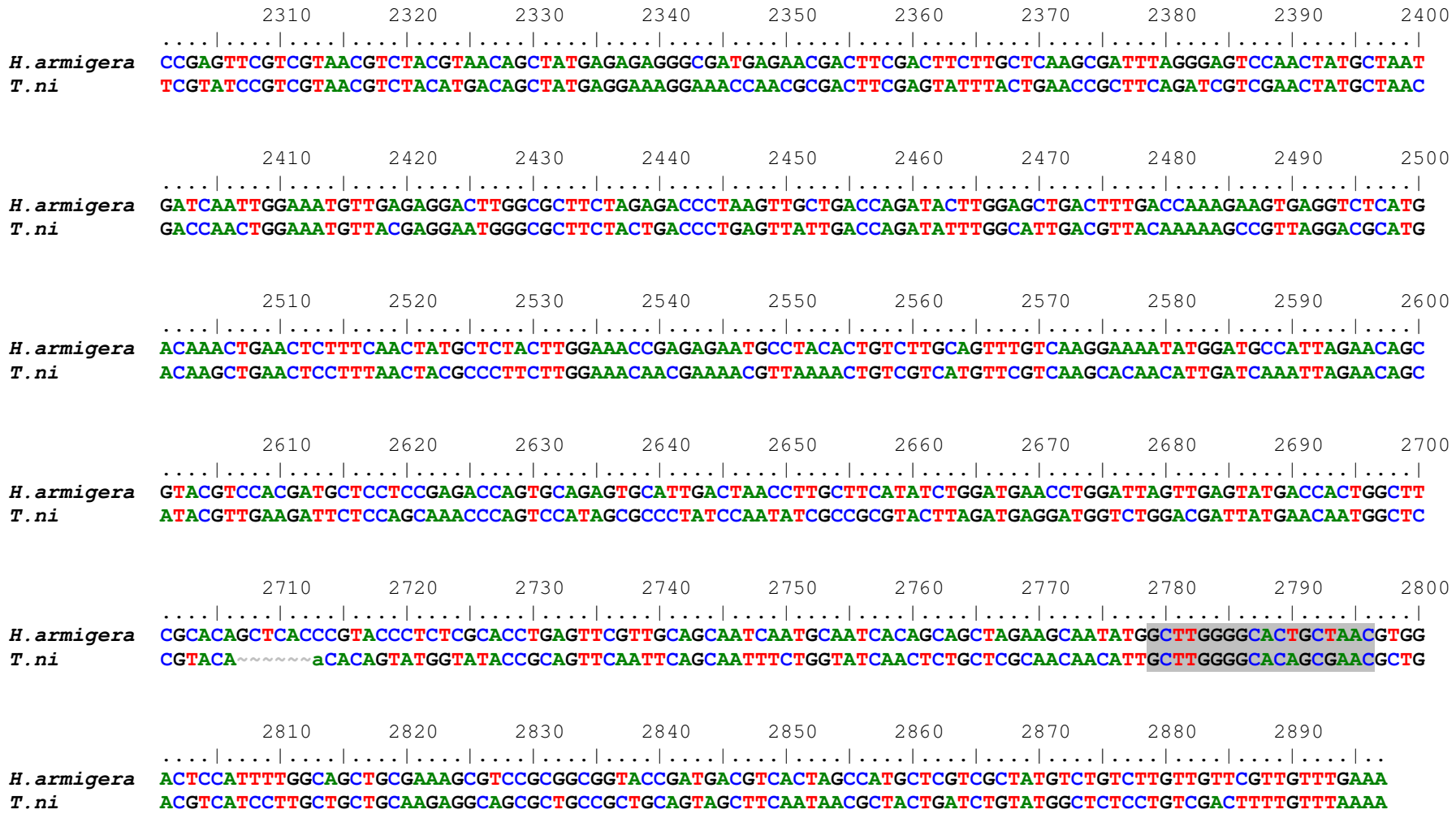
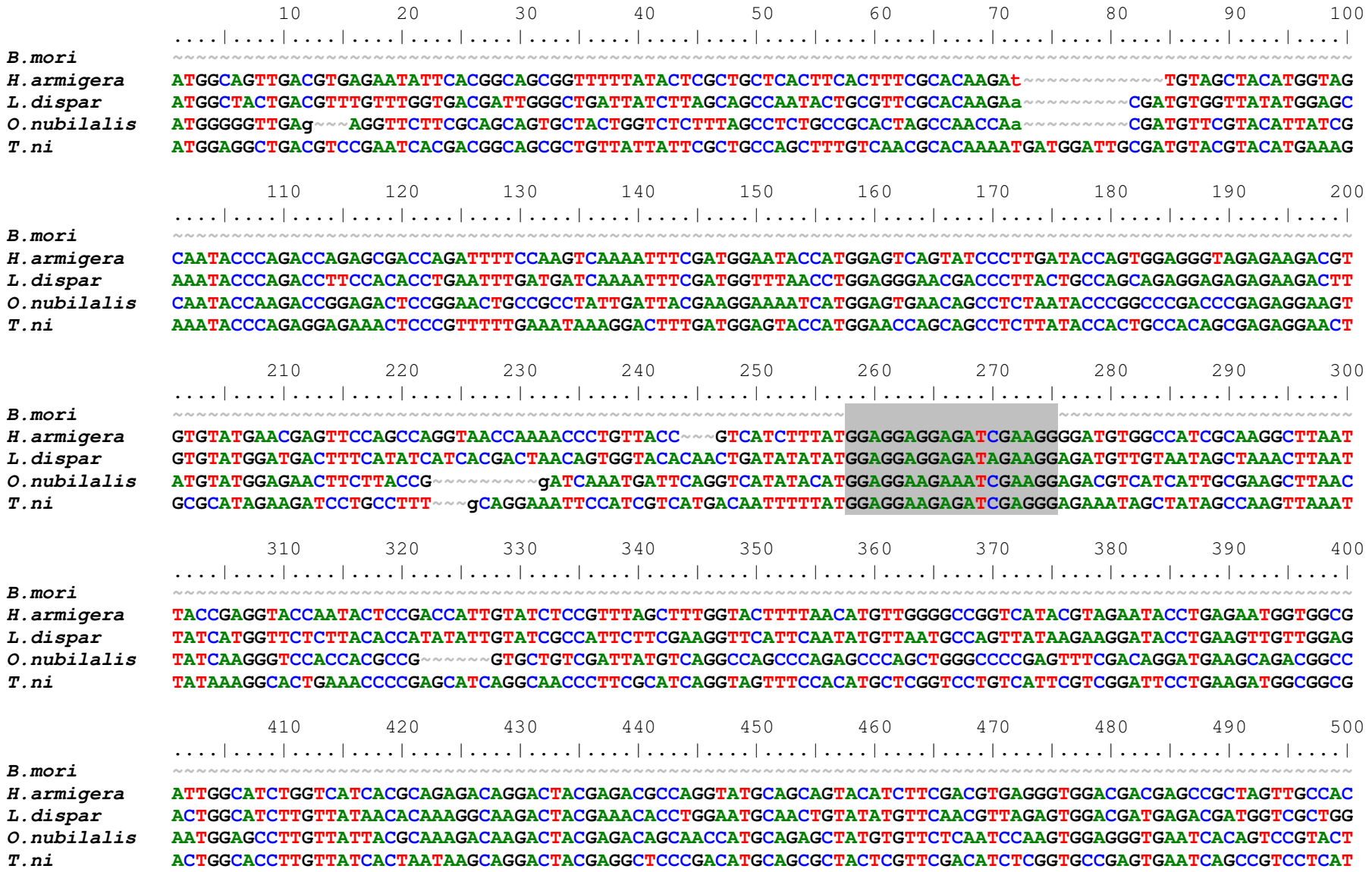
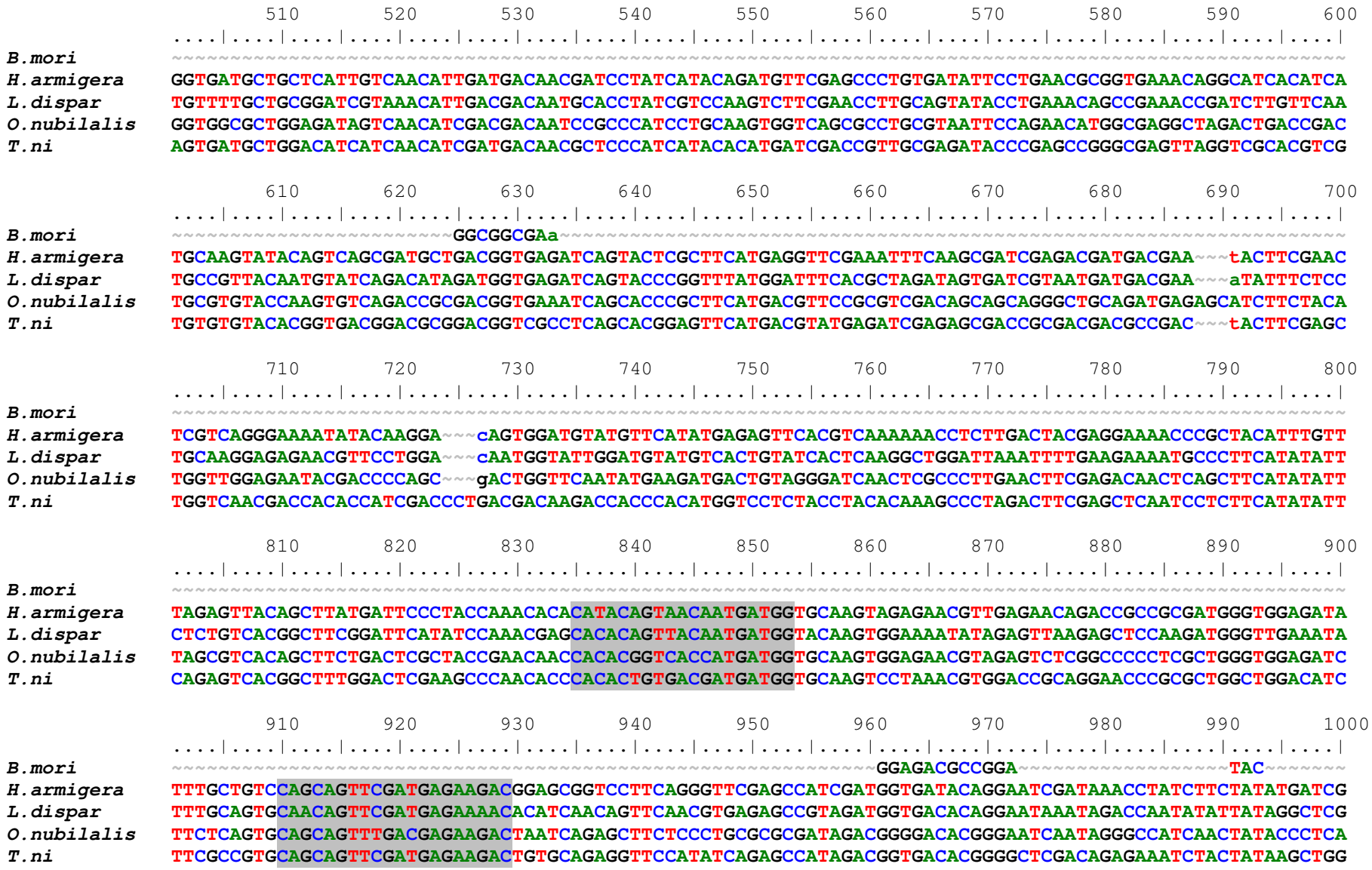


Figure B.6: DNA sequence alignment of *aminopeptidase N6* (*APN6*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Helicoverpa armigera* (170791084) and *Trichoplusia ni* (327082324).

B.7. *Cadherin* (*CAD*)

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B.mori
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L.dispar TGTTTTGCTGCGGATCGTAAACATTGACGACAATGCACCTATCGTCCAAGTCTTCGAACCTTGCAGTATACCTGAAACAGCCGAAACCGATCTTGTTCAA
O.nubilalis GGTGGCGCTGGAGATAGTCAACATCGACGACAATCCGCCCATCCTGCAAGTGGTCAGCGCCTGCGTAATTCCAGAACATGGCGAGGCTAGACTGACCGAC
T.ni AGTGATGCTGGACATCATCAACATCGATGACAACGCTCCCATCATACACATGATCGACCGTTGCGAGATACCCGAGCCGGGCGAGTTAGGTCGCACGTCG
610 620 630 640 650 660 670 680 690 700
B.mori GGCGGCGAa
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L.dispar TGCCGTTACAATGTATCAGACATAGATGGTGAGATCAGTACCCGGTTTATGGATTTCACGCTAGATAGTGATCGTAATGATGACGAA~~~aTATTTCTCC
O.nubilalis TGCGTGTACCAAGTGTCAGACCGCGACGGTGAAATCAGCACCCGCTTCATGACGTTCCGCGTCGACAGCAGCAGGGCTGCAGATGAGAGCATCTTCTACA
T.ni TGTGTGTACACGGTGACGGACGCGGACGGTCGCCTCAGCACGGAGTTCATGACGTATGAGATCGAGAGCGACCGCGACGACGCCGAC~~~tACTTCGAGC
710 720 730 740 750 760 770 780 790 800
B.mori
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L.dispar TGCAAGGAGAGAACGTTCCTGGA~~~cAATGGTATTGGATGTATGTCACTGTATCACTCAAGGCTGGATTAAATTTTGAAGAAAATGCCCTTCATATATT
O.nubilalis TGGTTGGAGAATACGACCCCAGC~~~gACTGGTTCAATATGAAGATGACTGTAGGGATCAACTCGCCCTTGAACTTCGAGACAACTCAGCTTCATATATT
T.ni TGGTCAACGACCACACCATCGACCCTGACGACAAGACCACCCACATGGTCCTCTACCTACACAAAGCCCTAGACTTCGAGCTCAATCCTCTTCATATATT
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B.mori
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O.nubilalis TAGCGTCACAGCTTCTGACTCGCTACCGAACAACCACACGGTCACCATGATGGTGCAAGTGGAGAACGTAGAGTCTCGGCCCCCTCGCTGGGTGGAGATC
T.ni CAGAGTCACGGCTTTGGACTCGAAGCCCAACACCCACACTGTGACGATGATGGTGCAAGTCCTAAACGTGGACCGCAGGAACCCGCGCTGGCTGGACATC
910 920 930 940 950 960 970 980 990 1000
B.mori GGAGACGCCGGA TAC
H.armigera TTTGCTGTCCAGCAGTTCGATGAGAAGACGGAGCGGTCCTTCAGGGTTCGAGCCATCGATGGTGATACAGGAATCGATAAACCTATCTTCTATATGATCG
L.dispar TTTGCAGTGCAACAGTTCGATGAGAAAACACATCAACAGTTCAACGTGAGAGCCGTAGATGGTGACACAGGAATAAATAGACCAATATATTATAGGCTCG
O.nubilalis TTCTCAGTGCAGCAGTTTGACGAGAAGACTAATCAGAGCTTCTCCCTGCGCGCGATAGACGGGGACACGGGAATCAATAGGGCCATCAACTATACCCTCA
T.ni TTCGCCGTGCAGCAGTTCGATGAGAAGACTGTGCAGAGGTTCCATATCAGAGCCATAGACGGTGACACGGGGCTCGACAGAGAAATCTACTATAAGCTGG

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                       1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
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L.dispar       AAAACGACGAATCA~~~GACACTTTCTTTCATATTGAAACAATAGAAGGTGGTAGAGACGGTGCTATATTTTATGTAGATCCTATAGATAGAGATACCTT
O.nubilalis    TCAGGGATGACGCT~~~GACGACTTCTTTTCCCTGGAGGTGATTGAA~~~~~~~~~gACGGAGCTATTCTGCACGTGACTGAGATCGACCGCGACACGCT
T.ni           AAGCGGACGAAGAA~~~GATACATTCTTCTCCCTGGAGCCAATCGCGGGAGATCGCAGCGGCGCCACATTAGTTGTTGACAAGATAGACAGAGACACTCT

                       1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     TGAGAAGGAAGTTTTCTACGTGTCCATAATAGCGTACAAATATGGCGATAATGACGTGGAAGGCAGTTCGTCGTTCCAGTCGAAGACCGATGTGGTCATC
L.dispar       AGAGCGTGAAGTATTCCAATTGGCTATAATAGCATACAAGAACGACGAATATGATAGAGAAATCAATTCa~~~~~~~~~ACCACAGCGAATGTCGTCATA
O.nubilalis    TGAAAGAGAGCTTTTCAACCTCACCATCGTTGCTTACAAATCTACTGACGCGAACTTTGCa~~~~~~~~~~~~~~~~~~ACAGAGGCCCATATTTTCATC
T.ni           ACAGCGGGAAGTGTTCCAGCTGTCCATAGTAGCGTATAAGTACGGTATCGATGATAAAGAGGGGAAGAACCCCTTCGAGACCAGAGCCAACATCGTGATC

                       1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     ATCGTGAACGATGTCAATGATCAGGCTCCGTTGCCTTTCCGGGAAGAGTACTCCATTGAAATTATGGAGGAAACTGCAATGACACTGAATTTa~~~GAAG
L.dispar       ATTGTCAACGATATTAACGATCAGAGACCAGAGCCTTTCCACAAAGAATACACAATTAACATAATGGAAGAAACTGCGATGACGTTGAATTTa~~~GAAG
O.nubilalis    ATCGTCAACGACGTCAACGATCAGCGACCCGAGCCGCTGCATAAAGAATACAGTATTGATATCATGGAGGAAACTCCAATGACTCTAAACTTCAATGAAG
T.ni           ATCGTCAATGATGTCAACGATCAAAGGCCTCTCCCTTTCAAGAATACCTACACAATAGAAATAGACGAAGAGACGCCCATGACCCTCAATTTa~~~GAAG

                       1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~GACCTG~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     ACTTTGGTTTCCATGATAGAGATCTTGGTCCTCACGCCCAATACTCAGTGCACCTAGAAAGCATCTATCCCCCCCGAGCTCACGAGGCGTTCCACATCGC
L.dispar       AATTCGGATTTCATGATCGCGATCTGGGAGAAAATGCACAATATATGGTGCATTTAGAGGCTGTATTTCCGCCAAATGCTGAGGAGGCATTCTACATTGC
O.nubilalis    AATTTGGATTCCATGATCGAGATTTGGGTGAAAACGCTCAATACACAGTGGAACTTGAGGACGTGTTCCCCCCAGGGGCGGCGTCCGCATTCTACATCGC
T.ni           ACTTTGGGTTCCACGACATAGATCTCGGTGAAAACGCTCAATATGAAGTGTTCTTGGAGAGTGTATACCCTGAAGGTGCCGAGGAAGCTTTCATGATCTC

                       1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CATGTCCTT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     ACCGGAGGTTGGCTACCAGCGCCAGTCTTTCATCATGGGCACGCAGAACCATCACATGCTGGACTTCGAAGTGCCAGAGTTCCAGAATATACAGCTAAGG
L.dispar       GCCAGAGGTGGGGTACCAGCGTCAAAGTTTTATAATGGGGACATTAAATCATCGTTTGTTGGACTTTGAAGTGCCTGAGTATCAATCTATCATGCTGAGG
O.nubilalis    GCCGGGGAGCGGCTACCAGAGGCAGACCTTCATTATGGGCACCATAAACCACACCATGCTGGATTACGAAGATGTCGTTTTTCAGAACATCATCATTAAG
T.ni           CCCGACGAGGGGATACCAGGAACAATCGTTCATAGTGTCGACGAGAAACCACCACCTTCTGGACTATGAAGTGGAAAAATATCAAAACATTCAGCTAAAG
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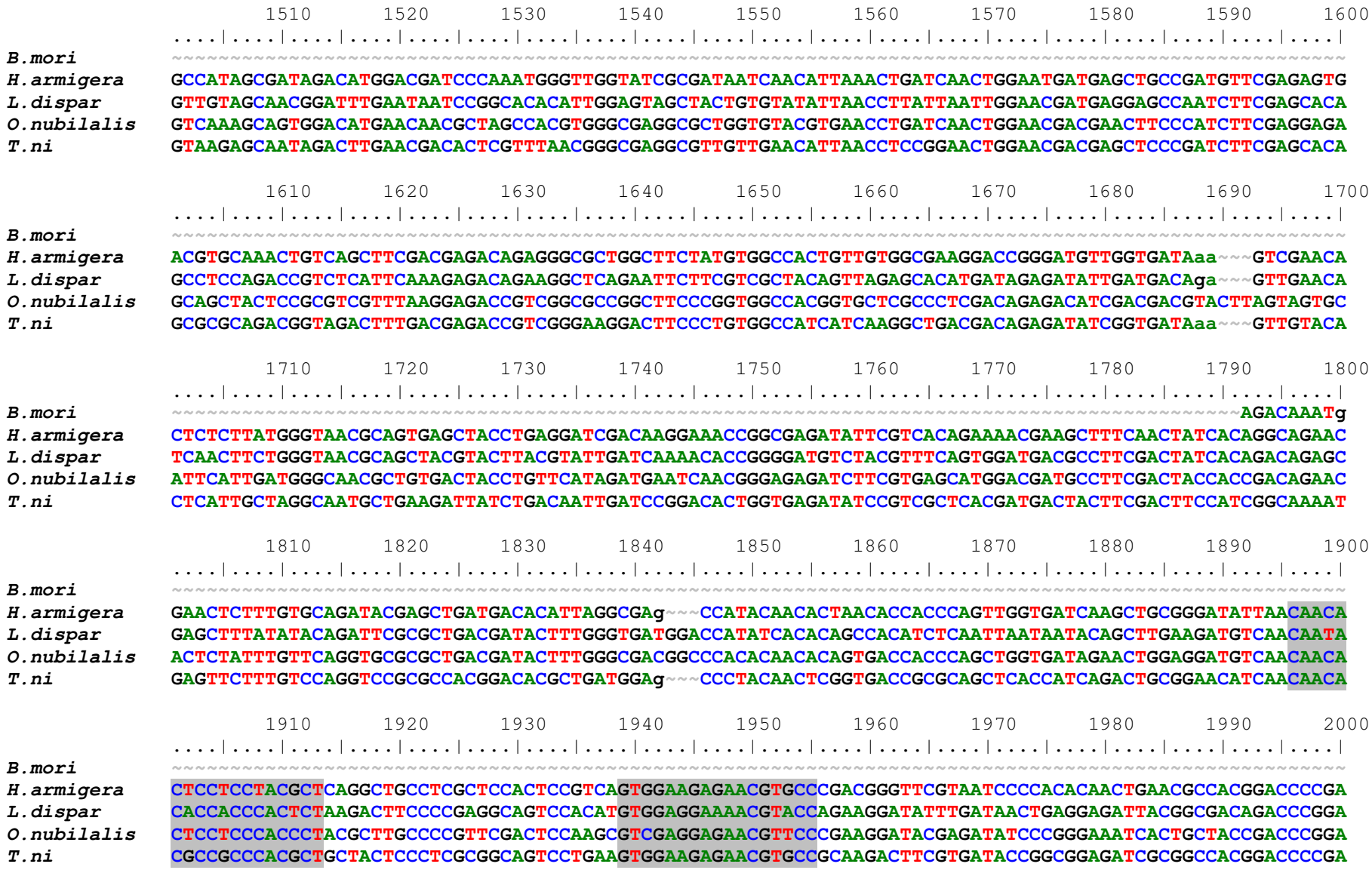
1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
B.mori
H.armigera GCCATAGCGATAGACATGGACGATCCCAAATGGGTTGGTATCGCGATAATCAACATTAAACTGATCAACTGGAATGATGAGCTGCCGATGTTCGAGAGTG
L.dispar GTTGTAGCAACGGATTTGAATAATCCGGCACACATTGGAGTAGCTACTGTGTATATTAACCTTATTAATTGGAACGATGAGGAGCCAATCTTCGAGCACA
O.nubilalis GTCAAAGCAGTGGACATGAACAACGCTAGCCACGTGGGCGAGGCGCTGGTGTACGTGAACCTGATCAACTGGAACGACGAACTTCCCATCTTCGAGGAGA
T.ni GTAAGAGCAATAGACTTGAACGACACTCGTTTAACGGGCGAGGCGTTGTTGAACATTAACCTCCGGAACTGGAACGACGAGCTCCCGATCTTCGAGCACA
1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
B.mori
H.armigera ACGTGCAAACTGTCAGCTTCGACGAGACAGAGGGCGCTGGCTTCTATGTGGCCACTGTTGTGGCGAAGGACCGGGATGTTGGTGATAaa~~~GTCGAACA
L.dispar GCCTCCAGACCGTCTCATTCAAAGAGACAGAAGGCTCAGAATTCTTCGTCGCTACAGTTAGAGCACATGATAGAGATATTGATGACAga~~~GTTGAACA
O.nubilalis GCAGCTACTCCGCGTCGTTTAAGGAGACCGTCGGCGCCGGCTTCCCGGTGGCCACGGTGCTCGCCCTCGACAGAGACATCGACGACGTACTTAGTAGTGC
T.ni GCGCGCAGACGGTAGACTTTGACGAGACCGTCGGGAAGGACTTCCCTGTGGCCATCATCAAGGCTGACGACAGAGATATCGGTGATAaa~~~GTTGTACA
1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
B.mori AGACAAATg
H.armigera CTCTCTTATGGGTAACGCAGTGAGCTACCTGAGGATCGACAAGGAAACCGGCGAGATATTCGTCACAGAAAACGAAGCTTTCAACTATCACAGGCAGAAC
L.dispar TCAACTTCTGGGTAACGCAGCTACGTACTTACGTATTGATCAAAACACCGGGGATGTCTACGTTTCAGTGGATGACGCCTTCGACTATCACAGACAGAGC
O.nubilalis ATTCATTGATGGGCAACGCTGTGACTACCTGTTCATAGATGAATCAACGGGAGAGATCTTCGTGAGCATGGACGATGCCTTCGACTACCACCGACAGAAC
T.ni CTCATTGCTAGGCAATGCTGAAGATTATCTGACAATTGATCCGGACACTGGTGAGATATCCGTCGCTCACGATGACTACTTCGACTTCCATCGGCAAAAT
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
B.mori
H.armigera GAACTCTTTGTGCAGATACGAGCTGATGACACATTAGGCGAg~~~CCATACAACACTAACACCACCCAGTTGGTGATCAAGCTGCGGGATATTAACAACA
L.dispar GAGCTTTATATACAGATTCGCGCTGACGATACTTTGGGTGATGGACCATATCACACAGCCACATCTCAATTAATAATACAGCTTGAAGATGTCAACAATA
O.nubilalis ACTCTATTTGTTCAGGTGCGCGCTGACGATACTTTGGGCGACGGCCCACACAACACAGTGACCACCCAGCTGGTGATAGAACTGGAGGATGTCAACAACA
T.ni GAGTTCTTTGTCCAGGTCCGCGCCACGGACACGCTGATGGAg~~~CCCTACAACTCGGTGACCGCGCAGCTCACCATCAGACTGCGGAACATCAACAACA
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
B.mori
H.armigera CTCCTCCTACGCTCAGGCTGCCTCGCTCCACTCCGTCAGTGGAAGAGAACGTGCCCGACGGGTTCGTAATCCCCACACAACTGAACGCCACGGACCCCGA
L.dispar CACCACCCACTCTAAGACTTCCCCGAGGCAGTCCACATGTGGAGGAAAACGTACCAGAAGGATATTTGATAACTGAGGAGATTACGGCGACAGACCCGGA
O.nubilalis CTCCTCCCACCCTACGCTTGCCCCGTTCGACTCCAAGCGTCGAGGAGAACGTTCCCGAAGGATACGAGATATCCCGGGAAATCACTGCTACCGACCCGGA
T.ni CGCCGCCCACGCTGCTACTCCCTCGCGGCAGTCCTGAAGTGGAAGAGAACGTGCCGCAAGACTTCGTGATACCGGCGGAGATCGCGGCCACGGACCCCGA

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                          2010      2020      2030      2040      2050      2060      2070      2080      2090      2100
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera        CACTACAGCCGAGCTGCGCTTCGAGATCGACTGGGAGAACTCCTATGCCACCAAGCAGGGACGGAATACTGACTCTAAGGAGTATATCGGTTGTATAGAA
L.dispar          TACTACTGCAGAACTTCGGTTTGAAATCGATTGGGAAAGCTCGTATGCCACAAAGCAAGGACGAGAGACACCACCTGAGGAGTACCATCATTGCGTAGAT
O.nubilalis       CACCAGCGCCTACCTGTGGTTCGAGATCGACTGGGACTCCACCTGGGCCACCAAGCAGGGCAGAGAGACCAACCCTACTGAATACGTCGGGTGTATAGTT
T.ni              CCTCGACGCACAACTGGAGTTCGAAATAGACTGGGAGAGCTCGTACGCGACCAAGCAGGGAAGACCAGCTCCCGATGTTGAGTTCCATAAATGCGTGGAA

                          2110      2120      2130      2140      2150      2160      2170      2180      2190      2200
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera        ATCGAGACGATATACCCGAATATAAACCAGAGAGGCAACGCCATCGGCCGCGTCGTAGTGCGCGAGATCCGGGACGGCGTCACCATAGACTATGAGATGT
L.dispar          ATTGAAACTATATACCCGCAAGCCACTGATCGTGGAACAGCTGTCGGCAGGATTATAGTAAGACAGATAAGACCAGGCGTCACCATCGACTACGAGGAAT
O.nubilalis       ATCGAAACGATATACCCCAACAAGGGCAACCGGGGTTCCGCCATCGGGCGCCTCGTGGTGCAAGAGATCCGGGACAACGTCACCATCGACTTCGAGGAAT
T.ni              ATAATAACCATCCCCACGGAGACCCGTCACCGc~~~~~~GTCATCGGGCGCCTCGACGTGAGGACCATCAGAGAGGGAGTCACCATCGACTACGAGGAGT

                          2210      2220      2230      2240      2250      2260      2270      2280      2290      2300
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera        TTGAGGTTCTATACCTCACCGTCATCGTGAGGGATCTCAACACCGTTATTGGGGAA~~~GACCATGATATATCCACATTCACGATCACGATAATAGACAT
L.dispar          TCGAAATGCTGTACCTCACTGTGAGGGTCAGGGATCTCAATACTATCATAGGCGAG~~~GATTACGATGAATCGACATTCACAATTACAATAGTGGATAT
O.nubilalis       TCGAGATGCTTTACCTCACCGTCCGCGTGAGGGACCTCAACACTGTCATCGGAGAT~~~GACTACGATGAGGCGACGTTCACGATCACAATAATCGACAT
T.ni              TCGAGATCCTGTACCTCAGCATCAAGGTCTATGACAGGAATACTGTGGCTGGTGCTATCGATCATGCTGAATCGATCCTGGCCATCAACATAATCGACAT

                          2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera        GAACGACAATCCTCCCCTGTGGGTGGAAGGCACCCTCACTCAGGAGTTCCGCGTGCGAGAGGTGGCAGCCTCAGGAGTCGTTATAGGATCCGTACTGGCT
L.dispar          GAATGATAATCCACCTGTATGGGTAGAAGGTACGTTGGAGCAAGAGTTTAGAGTAAGAGAGATGTCGAGCAGTGGCGTCGTTATTGGATCAGTACTGGCC
O.nubilalis       GAACGACAACGCGCCGATCTTCGCGAACGGCACGCTGACGCAGACGATGCGCGTGCGCGAGCTGGCGGCCAGCGGCACGCTCATCGGCTCCGTGCTCGCC
T.ni              GAACGACAACCCGCCGGTGTGGGCGGCGGGACAGCTGCGGCAGGCGCTGCGCGTGCGCGAGGGCTCTCCCGCCGGCGGGATCATCGGCTCACTGCTCGCC

                          2410      2420      2430      2440      2450      2460      2470      2480      2490      2500
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            ~~~~~~TACGACGGACCGCCt~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera        ACTGATATCGACGGACCCTTGTATAATCAAGTGCGGTATACTACCACTCCCAGACTCGATACTCCAGAAGACCTAGTGGACATAGACTTCAACACGGGTC
L.dispar          ACTGATATCGACGGACCACTTTATAATCAAGTGCGGTACACCATATTTCCAAGACAAGACACCCCTGAAGATCTTGTGGACATCGATTTTTACACGGGCC
O.nubilalis       ACCGACATCGACGGCCCGCTCTACAACCAAGTGCGCTACACTATACAACCTAGAAACAACACTCCCGAGGGATTAGTGAAGATTGACTTCACAACTGGTC
T.ni              ACCGACATCGACGGCCCGCTCTACAATAAAGTGCGGTACTCCATACATCCTAAGCCAGGCACCAAAGAAGGCCTAGTAGCGATCGATCCCATATTGGGTC
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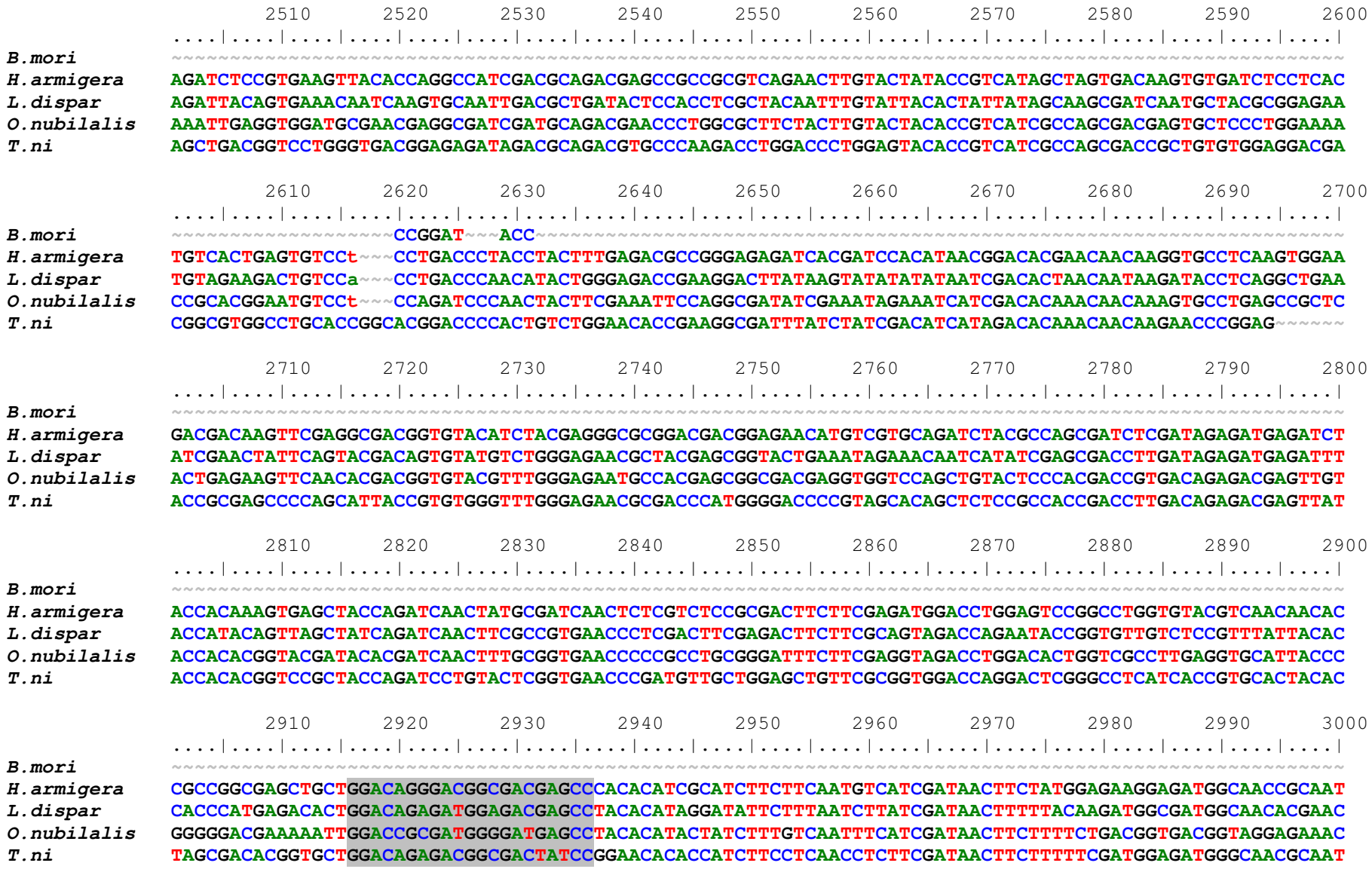
2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
B.mori
H.armigera AGATCTCCGTGAAGTTACACCAGGCCATCGACGCAGACGAGCCGCCGCGTCAGAACTTGTACTATACCGTCATAGCTAGTGACAAGTGTGATCTCCTCAC
L.dispar AGATTACAGTGAAACAATCAAGTGCAATTGACGCTGATACTCCACCTCGCTACAATTTGTATTACACTATTATAGCAAGCGATCAATGCTACGCGGAGAA
O.nubilalis AAATTGAGGTGGATGCGAACGAGGCGATCGATGCAGACGAACCCTGGCGCTTCTACTTGTACTACACCGTCATCGCCAGCGACGAGTGCTCCCTGGAAAA
T.ni AGCTGACGGTCCTGGGTGACGGAGAGATAGACGCAGACGTGCCCAAGACCTGGACCCTGGAGTACACCGTCATCGCCAGCGACCGCTGTGTGGAGGACGA
2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
B.mori CCGGAT ACC
H.armigera TGTCACTGAGTGTCCt CCTGACCCTACCTACTTTGAGACGCCGGGAGAGATCACGATCCACATAACGGACACGAACAACAAGGTGCCTCAAGTGGAA
L.dispar TGTAGAAGACTGTCCa CCTGACCCAACATACTGGGAGACCGAAGGACTTATAAGTATATATATAATCGACACTAACAATAAGATACCTCAGGCTGAA
O.nubilalis CCGCACGGAATGTCCt CCAGATCCCAACTACTTCGAAATTCCAGGCGATATCGAAATAGAAATCATCGACACAAACAACAAAGTGCCTGAGCCGCTC
T.ni CGGCGTGGCCTGCACCGGCACGGACCCCACTGTCTGGAACACCGAAGGCGATTTATCTATCGACATCATAGACACAAACAACAAGAACCCGGAG
2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
B.mori
H.armigera GACGACAAGTTCGAGGCGACGGTGTACATCTACGAGGGCGCGGACGACGGAGAACATGTCGTGCAGATCTACGCCAGCGATCTCGATAGAGATGAGATCT
L.dispar ATCGAACTATTCAGTACGACAGTGTATGTCTGGGAGAACGCTACGAGCGGTACTGAAATAGAAACAATCATATCGAGCGACCTTGATAGAGATGAGATTT
O.nubilalis ACTGAGAAGTTCAACACGACGGTGTACGTTTGGGAGAATGCCACGAGCGGCGACGAGGTGGTCCAGCTGTACTCCCACGACCGTGACAGAGACGAGTTGT
T.ni ACCGCGAGCCCCAGCATTACCGTGTGGGTTTGGGAGAACGCGACCCATGGGGACCCCGTAGCACAGCTCTCCGCCACCGACCTTGACAGAGACGAGTTAT
2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
B.mori
H.armigera ACCACAAAGTGAGCTACCAGATCAACTATGCGATCAACTCTCGTCTCCGCGACTTCTTCGAGATGGACCTGGAGTCCGGCCTGGTGTACGTCAACAACAC
L.dispar ACCATACAGTTAGCTATCAGATCAACTTCGCCGTGAACCCTCGACTTCGAGACTTCTTCGCAGTAGACCAGAATACCGGTGTTGTCTCCGTTTATTACAC
O.nubilalis ACCACACGGTACGATACACGATCAACTTTGCGGTGAACCCCCGCCTGCGGGATTTCTTCGAGGTAGACCTGGACACTGGTCGCCTTGAGGTGCATTACCC
T.ni ACCACACGGTCCGCTACCAGATCCTGTACTCGGTGAACCCGATGTTGCTGGAGCTGTTCGCGGTGGACCAGGACTCGGGCCTCATCACCGTGCACTACAC
2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
B.mori
H.armigera CGCCGGCGAGCTGCTGGACAGGGACGGCGACGAGCCCACACATCGCATCTTCTTCAATGTCATCGATAACTTCTATGGAGAAGGAGATGGCAACCGCAAT
L.dispar CACCCATGAGACACTGGACAGAGATGGAGACGAGCCTACACATAGGATATTCTTTAATCTTATCGATAACTTTTTTACAAGATGGCGATGGCAACACGAAC
O.nubilalis GGGGGACGAAAAATTGGACCGCGATGGGGATGAGCCTACACATACTATCTTTGTCAATTTCATCGATAACTTCTTTTTCTGACGGTGACGGTAGGAGAAAC
T.ni TAGCGACACGGTGCTGGACAGAGACGGCGACTATCCGGAACACACCATCTTCCTCAACCTCTTCGATAACTTCTTTTTTCGATGGAGATGGGCAACGCAAT

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                       3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     CAGAACGAGACACAAGTGCTGGTCGTGTTACTGGACATCAACGACAACTATCTGGAACTG~~~~~~CCTGAAACAATCCCATGGGCTATCTCTGAGAGCT
L.dispar       CAAAACACAACAGAGATATTCGTAATTCTGCTGGATGTAAATGATAATGCACCTGAATTGCCCACTCCAGATCAATTGTCCTGGAGCATATCAGAAAATA
O.nubilalis    CAGGACGAAGTTGAAATATTTGTCGTTCTATTGGATGTGAACGACAACGCTCCTGAGATGCCATCGCCTGATGAACTCCGGTTTGATGTTTCCGAAGGAG
T.ni           ATGGCGGAGAAGAGAGTGCTCGTGGTTCTGTTAGATGTGAACGACAACGCGCCCGAACTGCCGCTACCTGAAGAACTGTCCTGGTCTGTGTCTGAAGACG

                       3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     TAGAGCAGGGTGAGCGAGTACAGCCAGAAATCTTCGCCCGGGACCGCGACGAGCCAGGAACAGACAACTCTCGCGTCGCTTACGCCATCACCGGCCTCGC
L.dispar       TGGAAGCGGGAGTAAGACTAACACCGGATATCTACGCACCGGACCGGGATGAACCGGACACGGATAACTCACGCGTCGGTTATGCCATACTGGGCCTGAC
O.nubilalis    CAGTTGCTGGTGTCCGTGTACTCCCAGAAATCTACGCACCTGACAGGGATGAACCAGACACGGACAACTCGCGTGTCGGTTACGGAATCCTGGACCTCAC
T.ni           AGAGAGAAGAAGTACGCGTACTACCACATATCTACGCTCCGGACAGAGACGAGCCGGACACGGATAACTCTAGGGTCGGCTATGCGATTCTCGGCCTTAA

                       3210      3220      3230      3240      3250      3260      3270      3280      3290      3300
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera     CAGCGTCGACCGCGACATACAAGTGCCTGATCTCTTCAACATGATCACTATAGAGAGGGACAGAGGTATAGACCAGACTGGAATACTTGAGGCGGCTATG
L.dispar       CGTAACTAATCGAGATATCGACATACCTGAACTCTTCACCATGATACAGATACAG~~~~~~~~~~~~~~~aACGTCACTGGGGAGCTGGAGACTGTCAGA
O.nubilalis    GATCACCGACCGAGACATCGAGGTGCCGGATCTCTTCACCATGATCTCGATTGAA~~~~~~~~~~~~~~~aACAAAACTGGGGAACTTGAGACCGCTATG
T.ni           AGTGACCAACAGAGAGATCGAAGTCCCGGAGCTGTTCAACATGATTCAGATAGAG~~~~~~~~~~~~~~~aACAAGACAGGAGAGCTCGAGACCGCTCGC

                       3310      3320      3330      3340      3350      3360      3370      3380      3390      3400
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         ~~~~~~~~~~~~~~~~~~GGCGCTTAC~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CaaCAA~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~A
H.armigera     GATTTGAGAGGCTATTGGGGCACTTATGAAATTGATATTCAGGCGTACGACCATGGAATCCCACAAAGGATTTCGAATCAGAAGTACCCGCTGGTCATCA
L.dispar       CACCTTCAGGGTTATTGGGGCACTTATGATATACATATATGGGCATATGATCATGGTCACCCTCAACAGTCGAGTGACGAAACCTACCAGCTAGTCATCA
O.nubilalis    GACTTGAGGGGGTATTGGGGCACTTACGAAATATTCATTGAGGCCTTCGACCACGGCTACCCGCAGCAGAGGTCCAACGGGACGTACACACTGGTCATTC
T.ni           CATCTGAAAGGATTCTGGGGAACTTATAGTATACATATACAGGCGTACGACCACGGGATCCCTCAGCAGATATCTGAGGAGACGTACACCCTCATCATCC

                       3410      3420      3430      3440      3450      3460      3470      3480      3490      3500
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori         GA~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~AGG~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CTACTA~~~~~
H.armigera     GACCTTACAACTTCCACGACCCAGTGTTCGTGTTCCCTCAACCTGGATCTACTATCAGACTGGCAAAGGAGCGTGCAGTAGTCAATGGTATACTCGCTAC
L.dispar       GACCTTATAACTTCCACGATCCAGTGTTCGTATTCCCGCTGCCTGGCTCCACCATCAGACTCGCTAAGGAACGTATTTCGGTGAACGGGCTGTTGGTGAC
O.nubilalis    GCCCCTACAACTTCCACCACCCTGTGTTCGTGTTCCCGCAACCCGACTCCGTCATTCGGCTCTCTAGGGAGCGCGCAACAGAAGGCGGGGTCCTGGCGAC
T.ni           GCCCTTACAACTACCACGAGCCGGTGTTCGTGTTCCCACAGGCTGGCAACACCTTCAGATTGTCCAGGGAGCAGTCGACAGTGAACGGCGTGTTGGTCCG
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                      3510      3520      3530      3540      3550      3560      3570      3580      3590      3600
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TTA~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GAG~~~~~~
H.armigera    AGTGGATGGAGAATTTCTGGACAGAATCGTCGCCACCGACGAGGATGGTTTAGAGGCTGGACTCGTTACATTCTCTATCGCGGGAGATGATGAAGGTTCT
L.dispar      AACACAACTCGAGTTCTTGGAACGTATCCGAGCAACAGACGAGGACGGTCTTCACGCTGGCATTGTCACCTTCCACGTTGAGGGGAACGCTGAAGCGGTA
O.nubilalis   GGCTGCCAACGAGTTCCTGGAGCCGATCTACGCCACCGACGAGGACGGCCTCCACGCGGGCAGCGTCACGTTCCACGTCCAGGGAAATGAGGAGGCCGTT
T.ni          CGTGGACGGGCAGAGCTTCCCGCGCGTGTCGGCCACCGACGAGGACGGGCTGCACGCCGGCAGCGTCAGCTTCAGCGTCGTCGGGGCTGCC~~~~~~gCT

                      3610      3620      3630      3640      3650      3660      3670      3680      3690      3700
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    CAGTTCTTCGACGTa~~~~~~TTGAACGACGGAGTGAACTCGGGCTCTCTCACCCTCACGCGGCTCTTCCCTGAAGACTTCCGAGAGTTCCAGGTGACGA
L.dispar      CAGTTCTTCGAAGTg~~~~~~TCAAATGACGGGGAAAATGCTGGGTCACTTATCTTATTGAACACCTTCGATGAAGACATCAGAGAGTTTACGGTGGTAA
O.nubilalis   CAGTACTTTGATATAACTGAAGTGGGAGCAGGAGAAAATAGCGGGCAGCTTATATTACGCCAGCTTTTCCCAGAGCAAATCAGACAATTCAGGATCACGA
T.ni          GAATACTTCTCCATg~~~~~~CGTAACTTCGAGGACAACACTGGCGAGCTTTACCTGTCGCAGCCCTTGCCTCTAGAAGACGATGGATTTGATATCACCA

                      3710      3720      3730      3740      3750      3760      3770      3780      3790      3800
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~ACg~~~~~~~~~ACA~~~CCG~~~~~~~~~~~~~~~GACATATCA~~~~~~~~~~~~~~~~~~~~~~~~~~~GGG~~~~~~~~~TTC~~~~~
H.armigera    TTCGTGCTACGGACGGTGGAACTGAGCCTGGTCCAAGGAGTACTGACTGCTTGGTGACCGTAGTATTTGTTCCCACTCAGGGAGAGCCCGTGTTCGAGGA
L.dispar      TTCGCGGTACGGACGGCGGCACTGAACCTGGAACACGGCACACGGATTGTTCAGTCAGGCTGGTGTTCGTTCCCACTCAAGGAGAACCTACCTTCTCCGA
O.nubilalis   TCCGGGCCACAGACGGCGGCACGGAGCCCGGCCCGCTTTGGACCGACGTCACGTTTTCGGTGGTCTTCGTACCCACGCAGGGCGACCCAGTGTTCAGCGA
T.ni          TCCGCGGTTCTGACGCGGGCACAGAGCCGGGCTCGCTGTTCTCGGAGGTTTCCTTCAGACTGGTGTTCGTGCCCACGCACGGGGACCCTGTCTTCAGCGT

                      3810      3820      3830      3840      3850      3860      3870      3880      3890      3900
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CTG~~~~~~~~~~~~Gac~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    AAGTACCTACACAGTCGCTTTTGTTGAAAAAGATGAGGGTATGTTAGAGGAGGCGGAACTACCTCGCGCCTCAGACCCGAGGAACATCATGTGTGAAGAT
L.dispar      GAATACGGCGAGCGTCGCATTCTTTGAGCTAGAGGGCGGTATGACTGAACAGTTCCAACTGCCACTCGCTGACGATCCCAAGAACTATCTGTGTGAAGAC
O.nubilalis   AAATGCAGCTACTGTTGCCTTCTTCGAGGGTGAAGAAGGCCTCCGTGAGAGTTTTGAGCTGCCGCAAGCAGAAGACCTTAAAAACCACCTCTGCGAAGAT
T.ni          CAGCCAGTATACTGTCGCTTTTATAGAAAAAGAGGCTGGTTTACTGGAATCCCATCAACTGCCGAGAGCTGTGGACCCGAAGAACTACATGTGTGAGGAA

                      3910      3920      3930      3940      3950      3960      3970      3980      3990      4000
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    ~~~~~~~~~gATTGTCACGACACCTATTACAGCATTGTTGGAGGCAATTCGGGTGAACACTTCACAGTAGACCCTCGTACCAACGTGCAGACCCTCGCGA
L.dispar      ~~~~~~~~~gACTGCTTCAGCACTTATTACCTTATAATCGATGGCAACGCAGACGGCCACTTTGCTGTGAATCCAGTGACGAACGTAATCTATTTGGTGG
O.nubilalis   ~~~~~~~~~gACTGCCAAGATATCTACTACAGGTTTATTGACGGCAACAACGAGGGTCTGTTCGTGCTGGACCAGTCGAGCAACGTCATCTCCCTTGCGC
T.ni          ATGAACGAGCCTTGTCATGAGATATACTACAGTATTATCGATAACAACGAGGAGGGTTACTTCCAAGTGGACTCGACCACAAACGTGATCTCCCTATCCC
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                    4010      4020      4030      4040      4050      4060      4070      4080      4090      4100
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GATAAGAAGTC
H.armigera    AGCCGCTGGACCGCTCCGAACAGGAGACACACACCCTCATCATCGGCGCCAGCGACACT~~~~~~CCCAACCCGGCCGCCGTC~~~~~~CTGCAGGCTTC
L.dispar      AAGAGCTGGACCGCGAAGTAGAGGAAACCTACACTATCCTGGTGGCTGCTAGCAACTCC~~~~~~CCTGACAGCGTCAATGCg~~~~~~TTACCTTCTAA
O.nubilalis   AGGAGTTGGACCGCGAGGTTGCCACGTCTTACACGCTGCACATCGCGGCGAGCAACTCG~~~~~~CCCGACGCCACTGGGATC~~~CCTCTGCAGACTTC
T.ni          GCGAGCTGGAGCGGGCCTCGCAGGCCAGTCACGTGGTCCGCGTGGCGGCCTCCAACACTCTGCTGGACCCGGCCGCGCCGCCGCCGCTGCTGCCCTCCTC

                    4110      4120      4130      4140      4150      4160      4170      4180      4190      4200
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        g~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    TACACTCACCGTCACTGTTAATGTTCGAGAAGCGAACCCGCGACCAGTGTTCCAGAGAGCACTCTACACAGCTGGCATCTCTGCTGGCGATTTCATCGAA
L.dispar      TACTCTTACGGTCACTGTAAATGTGAGGGAAGCGAATCCAAGGCCCATGTTTACGAGCGAAGAATATATGGCTGGTATCTCCACTTCCGATAACATCAAC
O.nubilalis   CATCCTCGTTGTCACGGTCAATGTAAGAGAAGCGAACCCGCGCCCAATTTTCGAGCAGGACCTTTACACAGCGGGCATTTCGACGTTGGACAGCATTGGC
T.ni          CACCTTCCTGCTCACCATCAATGTCCGTGAGGCTGACCCGCGGCCAGTATTCGAGAGAGAGATCTACACCGCCGGCATCTACGAGACGGATACATCCAAC

                    4210      4220      4230      4240      4250      4260      4270      4280      4290      4300
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    AGAAACCTGCTGACTGTAGTAGCGACACATTCAGAAGGTCTGCCCATCACTTACACTCTGATACAAGAGTCCATGGAGGCAGACCCCACACTCGAAGCTG
L.dispar      AGAGTACTACTAACTGTACAGGCAACACACAGCGAGGGCGCTCCAGTCACATATGAAATAGATCATAGCACGATGATAGTAGATCCCACATTAGAAGCCG
O.nubilalis   CGGGAATTGCTTACCGTCAGGGCGAGCCACACAGAAGACGACACCATCACGTACATCATAGACCGTGCGAGCATGCAGCTGGACAGCAGCCTAGAAGCCG
T.ni          AGGGAACTACTCACTGTTCATGCGACACATACCGAAGGCCTAGACATAACGTACACGATGGACCTGGACACGATGGTGGTGGACCCCTCGCTGGAGGGCG

                    4310      4320      4330      4340      4350      4360      4370      4380      4390      4400
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GTGTTG~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    TTCAGGAGTCAGCCTTCATCCTCAACCCTGAGACTGGAGTCCTGTCCCTCAACTTCCAGCCAACCGCCGCCATGCACGGCATGTTTGAGTTCGAAGTCGA
L.dispar      TCAAGGACACCGCCTTCGTCCTAAACTCTCAAACAGGAGTTCTCACATTAAACATGCAGCCCACAGCATTCATGCATGGAAATTTCGAGTTTAAAGTCGT
O.nubilalis   TGCGCGACTCGGCCTTCACGCTGCATGCGACCACCGGCGTGCTTTCGCTCAATATGCAGCCCACCGCTTCCATGCACGGCATGTTCGAGTTCGACGTCAT
T.ni          TGAGGGAGTCCGCCTTCACGCTGCACCCGAGCAGCGGCGTCCTGTCCCTCAACATGAACCCGCTCGACACCATGGTCGGCATGTTCGAGTTCGATGTCGT

                    4410      4420      4430      4440      4450      4460      4470      4480      4490      4500
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori        ~~~~~~~GAT~~~~~~~~~~~~~~~~~~CGC~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera    AGCCACTGATTCAAGGACAGAAACTGCTCGCACGGAAGTGAAGGTGTACCTGATATCGGACCGCAACCGAGTGTTCTTCACGTTCAACAACCCGCTGCCT
L.dispar      TGCTACTGACCCAAGTGAAGCCACCGACCGCGCTGCTGTTAAGATATACCTTATATCGTCACTCAACAGAGTCACATTCACGTTCAGAAATACGCTGCAG
O.nubilalis   CGCTACGGATACAGCATCTGCAATCGACACAGCTCGTGTGAAAGTCTACCTCATCTCATCGCAAAACCGCGTGTCCTTCATTTTCGATAACCAACTTGAG
T.ni          GGCTACAGACACCAGAGGTGCAGAAGCCCGTACTGACGTGAAGATCTATCTGATCACTCATCTCAACAGAGTCTACTTCTTGTTCAACAACACGCTGGAT
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                        4510      4520      4530      4540      4550      4560      4570      4580      4590      4600
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GAT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera      GAAGTCACGCCCCAGGAAGATTTCATAGCGGAGACGTTCACGGCATTCTTCGGCATGACGTGCAACATCGACCAGACGTGGTGGGCCAGCGATCCCGTCA
L.dispar        CAAATTAATGACAACGTGGCATTTATAGCGCATACATTCTCGATTGGCTTCGACATGGATTGCAACATCGATCAGGTCGTACCAGCTAGCGAt~~~TCGA
O.nubilalis     ACCGTTGAGCAGAACAGAAATTTCATAGCGGCCACGTTCAGCACCGGGTTCAACATGACGTGCAACATCGACCAGGTGGTGCCGTTCAGCGAc~~~AGCA
T.ni            GTCGTTGACTCCAACAGAGCTTTCATAGCGGACACGTTCTCGTCGGTGTTCAGCCTGACGTGCAACATCGACGCGGTGCTGCGCGCGCCGGAc~~~AGCA

                        4610      4620      4630      4640      4650      4660      4670      4680      4690      4700
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera      CCGGCGCCACCAGGGACGACCAGACTGAAGTCAGGGCTCATTTCATCAGGGACGACTTACCCGTGCCTGCTGAGGAGATTGAACAGTTGCGCGGTAACCC
L.dispar        ATGGTGTCGCGTTGGATTACTTGACAGATGTAATGGCACACTTCATCAAGGACAACGAGCCTGTTTCGGCTGAAGAAATAGAACAGTTACGAAGTGACAC
O.nubilalis     GCGGCGTGGCACAAGACGACACCACCGAGGTGCGCGCGCACTTCATCCGGGACAACGTGCCCGTGCAGGCACAAGAGGTCGAGGCCGTCCGCAGCGACAC
T.ni            GCGGCGCCGCGCGCGACGACCGCACCGAGGTGCGCGCGCACTTCATACGGAACCACGTGCCCGCCACCACCGACGAGATAGAGCAGCTCCGTAGTAACAC

                        4710      4720      4730      4740      4750      4760      4770      4780      4790      4800
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera      AACTCTAGTAAATAGCATCCAACGAGCCCTAGAGGAACAGAACTTACAGCTGGCCGACCTGTTCACGGGCGAGACGCCCATCCTGGGCGGCGACGCGCAG
L.dispar        TATACTTCTGCGAACGATCCAACAGACTCTCAACATGCAGCTGGTGACGTTAGAGGACTTCGTGACTGGTGCCAGCCCTGACATCAGTACGGATAGTGCA
O.nubilalis     GGTGCTGCTGCGCACCATCCAGCTGATGCTGAGCACCAACAGCCTGGTGCTGCAAGACCTGGTGACGGGCGACACTCCGACGCTAGGCGAGGAGTCTATG
T.ni            AATACTGCTGAGAGCGATTCAGGAAACCCTGTTAACTCGCGAGCTGCATCTGGAGGACTTTGTGGGTGGCTCCAGCCCAGAGCTGGGCGTGGACAACAGC

                        4810      4820      4830      4840      4850      4860      4870      4880      4890      4900
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera      GCGCGAGCTCTGTACGCGCTGGCGGCGGTGGCTGCTGCGCTGGCACTGATCGTCGTTGTGCTGCTTATTGTGTTCTTTGTTAGGACTAGGACACTGAACC
L.dispar        CAGATCATAGTATACGTCCTCATCGGACTAACTGTTGCGCTCGGCTTCTTATGTATTGTTCTACTACTTACCTGCATCATCAGGACTAGGATGCTGAACC
O.nubilalis     CAGATCGCGGTCTACGCACTAGCCGCGCTCTCCGCTGTGCTGGGCTTCCTCTGCCTCGTGCTGCTGCTCGCACTGTTCTGTAGAACAAGAGCCCTAAACC
T.ni            ~~~cTCACAATATACGTGCTGGGCGCGCTCGCCGCGCTGCTCGGCTTCCTGTGCGTGCTGCTACTCATCACCTTCATCGTCAGGACTAGAGCGCTCAACC

                        4910      4920      4930      4940      4950      4960      4970      4980      4990      5000
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
H.armigera      GACGCTTGCAAGTTCTGTCCATGACCAAGTACAGTTCGCAAGACTCGGCCCTGAACCGCGTGGGTCTGGCAGCGCCGGGCACCAACAAGCATGCTGTCGA
L.dispar        GTCGTCTGGAAGCTCTGTCAATGACGAGATACGGTTCCGTAGACTCCGGTCTAAACCGCGCCGGTATCGCCGCTCCGGGTACTAACAAACATGCCGTTGA
O.nubilalis     GGCAGCTGCAAGCGCTCTCCATGACGAAGTACGGCTCGGTGGACTCCGGGCTGAACCGCGCGGGGCTG~~~GCGCCGGGCACCAACAAGCACGCCGTCGA
T.ni            GTCGCCTGGAAGCCCTATCCATGACTAAATACGGCTCAGTGGACTCCGGGCTGAACCGCGTGGGTCTGGCCGCGCCCGGGACCAACAAGCACGCCGTCGA
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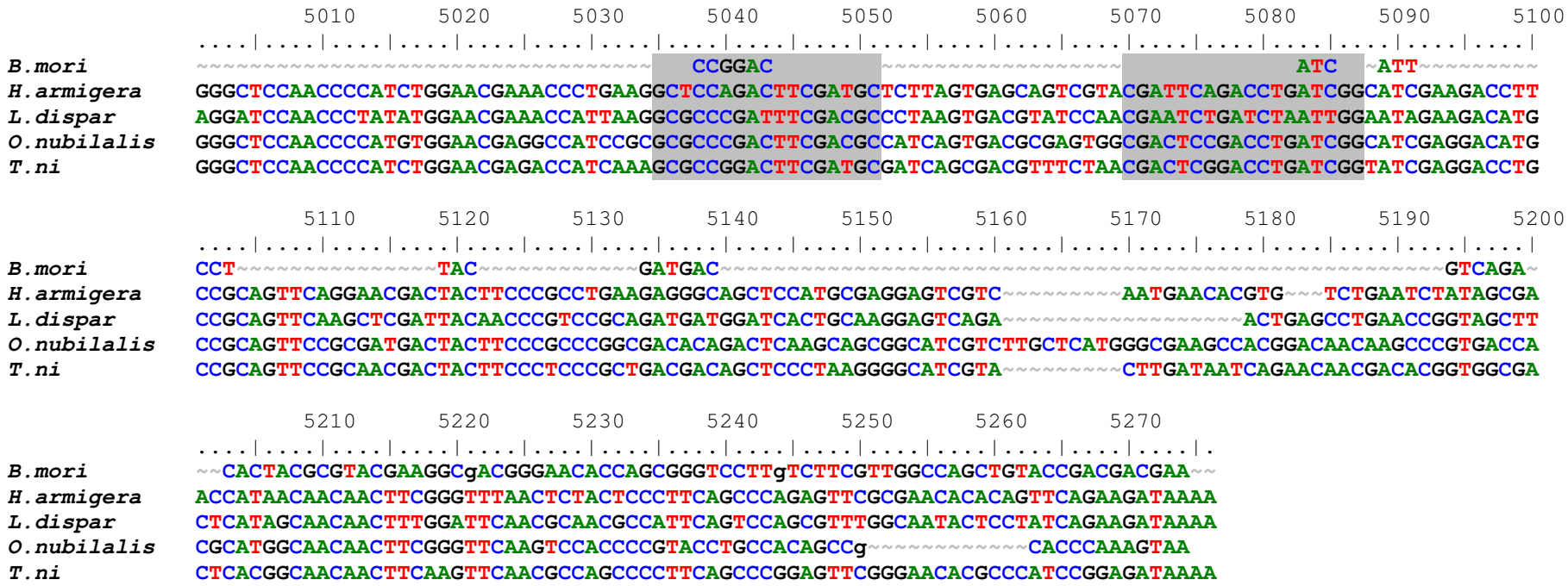
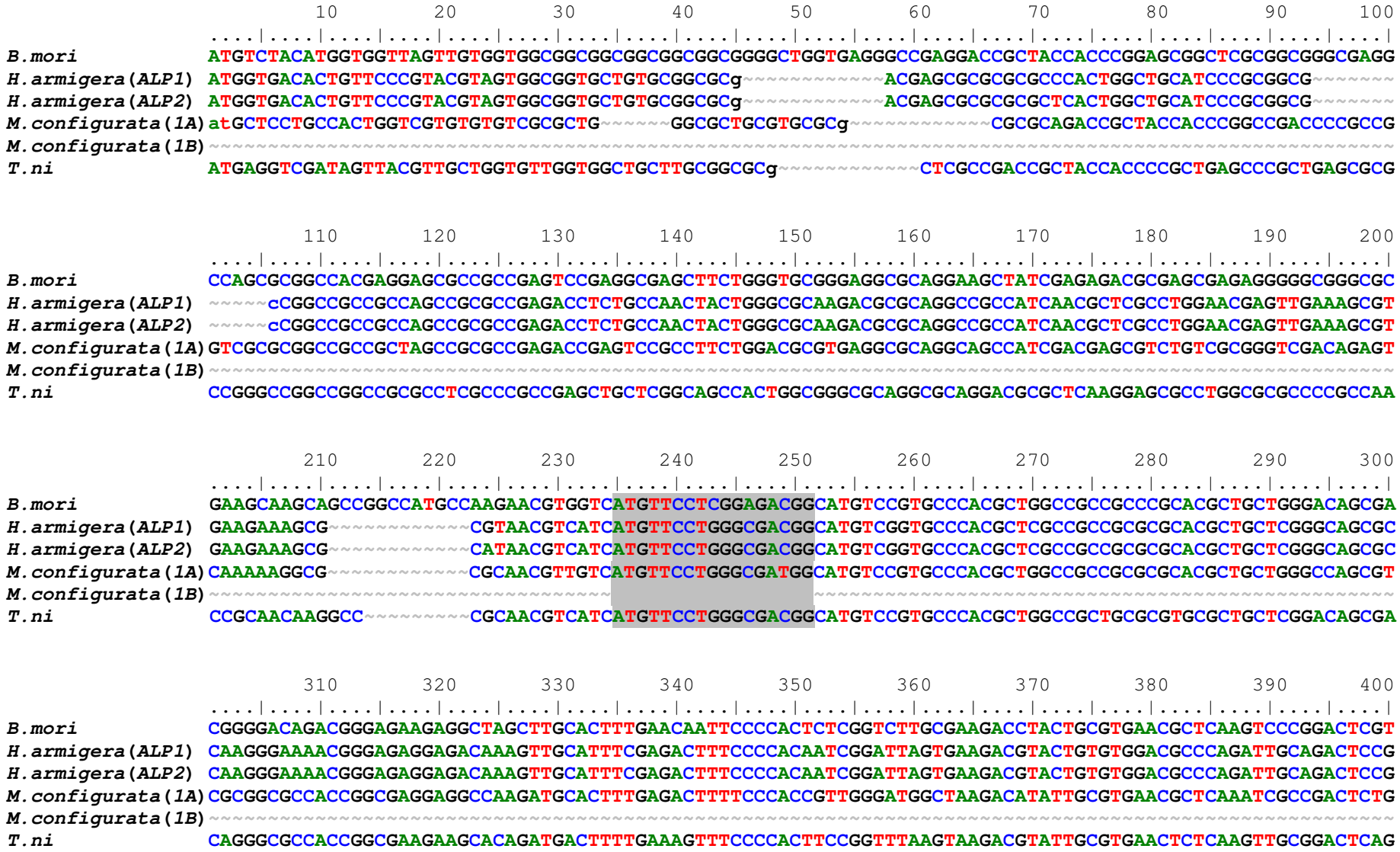
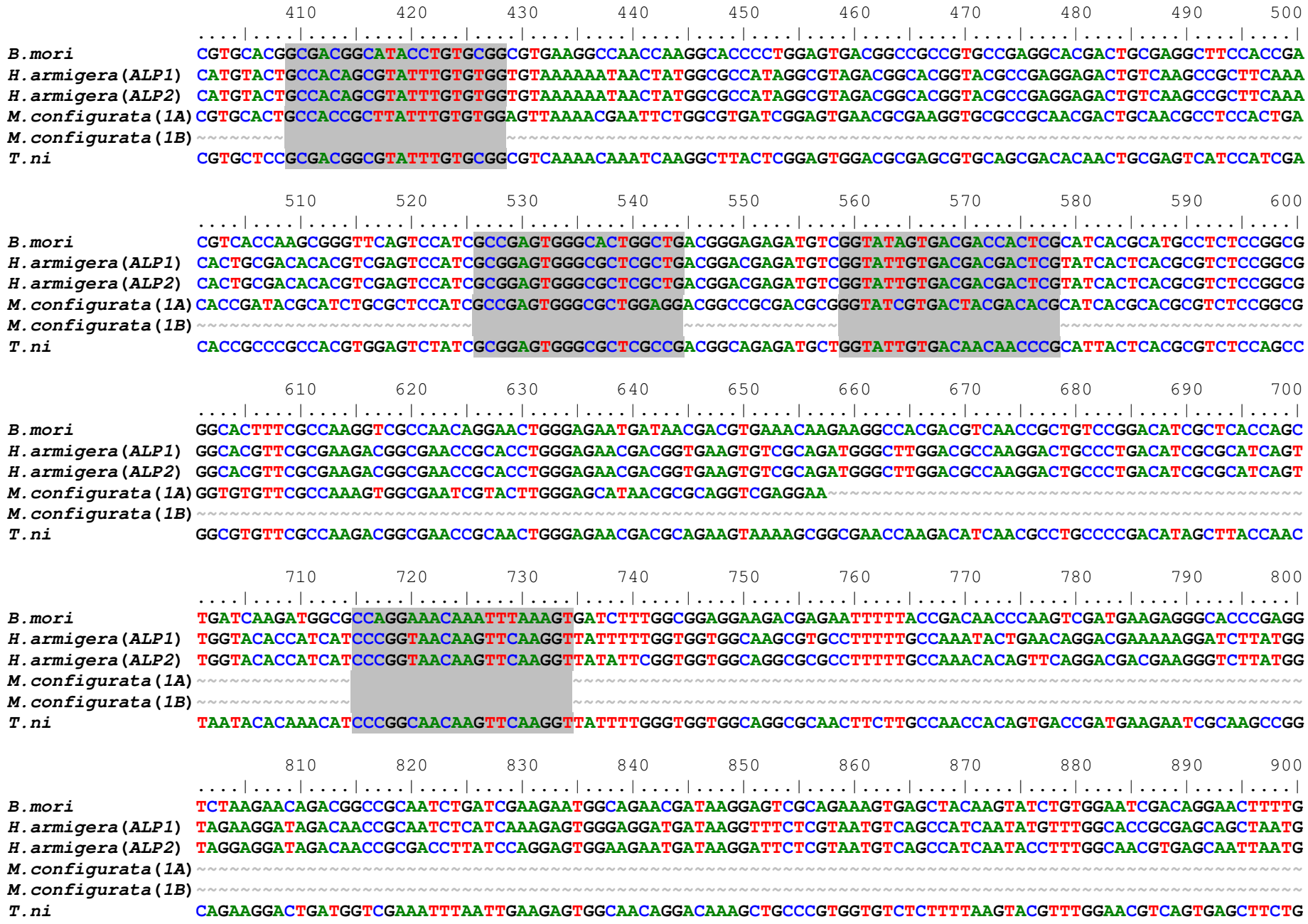
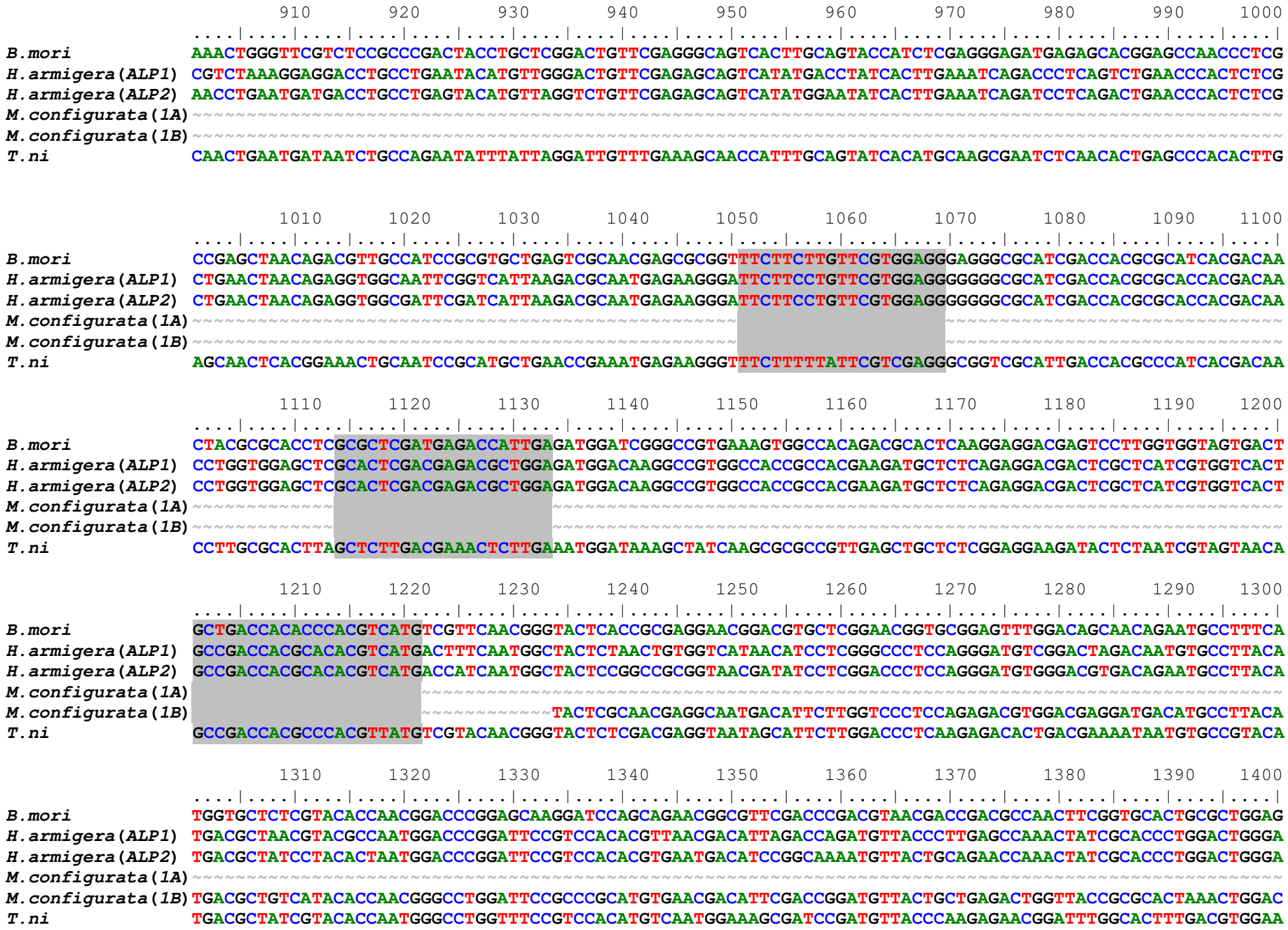


Figure B.7: DNA sequence alignment of *cadherin* for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (2381487), *Helicoverpa armigera* (40548429), *Lymantria dispar* (16588791), *Ostrinia nubilalis* (217315829) and *Trichoplusia ni* (327082289).

B.8. *Alkaline phosphatase* (*ALP*)




410 420 430 440 450 460 470 480 490 500
B.mori CGTGCACGGCGACGGCATACCTGTGCGGCGTGAAGGCCAACCAAGGCACCCCTGGAGTGACGGCCGCCGTGCCGAGGCACGACTGCGAGGCTTCCACCGA
H.armigera(ALP1) CATGTACTGCCACAGCGTATTTGTGTGGTGTAAAAAATAACTATGGCGCCATAGGCGTAGACGGCACGGTACGCCGAGGAGACTGTCAAGCCGCTTCAAA
H.armigera(ALP2) CATGTACTGCCACAGCGTATTTGTGTGGTGTAAAAAATAACTATGGCGCCATAGGCGTAGACGGCACGGTACGCCGAGGAGACTGTCAAGCCGCTTCAAA
M.configurata(1A) CGTGCACTGCCACCGCTTATTTGTGTGGAGTTAAAACGAATTCTGGCGTGATCGGAGTGAACGCGAAGGTGCGCCGCAACGACTGCAACGCCTCCACTGA
M.configurata(1B) ~~~~~~
T.ni CGTGCTCCGCGACGGCGTATTTGTGCGGCGTCAAAACAAATCAAGGCTTACTCGGAGTGGACGCGAGCGTGCAGCGACACAACTGCGAGTCATCCATCGA
510 520 530 540 550 560 570 580 590 600
B.mori CGTCACCAAGCGGGTTCAGTCCATCGCCGAGTGGGCACTGGCTGACGGGAGAGATGTCGGTATAGTGACGACCACTCGCATCACGCATGCCTCTCCGGCG
H.armigera(ALP1) CACTGCGACACACGTCGAGTCCATCGCGGAGTGGGCGCTCGCTGACGGACGAGATGTCGGTATTGTGACGACGACTCGTATCACTCACGCGTCTCCGGCG
H.armigera(ALP2) CACTGCGACACACGTCGAGTCCATCGCGGAGTGGGCGCTCGCTGACGGACGAGATGTCGGTATTGTGACGACGACTCGTATCACTCACGCGTCTCCGGCG
M.configurata(1A) CACCGATACGCATCTGCGCTCCATCGCCGAGTGGGCGCTGGAGGACGGCCGCGACGCGGGTATCGTGACTACGACACGCATCACGCACGCGTCTCCGGCG
M.configurata(1B) ~~~~~~
T.ni CACCGCCCGCCACGTGGAGTCTATCGCGGAGTGGGCGCTCGCCGACGGCAGAGATGCTGGTATTGTGACAACAACCCGCATTACTCACGCGTCTCCAGCC
610 620 630 640 650 660 670 680 690 700
B.mori GGCACTTTCGCCAAGGTCGCCAACAGGAACTGGGAGAATGATAACGACGTGAAACAAGAAGGCCACGACGTCAACCGCTGTCCGGACATCGCTCACCAGC
H.armigera(ALP1) GGCACGTTCGCGAAGACGGCGAACCGCACCTGGGAGAACGACGGTGAAGTGTCGCAGATGGGCTTGGACGCCAAGGACTGCCCTGACATCGCGCATCAGT
H.armigera(ALP2) GGCACGTTCGCGAAGACGGCGAACCGCACCTGGGAGAACGACGGTGAAGTGTCGCAGATGGGCTTGGACGCCAAGGACTGCCCTGACATCGCGCATCAGT
M.configurata(1A) GGTGTGTTCGCCAAAGTGGCGAATCGTACTTGGGAGCATAACGCGCAGGTCGAGGAA~~~~~~
M.configurata(1B) ~~~~~~
T.ni GGCGTGTTCGCCAAGACGGCGAACCGCAACTGGGAGAACGACGCAGAAGTAAAAGCGGCGAACCAAGACATCAACGCCTGCCCCGACATAGCTTACCAAC
710 720 730 740 750 760 770 780 790 800
B.mori TGATCAAGATGGCGCCAGGAAACAAATTTAAAGTGATCTTTGGCGGAGGAAGACGAGAATTTTTACCGACAACCCAAGTCGATGAAGAGGGCACCCGAGG
H.armigera(ALP1) TGGTACACCATCATCCCGGTAACAAGTTCAAGGTTATTTTTGGTGGTGGCAAGCGTGCCTTTTTGCCAAATACTGAACAGGACGAAAAAGGATCTTATGG
H.armigera(ALP2) TGGTACACCATCATCCCGGTAACAAGTTCAAGGTTATATTCGGTGGTGGCAGGCGCGCCTTTTTGCCAAACACAGTTCAGGACGACGAAGGGTCTTATGG
M.configurata(1A) ~~~~~~
M.configurata(1B) ~~~~~~
T.ni TAATACACAAACATCCCGGCAACAAGTTCAAGGTTATTTTGGGTGGTGGCAGGCGCAACTTCTTGCCAACCACAGTGACCGATGAAGAATCGCAAGCCGG
810 820 830 840 850 860 870 880 890 900
B.mori TCTAAGAACAGACGGCCGCAATCTGATCGAAGAATGGCAGAACGATAAGGAGTCGCAGAAAGTGAGCTACAAGTATCTGTGGAATCGACAGGAACTTTTG
H.armigera(ALP1) TAGAAGGATAGACAACCGCAATCTCATCAAAGAGTGGGAGGATGATAAGGTTTCTCGTAATGTCAGCCATCAATATGTTTGGCACCGCGAGCAGCTAATG
H.armigera(ALP2) TAGGAGGATAGACAACCGCGACCTTATCCAGGAGTGGAAGAATGATAAGGATTCTCGTAATGTCAGCCATCAATACCTTTGGCAACGTGAGCAATTAATG
M.configurata(1A) ~~~~~~
M.configurata(1B) ~~~~~~
T.ni CAGAAGGACTGATGGTCGAAATTTAATTGAAGAGTGGCAACAGGACAAAGCTGCCCGTGGTGTCTCTTTTAAGTACGTTTGGAACGTCAGTGAGCTTCTG


910 920 930 940 950 960 970 980 990 1000
B.mori AAACTGGGTTCGTCTCCGCCCGACTACCTGCTCGGACTGTTCGAGGGCAGTCACTTGCAGTACCATCTCGAGGGAGATGAGAGCACGGAGCCAACCCTCG
H.armigera(ALP1) CGTCTAAAGGAGGACCTGCCTGAATACATGTTGGGACTGTTCGAGAGCAGTCATATGACCTATCACTTGAAATCAGACCCTCAGTCTGAACCCACTCTCG
H.armigera(ALP2) AACCTGAATGATGACCTGCCTGAGTACATGTTAGGTCTGTTCGAGAGCAGTCATATGGAATATCACTTGAAATCAGATCCTCAGACTGAACCCACTCTCG
M.configurata(1A)
M.configurata(1B)
T.ni CAACTGAATGATAATCTGCCAGAATATTTATTAGGATTGTTTGAAAGCAACCATTTGCAGTATCACATGCAAGCGAATCTCAACACTGAGCCCACACTTG
1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
B.mori CCGAGCTAACAGACGTTGCCATCCGCGTGCTGAGTCGCAACGAGCGCGGTTTCTTCTTGTTCGTGGAGGGAGGGCGCATCGACCACGCGCATCACGACAA
H.armigera(ALP1) CTGAACTAACAGAGGTGGCAATTCGGTCATTAAGACGCAATGAGAAGGGATTCTTCCTGTTCGTGGAGGGGGGCGCATCGACCACGCGCACCACGACAA
H.armigera(ALP2) CTGAACTAACAGAGGTGGCGATTCGATCATTAAGACGCAATGAGAAGGGATTCTTCCTGTTCGTGGAGGGGGGCGCATCGACCACGCGCACCACGACAA
M.configurata(1A)
M.configurata(1B)
T.ni AGCAACTCACGGAAACTGCAATCCGCATGCTGAACCGAAATGAGAAGGGTTTCTTTTTATTCGTCGAGGGCGGTCGCATTGACCACGCCCATCACGACAA
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
B.mori CTACGCGCACCTCGCGCTCGATGAGACCATTGAGATGGATCGGGCCGTGAAAGTGGCCACAGACGCACTCAAGGAGGACGAGTCCTTGGTGGTAGTGACT
H.armigera(ALP1) CCTGGTGGAGCTCGCACTCGACGAGACGCTGGAGATGGACAAGGCCGTGGCCACCGCCACGAAGATGCTCTCAGAGGACGACTCGCTCATCGTGGTCACT
H.armigera(ALP2) CCTGGTGGAGCTCGCACTCGACGAGACGCTGGAGATGGACAAGGCCGTGGCCACCGCCACGAAGATGCTCTCAGAGGACGACTCGCTCATCGTGGTCACT
M.configurata(1A)
M.configurata(1B)
T.ni CCTTGCGCACTTAGCTCTTGACGAAACTCTTGAAATGGATAAAGCTATCAAGCGCGCCGTTGAGCTGCTCTCGGAGGAAGATACTCTAATCGTAGTAACA
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
B.mori GCTGACCACACCCACGTCATGTCGTTCAACGGGTACTCACCGCGAGGAACGGACGTGCTCGGAACGGTGCGGAGTTTGGACAGCAACAGAATGCCTTTCA
H.armigera(ALP1) GCCGACCACGCACACGTCATGACTTTCAATGGCTACTCTAACTGTGGTCATAACATCCTCGGGCCCTCCAGGGATGTCGGACTAGACAATGTGCCTTTACA
H.armigera(ALP2) GCCGACCACGCACACGTCATGACCATCAATGGCTACTCCGGCCGCGGTAACGATATCCTCGGACCCTCCAGGGATGTGGGACGTGACAGAATGCCTTTACA
M.configurata(1A)
M.configurata(1B) TACTCGCAACGAGGCAATGACATTCTTGGTCCCTCCAGAGACGTGGACGAGGATGACATGCCTTACA
T.ni GCCGACCACGCCCACGTTATGTCGTACAACGGGTACTCTCGACGAGGTAATAGCATTCTTGGACCCTCAAGAGACACTGACGAAAATAATGTGCCGTACA
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
B.mori TGGTGCTCTCGTACACCAACGGACCCGGAGCAAGGATCCAGCAGAACGGCGTTCGACCCGACGTAACGACCGACGCCAACTTCGGTGCACTGCGCTGGAG
H.armigera(ALP1) TGACGCTAACGTACGCCAATGGACCCGGATTCCGTCCACACGTTAACGACATTAGACCAGATGTTACCCTTGAGCCAAACTATCGCACCCTGGACTGGGA
H.armigera(ALP2) TGACGCTATCCTACACTAATGGACCCGGATTCCGTCCACACGTGAATGACATCCGGCAAAAATGTTACTGCAGAACCAAACTATCGCACCCTGGACTGGGA
M.configurata(1A)
M.configurata(1B) TGACGCTGTCATACACCAACGGGCCTGGATTCCGCCCGCATGTGAACGACATTCGACCGGATGTTACTGCTGAGACTGGTTACCGCGCACTAAACTGGAC
T.ni TGACGCTATCGTACACCAATGGGCCTGGTTTCCGTCCACATGTCAATGGAAAGCGATCCGATGTTACCCAAGAGAACGGATTTGGCACTTTGACGTGGAA

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                        1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            GACGCACACGGACGTCCCGCTGGACTCGGAGACGCACGGCGGAGATGACGTCACGGTGTTCGCATGGGGTGTGCACCACTGGATGTTCTCTGGTCTGTAC
H.armigera(ALP1)  GTCGCACGTGGACGTGCCGCTGGTGGACGAGACGCACGGCGGCGACGACGTGGCCGTGTTCGCGCGCGGGCCGCACCACTCCATGTTCACGGGGCTGTAC
H.armigera(ALP2)  GTCGCACGTGGACGTGCCGCTGGTGGACGAGACGCACGGCGGCGACGACGTGGCCGTGTTCGCGCGCGGGCCGCACCACTCCATGTTCACGGGGCTGTAC
M.configurata(1A) ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
M.configurata(1B) GTCTCACGTGGACGTGCCGCTGGACTCGGAGACGCACGGCGGCGACGACGTGGCGGTGTTCGCGCGCGGGCCGCACCACTCCATGTTCACGGGGCTGTAC
T.ni              ATCGCATGTCGACGTACCCCTAGACTCGGAAACACACGGCGGTGATGACGTGGCGGTGTTCGCGCGCGGTCCTTACCATATGCTTTTCACGGGGCTGTAC

                        1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            GAGCAGACGCACGTGCCACACCGCATGGCGTGGGCAGCCTGCATGGGCCCGGGCCGCCACGTCTGCGTCTCGGCTGCCACTGTGCCCACTGCg~~~~~~~
H.armigera(ALP1)  GAGCAGAGCCAGCTGCCGCACCTCATGGCCTACGCCGCCTGCATCGGCCCCGGCCGGCACGCCTGCGCCAGTGCCGCGCACTTGCCTAGCGCg~~~~~~~
H.armigera(ALP2)  GAGCAGAGCCAGCTGCCGCACCTCATGGCCTACGCCGCCTGCATCGGCCCCGGCCGGCACGCCTGCGCCAGTGCCGCGCACTTGCCTAGCGCg~~~~~~~
M.configurata(1A) ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
M.configurata(1B) GAGCAGAGCCAGCTGCCGCACCTCATGGCGTACGCCGCCTGCATCGGGCCCGGCCGCCACGCCTGCAGCAGCGCACATGTCGTGGCCGCGCCt~~~~~~~
T.ni              GAGCAGAATCAGATACCCCACCTTATGGCCTACGCCGCCTGCATCGGTCCCGGCCTGCACTCGTGTGCCGAGGCCGACACCACCTCCACGCCAGAGGCGA

                        1610      1620      1630      1640      1650      1660      1670      1680      1690      1700
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
B.mori            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GCGCTGCTTTCTCTCTTGCTCGCCGCCTTCATCACCTT
H.armigera(ALP1)  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CACTTCTTCGTAGCTCTGCTCGCTCTATT
H.armigera(ALP2)  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CACTTCTTCGTAGCTCTGCCCGCTCTATT
M.configurata(1A) ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
M.configurata(1B) ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ATCATCTTCTTTACCATATTTGTTCTACTCACTACTCT
T.ni              ACCCTCCAGAGGCTACTACCGCCAGCCCAACGACCGCTGAACCATCAGCTGCGGCGCCTGTCAGCGCTACGCTCGCACTTTTTGCTCTACTCACTACTCT

                        1710      1720
                  ....|....|....|....|...
B.mori            GCGGCATCAATGCTTTCTCTAAC
H.armigera(ALP1)  CACTTCCATTTTACTGCGATAAA
H.armigera(ALP2)  CATTTCCATTTTACTGCGATAAA
M.configurata(1A) ~~~~~~~~~~~~~~~~~~~~~~~
M.configurata(1B) TTTTATA~~~~~~~~~CAATGAA
T.ni              T~~~aCTTTGTTATTACACTAAA
```

Figure B.8: DNA sequence alignment of *alkaline phosphatase* (*ALP*) for several lepidopteran species. Conserved regions observed in the protein sequence alignment are highlighted in grey in the corresponding DNA sequence alignment. Species and GenBank Gen-Info Identifier (GI) numbers include: *Bombyx mori* (113208403), *Helicoverpa armigera* (ALP1) (194295555), *Helicoverpa armigera* (ALP2) (194295557), *Mamestra configurata* (ALP1A) (327420509), *Mamestra configurata* (ALP1B) (327420467) and *Trichoplusia ni* (334562422).