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Molecular Genetic Analysis of Cereal β-Amylase Genes Using Exon-Primed Intron-Crossing (EPIC) PCR

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Summary: The proteins encoded by cereal *β*-amylase genes *Bamy1* and *Bamy2* genes play an important role in seedling germination and in the brewing process. Here, we use exon-primed intron-crossing (EPIC) to analyse *Bamy1* and *Bamy2* genetic diversity among 38 accessions belonging to six *Poaceae* tribes. DNA sequence alignment of multiple *Poaceae* species *β*-amylase sequences allowed design of EPIC primers that simultaneously amplify *Bamy1* and *Bamy2* in all the cereal species investigated. The genetic variation observed in the samples investigated is analysed and discussed, and illustrates the effectiveness of this approach for intra- and interspecific analysis in plant species.
Keywords: *β*-amylase, barley, genetic diversity, intron-length polymorphism, *Poaceae*, rye, wheat

Abbreviation: Exon-Primed Intron-Crossing (EPIC)

Introduction

Due to the growing volume of data provided by genome sequencing projects, and the presence of regulatory regions within introns, the study of intronic polymorphism is emerging as an important aspect of genetic research in agricultural crops (Braglia et al. 2010). Compared to exonic regions, introns are more variable due to reduced selective pressure, i.e. the rate of mutation accumulation in these regions is comparatively high (Ludwig 2002, Morello & Breviario 2008, Yang et al. 2007). Introns often contain a variety of functional elements, including enhancers, silencers, regulators of alternative splicing, trans-splicing elements and other regulatory elements (some of which can be found in the expanded introns). Furthermore, intron characteristics such as length variability, position within the gene, dependency on the length of the exons, can also modulate gene properties such as expression, transcription, splicing and microRNA lifetime (Braglia et al. 2010, Ludwig 2002, Rose & Beliakoff 2000). In plants, investigation into intron function has highlighted their role in gene expression, and specifically in tissue- or stage-specific expression (Morello & Breviario 2008). Analysis of plant intron sequences has been applied in numerous studies (Chetelat et al. 1995, Fridman et al. 2000, Holland et al. 2001, Hongtrakul V 1998), with a well-studied example in cereal species being the role of putative *cis*-elements in intron 1 of vernalization response (*Vrn)* genes. Deletions spanning this region are thought to result in vernalization non-responsiveness (Cockram et al. 2007), and underlie the creation of globally important spring-sown cereal cultivars.

The polymerase chain reaction (PCR) based technique, 'exon-primed intron-crossing' (EPIC-PCR) (Palumbi & Baker 1994) has gained favour in plant and animal studies, and relies on design of primers selected to anneal to highly-conserved regions of the exons. For example, it has been used to study conserved regions within eukaryotic 18S and 28S ribosomal genes and prokaryotic 16S and 23S ribosomal genes, for amplification of variable intergenic regions known as internal transcribed spacers (ITS), containing 5.8S ribosomal gene (Gardes 1993).

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EPIC has a number of experimental advantages, including, (i) primers complementary to conserved regions within exons can be used across a wide taxonomic range, (ii) homologous amplified sequences can be easily defined by comparing their exonic and intronic regions depending on genetic distance between the taxons, and (iii) exonic and intronic fragments can help in the simultaneous studies of genetic variety at intraspecific and interspecific levels (Bierne et al. 2000, Ishikawa et al. 2007, Lessa 1992, Li et al. 2010). A further advantage of EPIC is that if avoids the need to develop DNA markers on a species -by-species basis, which can be costly and time consuming.

In cereal species (tribe: *Triticeae*), *β*-amylase hydrolyses starch by splitting α-1,4-glycosidic bonds, resulting in the formation of the high molecular weight dextrin and maltose - a disaccharide, that easily diffuses and can be used by the growing embryo (James et al. 2003). It is also an important component of the mash, a process used in brewing by which grain and water are combined and heated allowing enzymatic degradation of starch into sugars for fermentation (Priest 1986). Numerous species within the *Triticeae* can be used for brewing, including barley, rye, triticale, wheat, oat, and millet (Briggs 1998). Only members of the *Triticeae* are reported to have two different forms of *β*-amylase (Mason-Gamer 2005), differing significantly in their patterns of gene expression: *Bamy1* is specific to the endosperm (endospermal *β*-amylase), while *Bamy2* is expressed in all tissues (ubiquitous *β*-amylase). These genes are paralogs, and both consist of 7 exons and 6 introns.

In this study, EPIC-PCR was used to investigate *Bamy1* and *Bamy2* intronic genetic variation within representative species belonging to the *Poaceae*. Due to their importance within cereal species, molecular genetic characterisation of cereal *Bamy1* and *Bamy2* will provide new information on the range of inter- and intra-specific genetic variation present. This data will help inform how *Triticeae* germplasm resources may be used as important sources of qualitative character enhancement, allowing enhancement of *β*-amylase activity in cereals.

Materials and Methods

Germplasm and DNA extraction

The 38 accessions investigated, belonging to six *Poaceae* tribes, are listed in Table 1.

Table 1. *Poaceae* germplasm used in this study

Plant material	Source	Cultivars/Lines
Accessions used for interspecific analyses		
38 species of the family *Poaceae*	Botanical Institute of the V.L. Komarova, RAS; Plant Breeding and Genetics Institute, Odessa	*Hordeum vulgare* (Djau Kabutak), *H. vulgare* (Pallidum 107), *H. vulgare* (Odesskiy 17), *H. vulgare* (Odesskiy 31), *H. spontaneum*, *H. murinum*, *H. marinum*, *H. brachyantherum*, *H. leporinum*, *Triticum durum* (AABB), *Aegilops speltoides*, *Agropyron cristatum*, *Amblyopyrum muticum*, *Comopyrum comosum*, *Crithodium monococcum*, *Crithopsis delileana*, *Dasypyrum villosum*, *Eremopyrum distans*, *Henrardia persica*, *Heteranthelium piliferum*, *Lophopyrum elongatum*, *Peridictyon sanctum*, *Pseudoroegneria spicata*, *Secale strictum*, *Taeniatherum caput-medusae*, *Thinopyrum bessarabicum*, *Psathyrostachys fragillis*, *fragilis*, *Psathyrostachys fragillis*, *villosus*, *Festucopsis serpentinii*, *Elymus repens*, *Phleum pretense*, *Zingeria biebersteiniana, Z. trichopoda*, *Colpodium versicolor*, *Spartina alterniflora*, *Bromus sterilis*, *Avena sativa*, *Brachypodium distachyon*
Accessions used for intraspecific analyses		
25 cultivars of spring triticale (x *Triticosecale* Witt.)	Genbank Slovakia	Arc en Ciel, Fronteiro, Tentudia, Sierra de Almaraz, Sierra de Arroyo, Sierra de Lobos, Alter, Camarma, Cume, Curtido, Vrodi, Thisbi, Vrito, Legalo, Senatrit, Trimour, Dublet, Wanad, Gabo, Logo, Matejko, Nobi, Noe, Somtri, Sierra de Villuercas
36 cultivars of winter triticale		Benetto, NE 422T, UCRTCL 1, Greneder, Plains, Terreland 22, Mungis, Leontino, Constant, Trizeps, Tribeca, Dusi, Alekto, Cosinus, Kandar, Largus, Magistral, Noe, Pletomax, Tatra, Massimo, Nutriseeds 1-18, Bienvenu, Trismart, Trimmer, Wilfried, Pizarro, Aprim, Innoval, Amarillo 105, Blenio, Kinerit, UCRTCL3, Algoso, UCRTCL2, Flavius
Five cultivars of rye (*Secale cereale* L.)	MTT Agrifood Research, Finland	Riihi, Akusti, Iissavaara, Loppi, Hirvessalmi;
Aegilops speltoides syn. *Sitopsis speltoides* (Tausch)Á.Löve	The Institute of Evolution, Haifa	Seeds are selected randomly from the population received
Triticum turgidum subsp. *Dicoccoides*		

Genomic DNA was extracted from 5-day-old etiolated seedling or green leaves using CTAB buffer following established protocols (http://primerdigital.com/dna.html). DNA concentration was estimated by spectrophotometric analysis; DNA quality was determined by agarose gel electrophoresis.

PCR-amplification

PCR primers were designed using genomic β-amylase sequences retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/nuccore/). DNA multiple sequence alignments were performed using the program, Multain (Corpet 1988), while primer design was performed using FastPCR (Kalendar et al. 2011). After comparison of the amplification effectiveness of primer pair options, pairs 3162 and 3816 were selected: 3162 (5'-TCCAAGTCTACGTCATGCTCC-3', which anneals to *Bamy1* exon 1 position 1389-1409 bp (FJ161080), and *Bamy2* exon 1 position 54-74 bp (DQ889983) and 3816 (5'-GCTGCTGCTGCTTTGAAGTCTGCT-3', which anneals to *Bamy1* exon 1 position 3660-3683 bp (FJ161080), and *Bamy2* exon 1 position 1386-1409 bp (DQ889983).

PCRs were performed in 25 µl reaction mixtures, containing 25 ng genomic DNA, 1x DreamTaq buffer, 0.3 µM each primer, 0.2 mM each dNTP, 1 U DreamTaq DNA polymerase (Thermo Scientific). PCR cycling profiles were as follows: initial denaturation 95°C - 3 min, subsequently 32 cycles: 15 sec at 95°C, 60 sec at 65°C and 2 min at 72 °C, final elongation - 5 min at 72°C. Electrophoresis of the amplified products was performed using 1.3% ethidium bromide stained agarose gels and amplicons visualized using a FLA-5100 imaging system (Fuji Photo Film GmbH., Europe).

Results and Discussion

Design of EPIC primers for cereal β-amylase genes

Primer design was performed according to the complete nucleic acid sequences of the *Bamy1* and *Bamy2* genes of the barley, and from genomic gene fragments and cDNA of *Bamy1* and *Bamy2* of barley, soft wheat, rye, sorghum and maize, identified in the NCBI database (http://www.ncbi.nlm.nih.gov/nuccore/). Altogether, 45 nucleic acid sequences were identified and used for subsequent analyses, GenBank accessions: AB048949, AB306504, AF012343, AF061203, AF061204, AF300799, AF300800, AF414081, AF414082, AF470353, AJ301645, AK365629, AK375028, AY111979, AY454398, AY835429, AY835430, D21349, D49999, D63574, DQ889983, EF175466, EF175467, EF175468, EF175469, EF175470, EF175471, EF175472, EF175473, EU589327, EU589328, FJ161078, FJ161079, FJ161080, FJ936153, FN179393, FN179394, GU017481, NM_001112026, NM_001188314, X52321, X56785, X98504, XM_003562919, Z11772.

DNA multiple sequence alignments of the β-amylase genes identified regions of DNA conservation within exons, as well as variability within intronic regions (Supplemental File). The sequence alignment was used in conjunction with the program FastPCR (Kalendar et al. 2011) to design EPIC-primers which target the most conserved protein-coding regions of *Bamy1* and *Bamy2*. It should be noted that the exonic regions chosen for primer annealing show high levels of conservation not only for cereals, but also for more distant plant species, thus demonstrating the flexibility of EPIC for plant molecular studies.

Interspecific Poaceae Bamy1 and Bamy2 genetic variation

To study interspecific genetic variability in *Bamy1* and *Bamy2*, 38 *Poaceae* species were analysed (Tab. 2). Detection of β-amylase genetic polymorphism was performed using EPIC primers 3162 and 3816 (complementary to exons 1 and 4 in barley, respectively), allowing simultaneous study of *Bamy1* and *Bamy2* amplicons from multiple species. Analysis of EPIC PCR products amplified from all species found a wide range of genetic variation (Fig. 1). The expected amplicon size of barley PCR amplicons fragments from *H. vulgare* was 2295 or 2196 bp for *Bamy1* (due to a 126 bp MITE insertion in intron 3 (Erkkilä 1999), and 1365 bp for *Bamy2*. Amplification products of these sizes were identified in *Hordeum vulgare* (Fig. 1, samples 1-4), as well as for *H. spontaneum*, *H. murinum* and *T. durum*. Amplicons from the remaining species differed from these, ranging between 1700 and 2500 bp for *Bamy1*, and between 1300 and 1500 bp for *Bamy2* (Fig. 1). Species belonging to the tribes *Aveneae*, *Poeae* and *Brachypodieae* (*Phleum pratense, Zingeria biebersteiniana, Colpodium versicolor, Spartina alterniflora, Avena sativa, Brachypodium distachyon*; samples 31-34, 36-37) contain just one β-amylase gene, *Bamy2*. Similarly, for *Bromus sterilis* (which belongs to the Bromeae tribe), searches of public DNA databases found sequences for *Bamy1* alone (HE565904, HE565905). However, the EPIC analysis undertaken here found *B. sterilis* to possess both *Bamy1* and *Bamy2* (Fig 1, sample 35), indicating that the presence of endospermal β-amylase genes is not exclusive to the Triticeae tribe.

In total, ten different *Bamy1* alleles (from one to three amplicons per sample) and four *Bamy2* alleles (one amplicon per sample) were identified. EPIC-PCR analyses revealed both inter- and intraspecific variation in the samples analysed. Accordingly, the EPIC approach is appropriate for rapid analysis of genetic variety within the *Poaceae* family.

Triticeae β-amylase genetic variation

EPIC analyses of β-amylase intraspecific genetic variation for four members of the Triticeae tribe were undertaken: *Triticum dicoccoides, Aegilops speltoides,* rye and triticale.

Table 2. Analysis of *Bamy1* and *Bamy2* genes in species of the *Poaceae* family

no	Species	Tribe	*Bamy1*	*Bamy2*
1	*Hordeum vulgare*	*Triticeae*	+	+
2	*Hordeum spontaneum*	*Triticeae*	+	+
3	*Hordeum murinum*	*Triticeae*	+	+
4	*Hordeum marinum*	*Triticeae*	+	+
5	*Hordeum brachyantherum*	*Triticeae*	+	+
6	*Hordeum erectifolium*	*Triticeae*	+	+
7	*Triticum durum*	*Triticeae*	+	+
8	*Aegilops speltoides*	*Triticeae*	+	+
9	*Agropyron cristatum*	*Triticeae*	+	+
10	*Amblyopyrum muticum*	*Triticeae*	+	+
11	*Comopyrum comosum*	*Triticeae*	+	+
12	*Crithodium monococcum*	*Triticeae*	+	+
13	*Crithopsis delileana*	*Triticeae*	+	+
14	*Dasypyrum villosum*	*Triticeae*	+	+
15	*Eremopyrum distans*	*Triticeae*	+	+
16	*Henrardia persica*	*Triticeae*	+	+
17	*Heteranthelium piliferum*	*Triticeae*	+	+
18	*Lophopyrum elongatum*	*Triticeae*	+	+
19	*Peridictyon sanctum*	*Triticeae*	+	+
20	*Pseudoroegneria spicata*	*Triticeae*	+	+
21	*Secale strictum*	*Triticeae*	+	+
22	*Taeniatherum caput-medusae*	*Triticeae*	+	+
23	*Thinopyrum bessarabicum*	*Triticeae*	+	+
24	*Psathyrostachys fragillis, villosus*	*Triticeae*	+	+
25	*Psathyrostachys stoloniformis*	*Triticeae*	+	+
26	*Festucopsis serpentinii*	*Triticeae*	+	+
27	*Elymus repens*	*Triticeae*	+	+
28	*Bromus sterilis*	*Bromeae*	+	+
29	*Avena sativa* (cv. Vali)	*Aveneae*	-	+
30	*Zingeria biebersteiniana*	*Aveneae*	-	+
31	*Zingeria trichopoda*	*Aveneae*	-	+
32	*Colpodium versicolor*	*Poeae*	-	+
33	*Phleum pratense*	*Poeae*	-	+
34	*Spartina alterniflora*	*Zoysieae*	-	+
35	*Brachypodium distachyon*	*Brachypodieae*	-	+

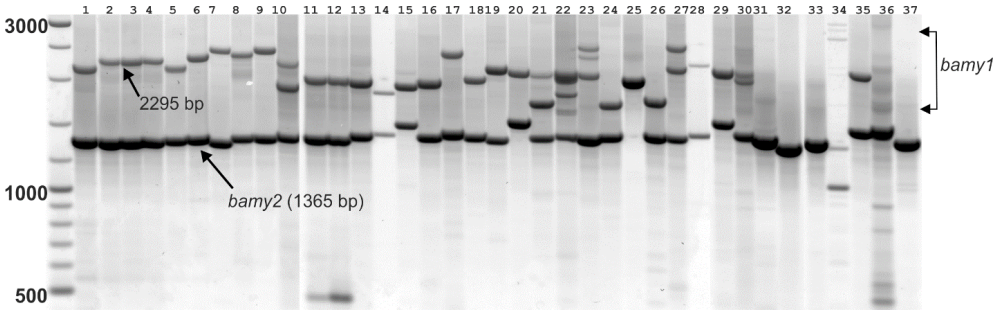

Figure 1. Electrophoregram products amplified from *Bamy1* and *Bamy2* in species of the family, *Poaceae*. M - DNA ladder (SM1173, Thermo Scientific);
1-4 *Hordeum vulgare*: 1 - cv. Djau Kabutak; 2 – cv. Pallidum 107; 3 – cv. Odesskiy 17; 4 – cv. Odesskiy 31; 5 - *H. spontaneum*; 6 – *H. murinum*; 7 – *H. marinum* ; 8 – *H. brachyantherum*; 9 – *H. leporinum*; 10 - *Triticum durum* (AABB); 11 - *Aegilops speltoides*; 12 - *Agropyron cristatum*; 13 - *Amblyopyrum muticum*; 14 - *Comopyrum comosum*; 15 - *Crithodium monococcum*; 16 - *Crithopsis delileana*; 17 - *Dasypyrum villosum*; 18 - *Eremopyrum distans*; 19 - *Henrardia persica*; 20 - *Heteranthelium piliferum*; 21 - *Lophopyrum elongatum*; 22 - *Peridictyon sanctum*; 23 - *Pseudoroegneria spicata*; 24 - *Secale strictum*; 25 - *Taeniatherum caput-medusae*; 26 - *Thinopyrum bessarabicum*; 27 - *Psathyrostachys fragillis*; *fragilis*; 28 - *Psathyrostachys fragillis, villosus*; 29 - *Festucopsis serpentinii*; 30 - *Elymus repens*; 31 - *Phleum pretense*; 32 - *Zingeria biebersteiniana;* 33 - *Colpodium versicolor*; 34 - *Spartina alterniflora*; 35 - *Bromus sterilis*; 36 - *Avena sativa*; 37 - *Brachypodium distachyon*.

These species were selected for the following reasons: *T. dicoccoides* (emmer or spelt wheat) is the tetraploid parent of the cultivated hexaploid wheat *T. aestivum* (2n = 6x = 42, AABBDD), while *A. speltoides* (2n = 2x = 14, BB or SS) carries valuable agronomic attributes exploited within the artificial hybridization with bread wheat. As well as being a valuable crop in its own right, rye is used to form a hybrid with wheat to produce triticale, a crop which combines the valuable characteristics of these genera (Lukaszewski 2006, Yang et al. 2011).

Three EPIC amplicons were found to be common for all four species investigated: products of 2295 bp (as expected for *Bamy1*), ~1900 bp (included in the allelomorphic spectrum of *Bamy1*) and 1356 bp (*Bamy2*) (Fig. 2, 3, 4). It should be noted that EPIC amplicons of size 2295 and 1356 bp were also present in the allelomorphic spectrum of *Hordeum* (samples 1 - 6) and *T. durum* (10) (Fig. 1). Analysis of *Bamy1* polymorphism in triticale and rye showed that their level of common polymorphism is higher than those of the cultivars of barley (Fig. 1), in which only two *Bamy1* alleles were found. Comparison of triticale and rye genotypes showed that the majority of the triticale allelomorphic spectrum was present in rye (including fragments of 2295 and 1356 bp). In addition, the allelomorphic spectrums of winter and spring triticale contained amplicons of ~2000 bp (Fig. 2 – samples no. 9, 17, 18, 22, 29 and 34). These were absent in rye, and as they were identified here in wheat, it is likely they originate from the wheat parent (Fig. 4 A). The 1850 bp amplicon found in the triticale samples was absent from rye and wheat, indicating this allele was not captured in the donor species samples screened in this study.

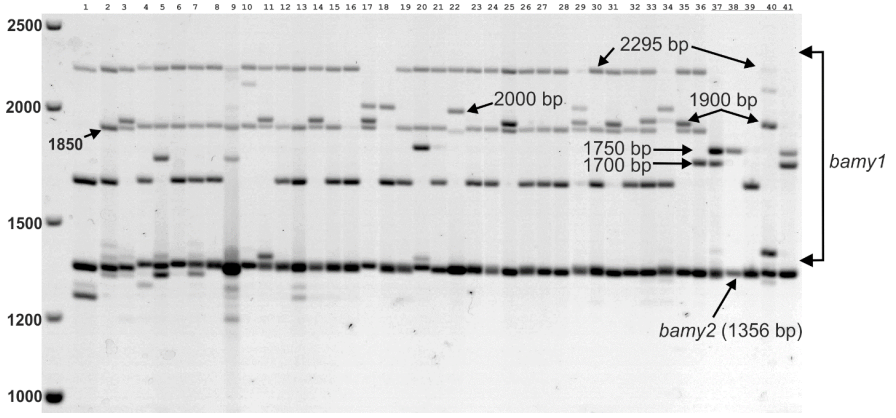

Figure 2. *Bamy1* and *Bamy2* genetic variation identified by EPIC in winter cultivars of triticale and rye. M - DNA ladder (SM1173);
1-36 cultivars of winter triticale: 1 - Benetto; 2 - NE 422T; 3 - UCRTCL1; 4 - Greneder; 5 - Plains; 6 - Terreland 22 7 - Mungis; 8 - Leontino; 9 - Constant; 10 - Trizeps; 11 - Tribeca; 12 - Dusi; 13 - Alekto; 14 - Cosinus; 15 - Kandar; 16 - Largus; 17 - Magistral; 18 - Noe; 19 - Pletomax; 20 - Tatra; 21 - Massimo; 22 - Nutriseeds 1 - 18; 23 - Bienvenu; 24 - Trismart; 25 - Trimmer; 26 - Wilfried; 27 - Pizarro; 28 - Aprim; 29 - Innoval; 30 - Amarillo 105; 31 - Blenio; 32 - Kinerit; 33 - UCRTCL3; 34 - Algoso; 35 - UCRTCL2; 36 - Flavius;
37-41 cultivars of rye: 37 - Riihi; 38 - Akusti; 39 - Iissavaara; 40 - Loppi; 41 - Hirvessalmi.

Up to eight *Bamy1* polymorphic PCR products (maximum per accession = 4) and three *Bamy2* variants (maximum per accession = 2) were detected in the samples of winter triticale analysed. For spring triticale, EPIC primers for *Bamy1* and *Bamy2* identified a total of six (maximum per accession = 3) and two amplicons (maximum per accession = 1), respectively. For triticale *Bamy1*, the frequency of the 2295 bp fragments varied with the seasonal growth habit (SGH) classification of the accessions studied: it is found in 86% of the winter cultivars, while it is present in just 48% of spring lines. The *Bamy1* 2000 bp fragment varied in frequency between SGH classes, being present in 52% of spring triticale, but found in just 13% of winter lines. Similarly, the 1900 bp *Bamy1* amplicon is found in 56% of spring triticale cultivars, and in 27% of winter lines (Fig. 2 and 3). EPIC PCR products of ~1700 bp (found in cultivars Plains, Constant and Flavius) and ~1750 bp (cultivar Tatra) were found only in winter triticale. The most common *Bamy2* variant identified in the triticale cultivars analysed was the 1356 bp fragment. For rye, six *Bamy1* (from one to three amplicons per cultivar) and two *Bamy2* (from one to two per cultivar) products were found.

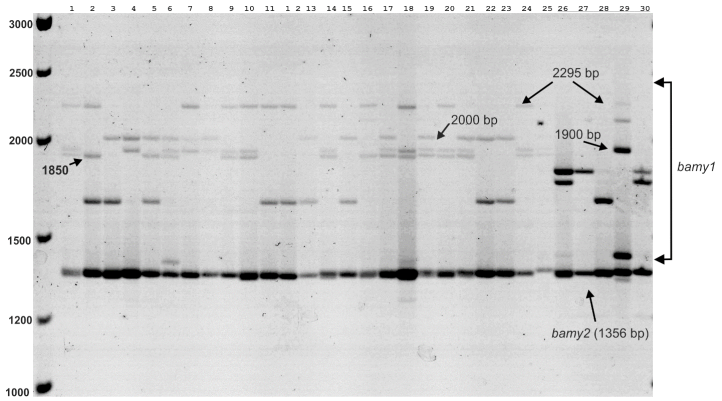

Figure 3. *Bamy1* and *Bamy2* genetic variation identified by EPIC in spring cultivars of triticale and rye.
M - DNA ladder (SM1173);
1-25 cultivars of spring triticale: 1 - Arc en Ciel; 2 - Fronteiro; 3 - Tentudia; 4 - Sierra de Almaraz; 5 - Sierra de Arroyo; 6 - Sierra de Lobos; 7 - Alter; 8 - Camarma; 9 - Cume; 10 - Curtido; 11 - Vrodi; 12 - Thisbi; 13 - Vrito; 14 - Legalo; 15 - Senatrit; 16 - Trimour; 17 - Dublet; 18 - Wanad; 19 - Gabo; 20 - Logo; 21 - Matejko; 22 - Nobi; 23 - Noe; 24 - Somtri; 25 - Sierra de Villuercas;
26-30 cultivars of rye: 26 - Riihi; 27 - Akusti; 28 - Iissavaara; 29 - Loppi; 30 - Hirvessalmi.

EPIC analysis of *β*-amylase genes from Emmer wheat revealed that *T. dicoccoides* lines commonly possess two *Bamy1* amplicons: the first (~ 1900 bp) was found to be present in all *T. dicoccoides* investigated, while amplicons of ~2000 bp and 2296 bp were observed in 79% and 16 % of samples, respectively (Fig. 4A). Among the 42 *Aegilops speltoides* accessions investigated, three and two amplicons were found for *Bamy1* and *Bamy2*, respectively. In addition to amplicons of 2295 and 1900 bp (found also in *T. turgidum*, triticale, rye and barley), products of ~2600 bp (not found in any of the other species investigated) were also identified in *A. speltoides*, where it was found in 63% of accessions (Fig. 4B). Comparison of the molecular profiles of *T. dicoccoides* and *Aegilops speltoides* highlighted the presence of common *Bamy1* amplicon sizes between these species, of 2295 and 1900 bp (Fig. 4B). The 1900 bp amplification fragment was found in 16% of *A. speltoides* accessions and in 100% of *T. dicoccoides* lines (Fig. 4A-B).

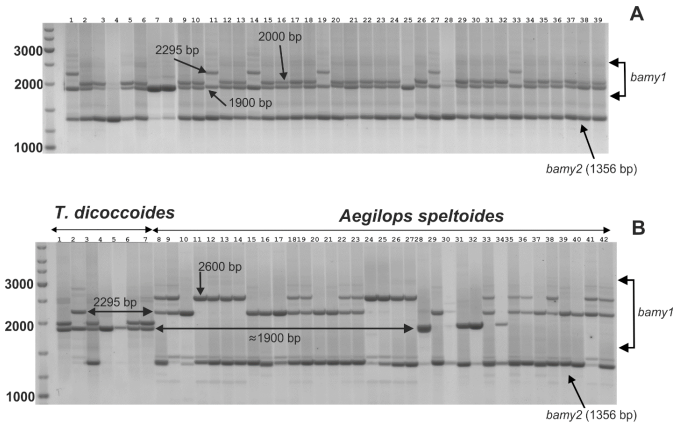

Figure 4. *Bamy1* and *Bamy2* genetic variation identified by EPIC in emmer wheat and *Aegilops speltoides* accessions. M - DNA ladder (SM1173);
A – wild wheat lines *T.turgidum* subsp. *dicoccoides*;
B – *T.dicoccoides* (1-7) and *A.speltoides* (8-38).

Conclusion

Conservation of exonic regions allowed EPIC to be used to analyse intra- and interspecific polymorphism within β-amylase genes from multiple cereal species. Here, the ability to use EPIC for the analysis of a wide taxonomic range within the *Poaceae* was demonstrated. Thus, genomic data obtained from one or more organisms can be effectively used to rapidly design EPIC-markers for additional related species. This work demonstrates how EPIC can be used to determine inter- and intra-specific genetic variation within plant taxonomic groups, and could represent a useful tool for marker-assisted selection of novel β-amylase alleles within breeding programmes.

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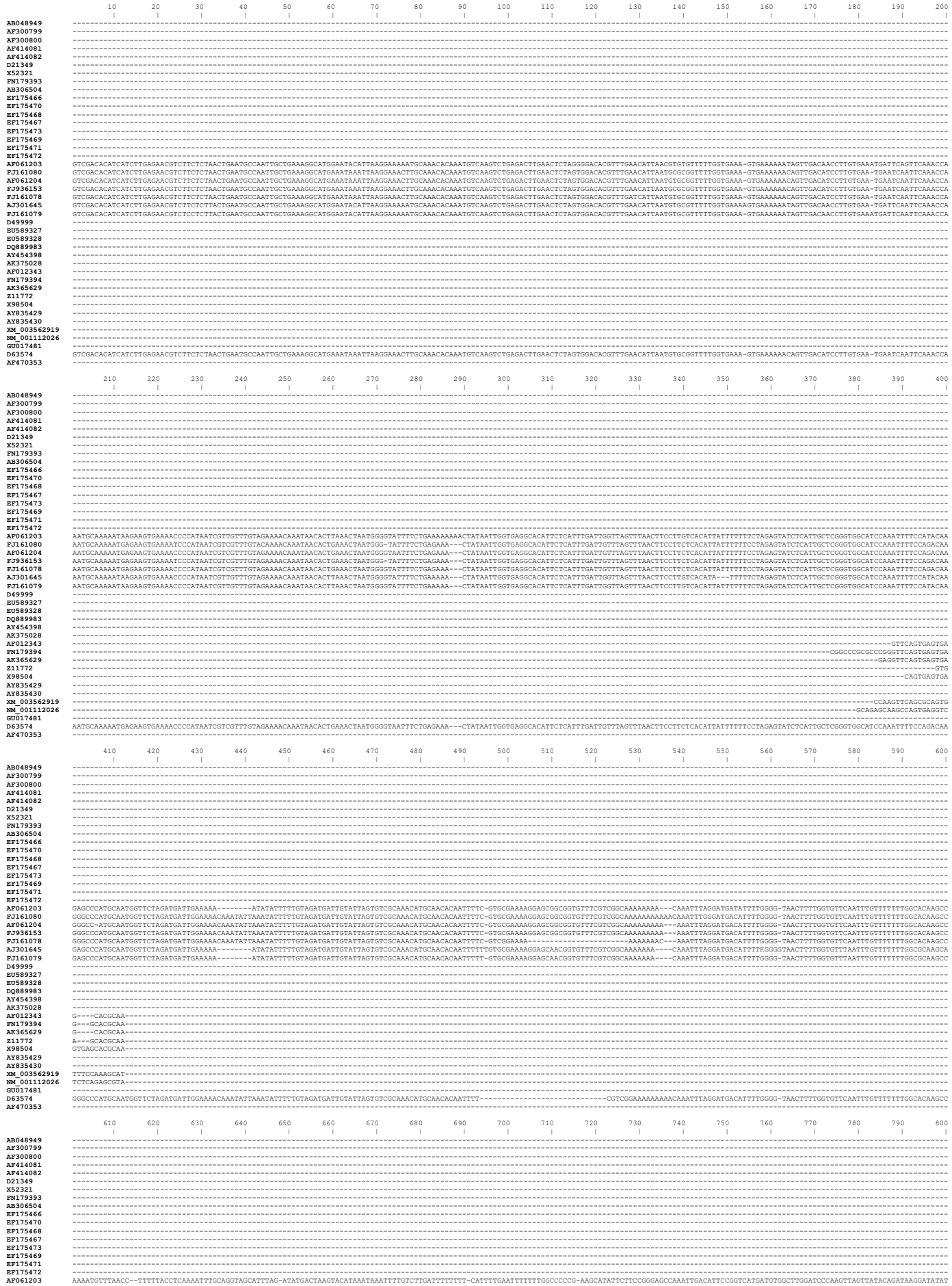
Molekularna genetička analiza gena za β-amilazu korišćenjem Exon-Primed Intron-Crossing (EPIC) PCR

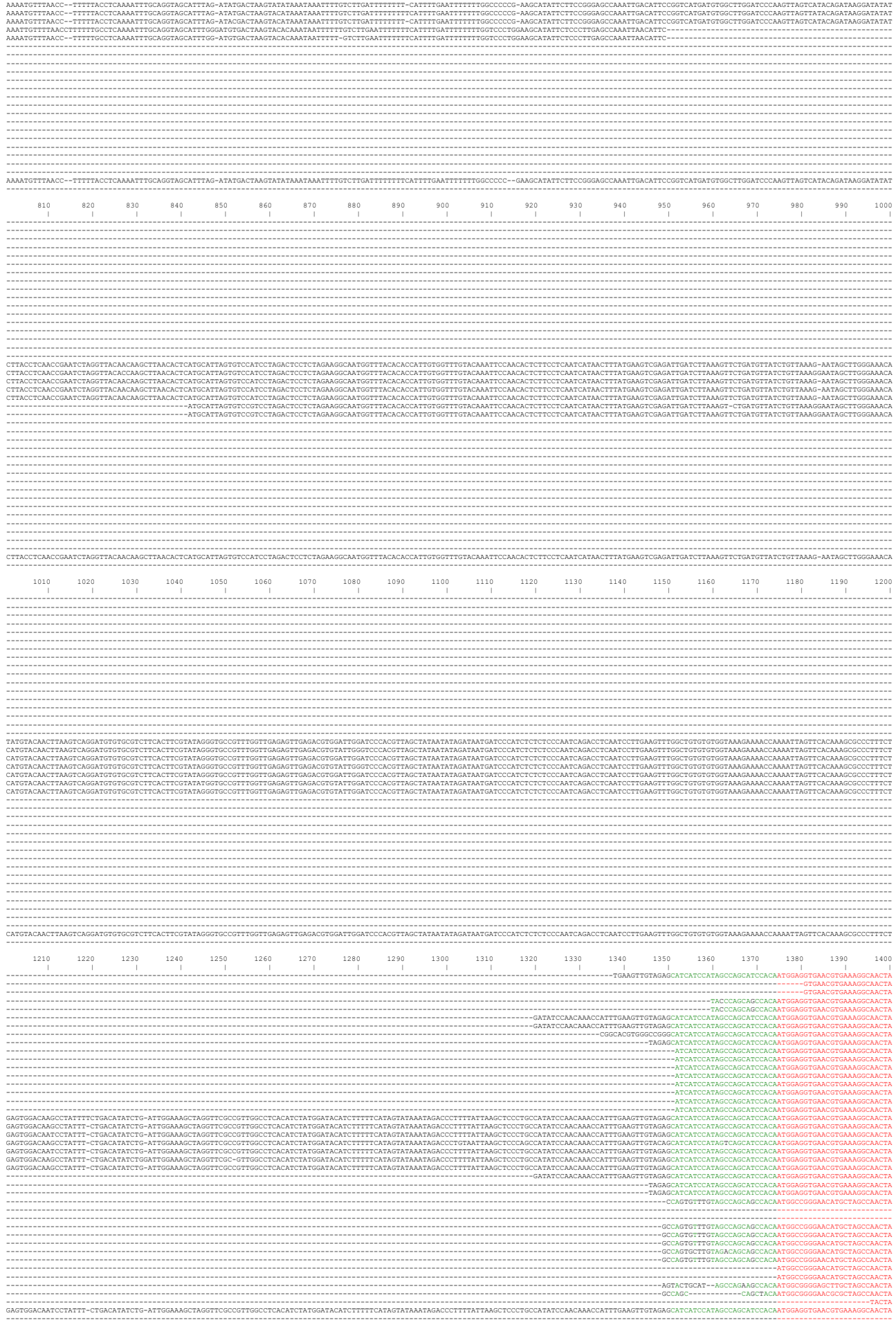
Olga Stratula · James Cockram · Ruslan Kalendar

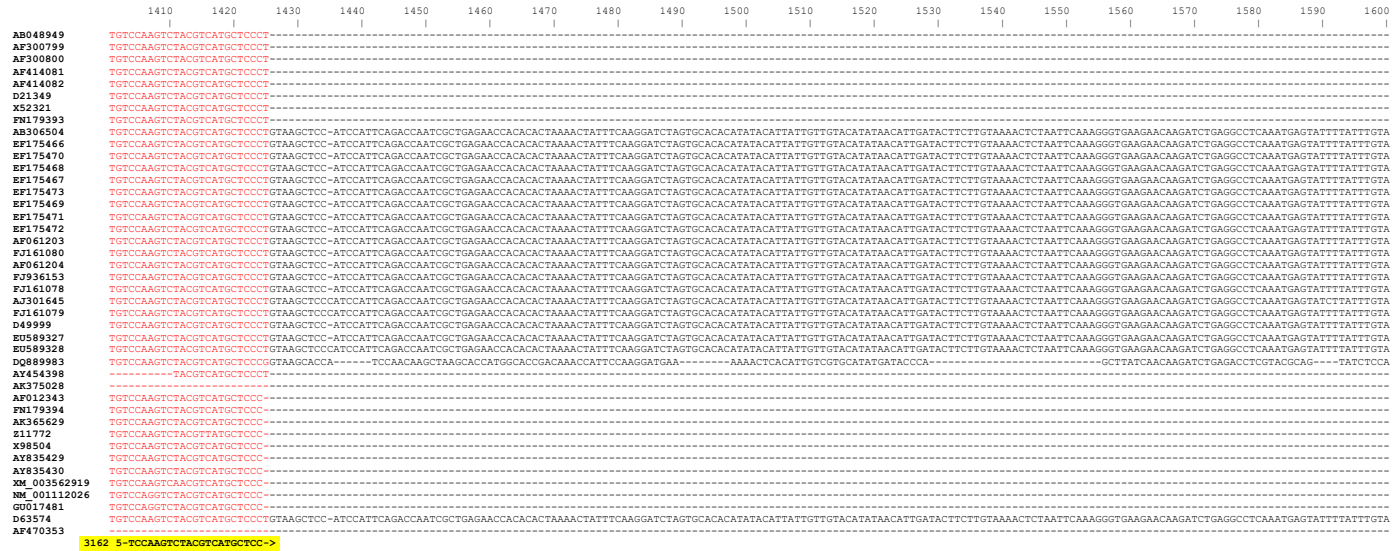
Sažetak: Proteini koje kodiraju geni za β-amilazu kod strnih žita *Bamy1* i *Bamy2*, igraju veoma značajnu ulogu u klijanju semena i procesu proizvodnje piva. U ovom radu, korišćeni su exon-primed intron-crossing (EPIC) markeri za analizu genetičke divergentnosti *Bamy1* i *Bamy2* gena između 38 genotipova, klasifikovanih u 6 tribusa familije *Poaceae*. Podudarnost DNK sekvenci za β-amilazu kod različitih *Poaceae* vrsta omogućila je dizajniranje EPIC prajmera koji istovremeno amplifikuju *Bamy1* i *Bamy2* sekvence u svim ispitivanim vrstama žitarica. Utvrđena genetička varijabilnost u ispitivanim uzorcima je analizirana i diskutovana i ilustruje efikasnost ovog pristupa za intra- i interspecies analize različitih biljnih vrsta.

Ključne reči: β-amilaza, genetska raznovrsnost, ječam, *Poaceae*, polimorfizam dužine introna, pšenica, raž

Figure S1 Multiple alignments of β-amylase genes sequences. Exons are marked in red.



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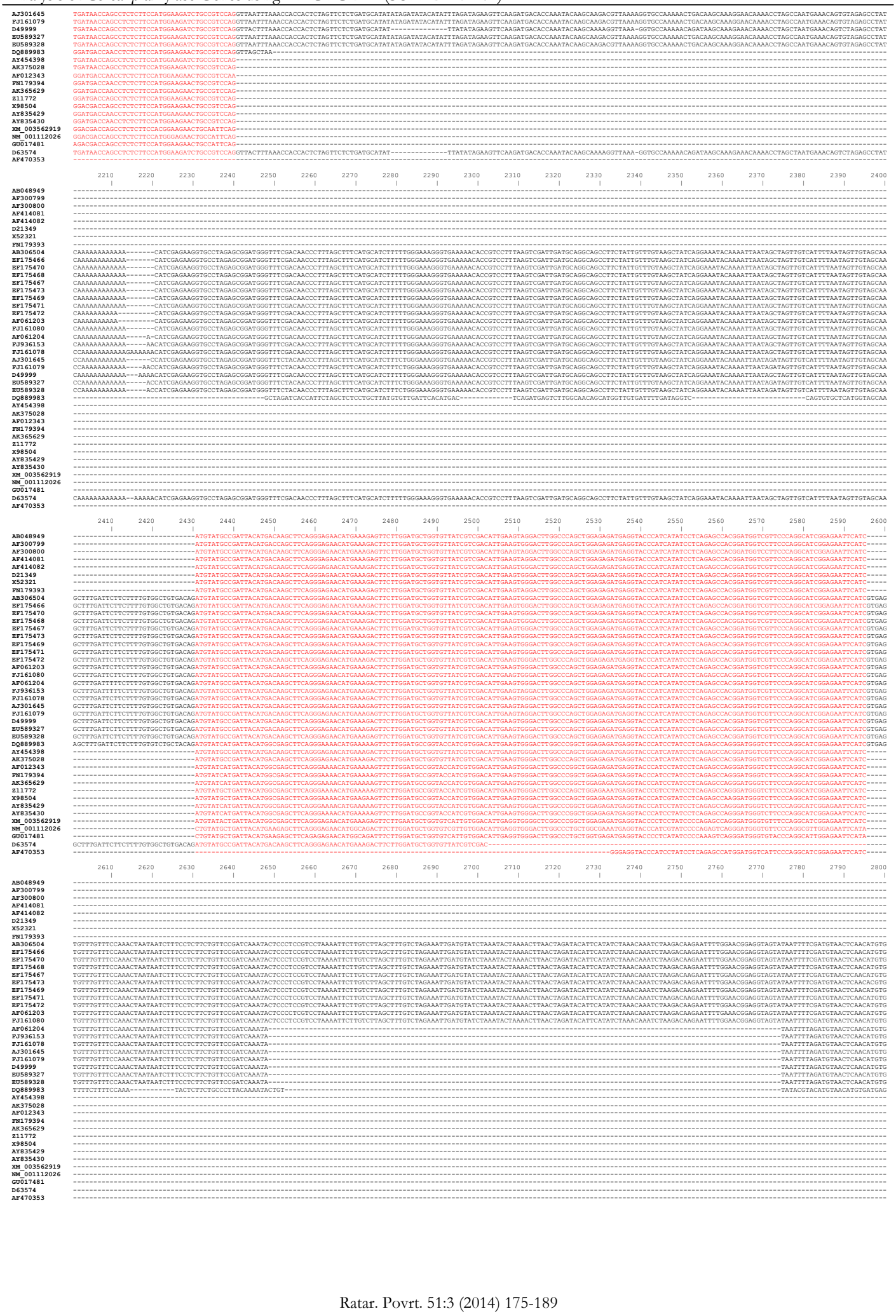

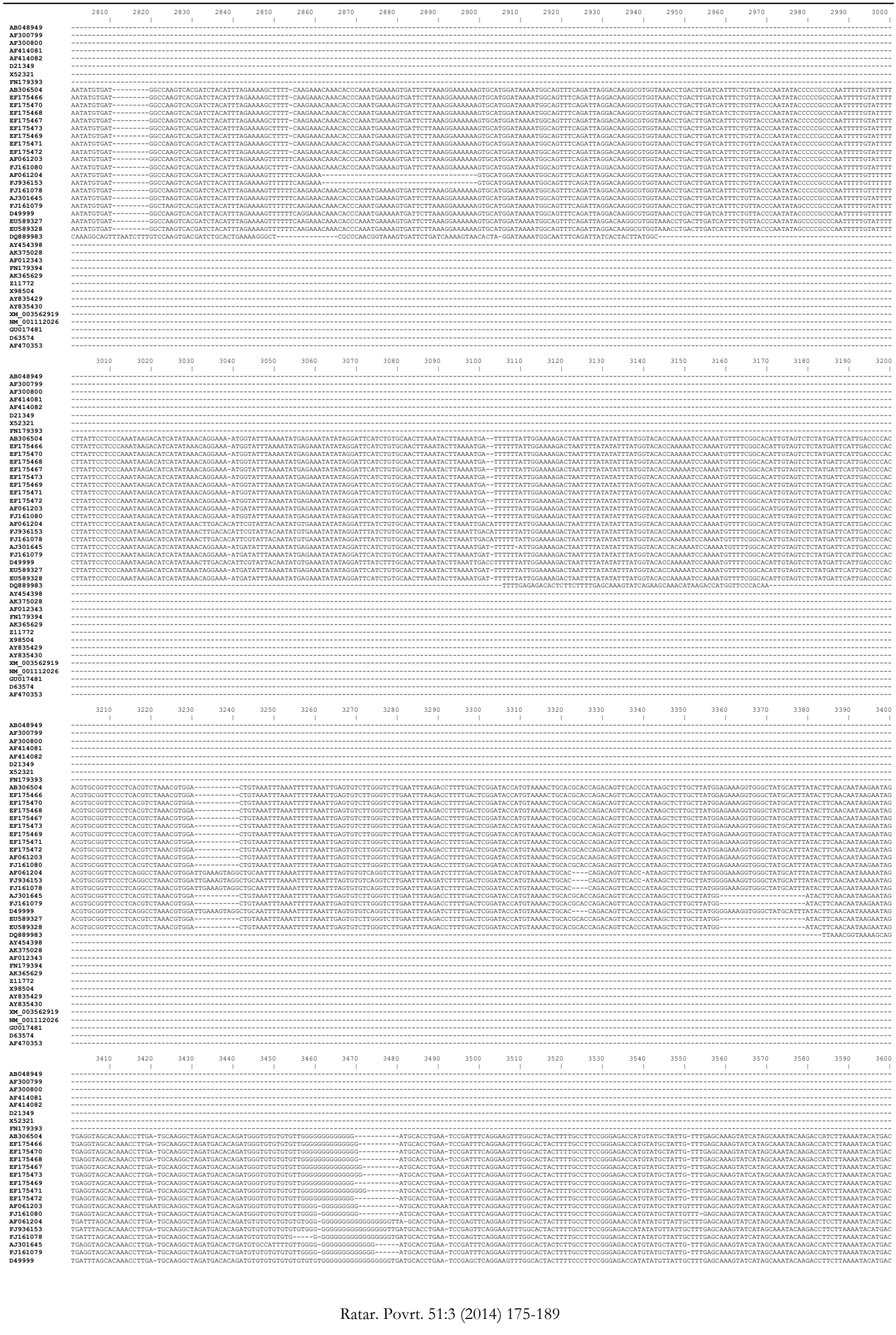

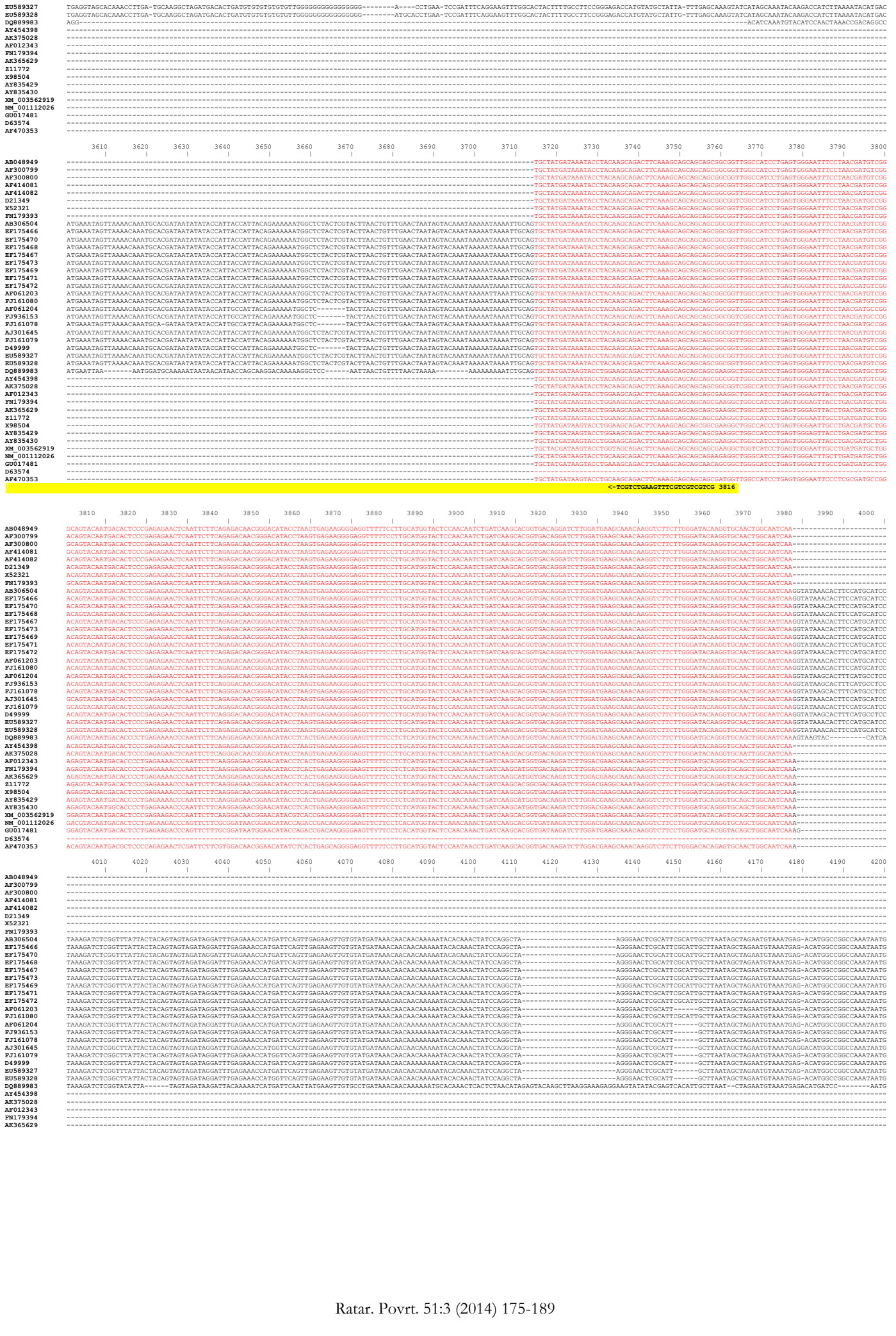
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