Ann. Bot. Fennici 52: 33–37 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 30 January 2015

Plantago schrenkii is *P. maritima*: morphological and molecular evidence

Alexey Shipunov

Department of Biology, Minot State University, Minot, North Dakota 58701, USA (dactylorhiza@gmail.com)

Received 19 July 2014, final version received 5 Sep. 2014, accepted 22 Oct. 2014

Shipunov, A. 2015: *Plantago schrenkii* is *P. maritima*: morphological and molecular evidence. — *Ann. Bot. Fennici* 52: 33–37.

Plantago schrenkii (dwarf plantain) from the Russian Arctic is frequently segregated at species level from *P. maritima* (sea plantain). Using simultaneous morphometric and molecular analyses, I came to the conclusion that *P. schrenkii* is just an ecological form of sea plantain and should not be recognized at any taxonomical level.

Introduction

Plantago maritima (sea plantain) is a highly variable species with a very large geographic distribution. The variability led to the segregation of multiple "species" such as *P. juncoides*, *P. decipiens*, *P. borealis*, *P. groenlandica* and others (Pilger 1937), which were subsequently included in *P. maritima* (Rahn 1996).

Arctic forms of *P. maritima* were often regarded as separate species. One example is *P. schrenkii* (dwarf plantain), first collected in 1837 from Waigatsch island (Russian Arctic). It was believed to have clear morphological differences from typical *P. maritima*, especially the small size of leaves and inflorescences.

Shipunov (2000), in the review of *Plantago* from European Russia, accepted *P. schrenkii* based on results of the statistical analysis first published in my dissertation (Shipunov 1998). However, that conclusion did not seem to be robust, and I always felt that the problem of *P. schrenkii* must be re-assessed for several reasons. First, characters which discriminate *P. schrenkii* are highly polymorphic and are often seen in *P. maritima* plants growing in stressful ecological conditions (for example, on poor substrates such as seaside rocks). In addition, it is well-known from the classic works of Gregor (1938, 1950) that typical *P. maritima* populations often include some dwarfish plants. In my dissertation (Shipunov 1998), I used morphometric data from fewer than 40 *P. schrenkii* plants, and the separation between them and typical *P. maritima* was not absolutely clear. Last, there was no possibility for a molecular analysis. Therefore, I decided to review the problem based on better sampling and analysis of relevant molecular markers.

Material and methods

All material was collected from the White Sea coast, near the Arctic circle. This area is well-known for the co-occurrence of *P. maritima* and *P. schrenkii* (Andreev 1930, Sergienko 1977). Typically, the latter could be found in the locations where rocky habitats are prevalent. A total of 394 plants were measured in order to obtain morphometric data. I collected 15 silica gel dried plant tissue samples from one measured plant per locality (28 localities in total) in order to extract and sequence the DNA.

The main characters measured were (1) length of lower photosynthetic leaf (abbreviation: LOWER), (2) length of leaf closest to the stalk (UPPER), (3) length of maximal stalk (STALK), (4) length of spike on the maximal stalk (SPIKE), and (5) total number of flowers on the measured spike (FLOWER). All these characters were believed to distinguish *P. maritima* from *P. schrenkii* (Shipunov 2000). In addition, distance to the nearest plantain, substrate type (reflecting the "hardiness" of substrate) and the distance from seashore were registered as "ecological variables". All metric measurements were in millimeters.

Statistical methods included principal component analysis (PCA) with ANOSIM testing on the cluster differences (Oksanen *et al*. 2013) and Spearman's correlation. For all statistic calculations, the R package (R Core Team 2014) was used.

The "logical discrimination" was applied to the collected data. Dichotomous identification keys allow for the step-by-step determination based on the series of negations and confirmations; this method was made algorithmic in R. For example, *P. schrenkii* characters were encoded as "(FLOWER < 10) & (SPIKE < 140) & (UPPER < 90)". I used this approach for the discrimination of samples in both morphometric and molecular parts of the study.

The DNA was extracted using a MO BIO PowerPlant DNA Isolation Kit (MO BIO Laboratories, Carlsbad, California, USA). Dry plant leaf material (~0.1 g) was ground using a sterile mortar and pestle and then processed in accordance with the supplied protocol. We sequenced the ITS2 region of the ribosomal intergeneric spacer, which has proven to be best for revealing species-level phylogenetic relationships among *Plantago* (Ronsted *et al*. 2002, Hoggard *et al*. 2003) using the primers ITS2_S2F (Chen *et al*. 2010) and ITS4 (White *et al*. 1990). PCR was carried out as follows: the reaction mixture in a total volume of 20 μl contained 5.2 μl of PCR Master Mix (components from QIAGEN Corporation, Germantown, Maryland), 1 μl of 10 μM forward and reverse primers, 1 μl of DNA solution from the extraction above and 11.8 μl purified water. Samples were incubated in a thermal cycler: 94° for 5 min, then 35 cycles of 94° for 1 min; 51° for 1 min, 72° for 2 min, and finally 72° for 10 min. Single band PCR products were sent for purification and sequencing to Functional Biosciences, Inc. (Madison, Wyoming) and sequenced there in accordance with standard protocol. Sequences were then assembled and edited using Sequencher™ 4.5 (Genes Codes Corporation, Ann Arbor, Michigan, USA) and then aligned with ClustalX (Thompson *et al*. 1997) using gap opening cost 9, gap extension cost 0.05 and IUB weight matrix.

Part of the DNA was sequenced with help of the Barcoding of Life Consortium, as a part of "SAPNA" ("*Plantago* of North America") project.Other barcoding fragments (for example, *rbc*L and *mat*K) were also sequenced within this project but ITS2 provided the best resolution.

Results

Morphological analysis

Logical discrimination resulted in two groups: putative *P. schrenkii* and more typical *P. maritima*. Among the DNA samples, five appeared to belong to the first group. Among all samples, 68 (17%) were determined as *P. schrenkii*.

The difference between these two groups was assessed using principal component analysis of morphological characters. The two first principal components combined for 85% of variation. However, those components were unable to separate *P. schrenkii* (Fig. 1). Analysis of (dis) similarity between clusters showed low values: ANOSIM $R = 0.05284$, $p = 0.06$.

Correlation between morphological characters and ecological variables was especially high for the UPPER and FLOWER: they correlated with the type of substrate (Spearman's $\rho = -0.4765257$, $p < 0.0001$) and distance from seashore (Spearman's $\rho = 0.7350455$, $p < 0.0001$), respectively. In other words, on more stressful substrates the leaves become shorter, and the number of flowers is higher on the plants growing farther from seashore.

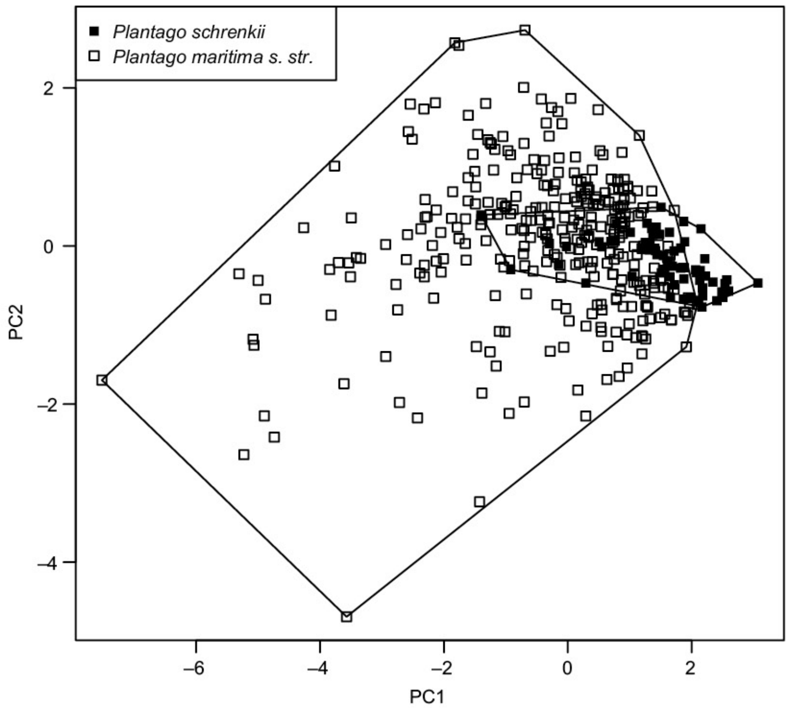

Fig. 1. Principal component analysis of morphological characters. Ordination hulls emphasize the intersection between groups.

Molecular analysis

Sequences of the whole ITS2 region had a length of 299–320 base pairs. Their alignment started from the 413 position of the full ITS1/5.8S/ITS2 region (*P. maritima*, GenBank AY101879) reflecting the absence of significant differences between two groups (*see* Appendix).

Discussion

Several authors have accepted *P. schrenkii* as a separate species (Andreev 1930, Sergienko 1977, Tzvelev 1979, Shipunov 2000). However, there is a clear trend in publications toward a broader concept of *P. maritima*, which now includes numerous previously distinguished forms (Kuiper & Boss 1992, Rahn 1996). The present study on *P. schrenkii* supports the broader concept. The dwarf Arctic plants should not be recognized at any taxonomical level, but represent just ecological modifications or forms.

The more is known about the genetic and ecological polymorphism in *Plantago*, the more specialists shift to wider taxonomic concepts at species level, not only in the *P. maritima* group but also in other widely distributed, frequently invasive plants such as *P. major*, *P. media*, *P. lanceolata* and *P. coronopus* (Rahn 1996, Shipunov 2000). It is likely that many of those taxa have not yet reached the stage of stabilization (Grant 1981) and "are caught" in the intermediate stage of the speciation process.

Acknowledgements

I am grateful to all participants and heads of the Moscow South-West High School White Sea Expedition for their support, and especially to P. Borisova, D. Zakharova, K. Aquilar and M. Kuzmina for their invaluable help. Many thanks also to the anonymous reviewer for the constructive comments.

References

Andreev V.N. [Андреев В.Н.] 1930: [Species of *Plantago* of the Arctic coast of Europe]. — *Zhurnal Russkogo Botanicheckogo Obschestva* 15: 291–304. [In Russian].

Chen S., Yao H., Han J., Liu C., Song J., Shi L., Zhu Y., Ma X., Gao T., Pang X., Luo K., Li Y., Li X., Jia X., Lin Y. & Leonand Ch. 2010: Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. — *PLoS ONE* 5: e8613, doi:10.1371/journal.pone.0008613.

Cho Y., Mower J.P., Qiu Y.L. & Palmer J.D. 2004: Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. — *Proceedings of the National Academy of Sciences of the United States of America* 101: 17741–17746.

Grant V. 1981: *Plant speciation*. — Columbia University Press, New York.

Gregor J.W. 1938: Experimental taxonomy II. Initial population differentiation in *Plantago maritima* L. of Britain. — *New Phytologist* 37: 15–49.

Gregor J.W. & Lang J.M.S. 1950: Intra-colonial variation in plant size and habit in *Plantago maritima*. — *New Phytologist* 49: 135–141.

Hoggard R.K., Kores P.J., Molvray M., Hoggard G.D. & Broughton D.A. 2003: Molecular systematics and biogeography of the amphibious genus *Littorella* (Plantaginaceae). — *American Journal of Botany* 90: 429–435.

Kuiper P.J.C. & Bos M. (eds.) 1992: *Plantago: a multidisciplinary study*. — Springer Verlag, Berlin.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H. & Wagnerand H. 2013: *Vegan: community ecology package*. — R package ver. 2.0-10, available at http://CRAN.R-project.org/package=vegan.

Pilger R. 1937: *Plantaginaceae*. — Verlag von Wilhelm Engelmann, Leipzig.

R Core Team 2014: *R: A language and environment for statistical computing*. — R Foundation for Statistical Computing, Vienna, Austria, available at http://www.R-project.org/.

Rahn K. 1996: A phylogenetic study of the Plantaginaceae. — *Botanical Journal of Linnean Society* 120: 145–198.

Ronsted N., Chase M.W., Albach D.A. & Bello M.A. 2002: Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid *trn*L-F sequence data. — *Botanical Journal of the Linnean Society* 139: 323–338.

Sergienko L.A. [Сергиенко Л.А.] 1977: [Notes about maritime species of genus *Plantago* L. on the north of European part of USSR]. — *Novosti Sistematiki Vysshikh Rastenij* 14: 209–216. [In Russian].

Shipunov A.B. [Шипунов А.Б.] 1998: [*The genera Plantago L. and Psyllium Mill. (Plantaginaceae Juss.) in the flora of European Russia and neighboring territories*].— Ph.D. thesis, Moscow State University, Moscow. [In Russian].

Shipunov A.B. [Шипунов А.Б.] 2000: [The genera *Plantago* L. and *Psyllium* Mill. (Plantaginaceae Juss.) in the flora of East Europe]. — *Novosti Sistematiki Vysshikh Rastenij* 32: 139–152. [In Russian].

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. & Higgins D.G. 1997: The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. — *Nucleic Acids Research* 25: 4876–4882.

Tsvelev N.N. [Цвелев Н.Н.] 1979: [Notes about plantain genus (*Plantago* L.) in European part of USSR]. — *Novosti Sistematiki Vysshikh Rastenij* 16: 169–175. [In Russian].

White T.J., Bruns T., Lee S. & Taylor J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. — In: Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds.), *PCR Protocols: a guide to methods and applications*: 315–322. Academic Press, New York.

Appendix. Alignment of ITS2 fragment sequences. Plants determined as *Plantago schrenkii* marked with "_s" postfix. First three sequences are from GenBank: AY101879 and HQ593837 were obtained from cultivated plants, geographic origin of AJ548986 is "North America" (Hoggard *et al.* 2003).

	·414 ·416 ·418 ·420 ·422 ·424 ·426 ·428 ·430 ·432 ·434 ·436 ·438 ·440 ·442 ·444 ·446 ·448 ·450 ·452 ·454 ·456 ·458 ·460 ·462 ·464 ·466 ·468 ·470 ·472 ·474 ·476 ·478 ·480 ·482 ·484 ·486 ·488 ·490 ·492 ·494 ·496 ·498 ·500 ·502 ·504 ·506	
AY101879_P_maritima	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	507
AJ548986_P_maritima	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
HQ593837_P_maritima	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p01_s	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p02	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p03_s	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p04_s	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p05	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p06_s	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p08	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p09	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p10	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p11	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p12_s	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p13	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95
p14	TGCAGAATCCCGTGAACCATCGAATCTTTGAACGCAAGTTGCGCCCGACGCCTTCGGGCTGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTC	95

	·508 ·510 ·512 ·514 ·516 ·518 ·520 ·522 ·524 ·526 ·528 ·530 ·532 ·534 ·536 ·538 ·540 ·542 ·544 ·546 ·548 ·550 ·552 ·554 ·556 ·558 ·560 ·562 ·564 ·566 ·568 ·570 ·572 ·574 ·576 ·578 ·580 ·582 ·584 ·586 ·588 ·590 ·592 ·594 ·596 ·598 ·600 ·602	
AY101879_P_maritima	GCCCCCTCCCAGTCCCTTGCGGATAGGTGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	602
AJ548986_P_maritima	GCCCCCTCCCAGTCCCTTGCGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
HQ593837_P_maritima	GCCCCCTCCCAGTCCCTTGCGGATAGGYGAYGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p01_s	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p02	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p03_s	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p04_s	GCCCCCTCCCAGTCCCTTGYGGATAGGYGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p05	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p06_s	GCCCCCTCCCAGTCCCTTGYGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p08	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p09	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p10	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p11	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p12_s	GCCCCCTCCCAGTCCCTTGYGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p13	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190
p14	GCCCCCTCCCAGTCCCTTGTGGATAGGCGATGGGGCGGAAAATGGCCTCCCGTTAGCTCGGTTGGCCCAAATGGGATCCCTCATCGACGGATGTC	190

	·604 ·606 ·608 ·610 ·612 ·614 ·616 ·618 ·620 ·622 ·624 ·626 ·628 ·630 ·632 ·634 ·636 ·638 ·640 ·642 ·644 ·646 ·648 ·650 ·652 ·654 ·656 ·658 ·660 ·662 ·664 ·666 ·668 ·670 ·672 ·674 ·676 ·678 ·680 ·682 ·684 ·686 ·688 ·690 ·692 ·694 ·696	
AY101879_P_maritima	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	697
AJ548986_P_maritima	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
HQ593837_P_maritima	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGYTGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p01_s	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p02	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p03_s	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p04_s	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p05	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p06_s	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p08	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p09	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p10	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p11	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p12_s	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p13	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285
p14	ACAACCAGTGGTGGTTGAAAGATCATTTGTGCTGTTGTGCTTCACGCTGTCGCTTGTGGTGGATCGCTCGGAACCAACGGCGTGTCATGCGCCTT	285