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Species-origin identification of the deer in the Purtier Placenta Live Stem Cell Therapy by DNA typing

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ABSTRACT

Purtier Placenta Live Stem Cell Therapy is a food supplement by Riway Singapore Pte. Ltd., claimed to contain living deer placenta stem cells together with 11 other active ingredients. It is also promoted as maintaining health and youthfulness and as being supported by numerous patents and research trials. Individual ingredients are accompanied by many other statements implying that Purtier Placenta can alleviate or cure diseases such as diabetes, cancer, and neurodegenerative diseases. However, none of these claims is traceable in clinical trial registries, and the mechanism allowing stem cells to be alive in the capsule and be effectively delivered to the body *via* the digestive tract does not seem plausible. Consequently, authorities in the United Arab Emirates, the Philippines, and Singapore have issued public warnings or imposed penalties in relation to misleading claims. Nevertheless, the product has gained popularity among Czech oncological patients, who perceive it as a magic cure. This paper describes a three-stage testing process designed to verify the species origin of the declared main ingredient. Using barcoding primer mixes, melting analysis, and Sanger sequencing with species-specific primers against seven reference species, we tested whether the capsule content corresponded to the red deer (*Cervus elaphus)* depicted on the official website (https://www.officialpurtier.com). Our analysis identified that DNA extracted from the 6th Edition capsule was primarily *Rusa unicolor/timorensis*, with minor components of *Dama dama* and *Cervus elaphus*. Although the main component was confirmed to be of deer origin, the lack of specific information and the wording of the marketing materials may be misleading.

1. Introduction

Placental extracts from various species (human, pig, deer, etc.) have been used in traditional medicine across many cultures for centuries [1]. The first written record of medicinal use of human placenta in Traditional Chinese Medicine (TCM) dates back to 1593, in the Compendium of Materia Medica by Li Shizhen [2]. The modern use of amniotic membranes as skin grafts dates back to 1910 [2], while placental extracts emerged during the 1930s in the form of Filatov's tissue therapy [1].

Human placental extract has been successfully used in modern-day clinical trials in ophthalmology [3], wound healing [4], management of the menstrual cycle and menopausal symptoms [1], reduction of chronic fatigue and vitality improvement, etc. However, the mechanisms of action are mostly unclear. Porcine, bovine, equine, caprine, or ovine placental extracts and amniotic membranes are also tested for their beneficial effects on wound healing and skin condition [2,5], liver regeneration [1], fatigue reduction, immune enhancement [5], etc.

Deer placenta is an important ingredient in TCM, centred mostly on symptoms connected to low energy and fatigue, insufficient immunity, and kidney problems [6]. Modern research highlights the potential of deer placenta for treating chemotherapy-induced hair loss [7], anti-ageing properties, induction of skin fibroblast proliferation [1,7], fatigue reduction, and anti-inflammatory effects [1].

The combination of emerging modern research and historical experience with traditional medicine has led to a growing interest in placental therapy among patients with a wide range of diagnoses. Consequently, the number of dietary supplement producers is increasing, operating within the knowledge gap between mechanisms of action and clinical effects and in grey areas by non-standardised

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extraction methodologies, unclear production, costs, and insufficient regulation [8].

Purtier Placenta Live Stem Cell Therapy 6th Edition is a food supplement produced by Riway Singapore Pte. Ltd. In 2022, the first European branch of Riway was opened in Prague, marking its spread among Czech customers, mainly oncological patients (https://web.riway.com/riway_news/riway-europe-branch-opening/). On the manufacturer's webpage (https://officialpurtier.com/home, accessed 14th April 2026), the first listed ingredient is 100 mg of a deer placenta, accompanied by an image of red deer (*Cervus elaphus*). Other ingredients are 100 mg of NucleiClavem™, 50 mg of lycopene oil, 50 mg of fucoidan extract, 100 mg of marine collagen peptides, 100 mg of squalene in the form of shark liver extract, 100 mg of evening primrose oil, 200 mg of borage oil, 10 mg of Aloe vera, 100 mg of avocado oil, 20 mg of dendrobium, and 20 mg of apple polyphenol. The species origin of many ingredients is not specified and can only be inferred from accompanying images. The use of *Cervus elaphus* imagery leads potential customers to reasonably believe that the supplements contains this ingredient. Even though Purtier Placenta claims no connection to TCM, the *Cervus elaphus* can still be considered highly-valued ingredient, as the deer material in TCM must be sourced exclusively from *Cervus elaphus* and *Cervus nippon* [9,10].

According to the manufacturer's website listed above, the capsule is "Utilising the restorative powers of Deer Placenta and active live cells through live-cell therapy …" and the NucleiClavem™ ingredient is "… backed by close to 10 million research trials …". No clinical trial supporting this product-specific claims was found in clinicaltrials.gov or clinicaltrialsregister.eu.

The biological mechanism through which an oral supplement could effectively deliver live stem cells to the body seems implausible. Moreover, a study by Santos et al. [11] found no evidence of stem cells in Purtier capsules.

Several websites can be perceived as manufacturers' official materials (www.officialpurtier.com, www.purtier.com, www.riway.live, www.riway.com, www.stemcellincapsule.com), all of which use slightly different wording. At the moment, the site www.officialpurtier.com cites that Purtier Placenta 6th Edition "maintains good health and youthfulness" and "boosts the growth and function of existing body tissues, [while] the live cells also activate dormant cells in the body and create new synapses and connective tissues". On the same page, the individual ingredients are listed as having anti-inflammatory and antioxidant effects, delaying ageing, boosting the immune system, inducing apoptosis in cancer cells, preventing diabetes, maintaining cholesterol levels, lowering blood pressure, improving blood circulation, improving digestive health, and lubricating joints. Testimonials provided by the manufacturer include patients with epidermolysis bullosa, gout, arthritic conditions, and psoriasis. It is also worth noting that Riway's marketing language changes dynamically. Some claims cited in various sources may therefore no longer appear on the webpage. The webpage, as accessed on the 14th April 2026, is available in the Supplementary materials.

During manuscript preparation, a new edition of Purtier Placenta was issued with a similar but expanded ingredient list. The www.stemcellincapsule.com webpage, concerning the Purtier Placenta 7th Edition, claims that the deer placenta has "the powerful anti-inflammatory effect". The www.riway.live webpage (also concerning the Purtier Placenta 7th Edition, automatically translated to English from Chinese by Google Translate) further expands this wording with claims that the "powerful anti-inflammatory effects [of deer placenta] help regenerate degenerated cells [and] promote cell regeneration", that the included fucoidan extracts "can induce apoptosis in cancer cells within 24 h" and that the processes started by capsule intake "naturally resolves many health issues". Again, no clinical trials supporting any of these claims were identified in the aforementioned registers.

In 2018, the Food and Drug Administration of the Philippines and the United Arab Emirates Ministry of Health and Prevention issued a public warnings against Purtier Placenta, describing the manufacturer's claims as unapproved and misleading [12,13]. The UAE Ministry further warned that the supplement could pose a high health risk.

On similar grounds, especially marketing claims that the product has an "anti-tumour" or "anti-cancer effect" and "can cure diabetes" [14], the Health Sciences Authority (HSA) in Singapore investigated the product in 2019. In 2021, Riway was convicted of making false claims in court and fined 3 000 SGD, the sum equal or less than the sum paid by a single customer.

In this paper, we set aside the question of whether placental stem cells could remain alive in the capsule, pass from the intestine to the bloodstream, and provide health benefits. Instead, we focused solely on determining the species origin of the declared main component, specifically whether the material is of deer origin, as implied by the marketing imagery. This information may help potential customers to better evaluate the vague or incomplete marketing claims.

Principles of species authentication in forensic food and drug safety centre on detecting economically motivated adulteration, namely the substitution of high-value ingredients with cheaper, undesirable, or illicit alternatives) [15–17], and on detecting falsifications of traditional medicine or pharmaceutical products [6,9,10,18,19]. DNA-based molecular techniques, especially DNA barcoding using mitochondrial markers such as *COX1*, *Cytb*, and 12S and 16S rRNA, are considered the gold standard in these fields [9,10,20,21]. This study used the mitochondrial DNA markers listed above in a three-staged experimental design combining qPCR, melting analysis, reference sample comparison, and Sanger sequencing.

2. Methods

2.1. Samples

The main testing material was DNA isolated from a pill labelled "Purtier Placenta Live Stem Cell Therapy, 6th Edition" (batch number: AL46242, manufactured 03/22, expiry date 03/25; DNA extraction was performed before this date), produced by Riway Singapore Pte. Ltd. The reference samples used in the study are listed in Table 1.

All biological material had been previously stored by the provider or was obtained during a veterinary procedure or euthanasia planned for reasons unrelated to this study. No animal was harmed for the purpose of obtaining the reference sample.

2.2. DNA extraction

The DNA from the Purtier Placenta pill was extracted using the QIAamp DNA Investigator Kit (Qiagen) according to the "Isolation of total DNA from small volumes of blood and saliva" protocol (QIAamp DNA Investigator Handbook, January 2020), with the following modifications:

A total of 800 µL of pill content was transferred by a sterile syringe

Table 1
List of reference samples and their source.

Species	Common name	Tissue	Source
Cervus elaphus	Red deer	Muscle	Commercial meat market
Dama dama	European fallow deer	Muscle	Commercial meat market
Dama mesopotamica	Persian fallow deer	Buccal swab	ZOO Olomouc
Cervus nippon pseudoaxis	Sika deer	Blood smear	ZOO Olomouc
Rangifer tarandus fennicus	Reindeer	Blood	ZOO Praha
Rusa timorensis	Rusa deer	Blood	ZOO Praha
Rusa alfredi	Philippine spotted deer	Blood	ZOO Ostrava

into a low-binding tube with 80 µL of proteinase K and 800 µL of AL buffer containing 2 µL of dissolved RNA carrier. After incubation for 10 min at 56 °C, another 40 µL of proteinase K was added, and the sample was incubated at 56 °C and 800 rpm overnight.

After overnight incubation, four layers formed: an oil layer, a viscous solution, an aqueous solution, and solid sediment (from top to bottom). A further 40 µL of proteinase K was added, the sample was incubated for 10 min at 70 °C and 800 rpm, and then centrifuged at 16,000*g* for 2 min. The aforementioned layers were divided into three tubes (oil, aqueous solution, and viscous solution with sediment). The aqueous layer was selected for subsequent extraction because it was considered the most likely to contain free DNA molecules. From an overall volume of approximately 660 µL of aqueous solution, 210 µL was used for extraction. The remainder was stored at −20 °C and used for subsequent extractions.

The subsequent procedures were performed exactly as described in the Qiagen protocol from step 7 onward, with an elution volume of 50 µL ATE buffer. The obtained DNA was subsequently purified using the MinElute PCR Purification Kit (Qiagen). The final concentration of extracted DNA ranged from 17.6 ng/µL to 2.4 ng/µL after prolonged storage of intermediate extraction products, as measured using the Qubit dsDNA High Sensitivity Assay (Invitrogen).

DNA from reference samples was extracted using the cobas DNA Sample Preparation Kit (Roche), without the deparaffinisation step for meat samples; using the QiAamp DNA Investigator Kit (Qiagen), according to the "Isolation of Total DNA from Surface and Buccal Swabs" protocol (QIAamp DNA Investigator Handbook, January 2020), for buccal-swab samples; and using the MagCore Genomic DNA Large Volume Whole Blood Kit 104 (RBC Bioscience) for the whole blood and blood-smears samples. Blood samples were diluted with PBS because of their high viscosity.

2.3. Experiment design

The experimental design is shown schematically in Fig. 1.

2.4. Primer design

Most primers were selected from the cited literature [10,22–27]; in-house primers were designed using Primer3 https://primer3.ut.ee/. From 18 universal and 9 species-specific primer pairs, 5 and 5 pairs, respectively, were selected after pilot screening with reference samples for testing with the pill DNA, based on reproducibility and the difference in Ct values between positive and non-template controls. The sequences of the final primer sets are listed in Table 2.

2.5. PCR assays

All qPCR assays were performed on the LightCycler 480 instrument in the touchdown mode (in which the annealing temperature is set above the primer melting point and gradually decreased during the initial cycles to the optimal annealing temperature to suppress nonspecific binding) and using GoTaq G2 Flexi polymerase (Promega). PCR conditions for the first stage of analysis are described in Table 3. Primer mix (PM) was prepared by diluting 1 µL of forward primer and 1 µL of reverse primer (both 0.1 mM stock concentration) in 18 µL of water.

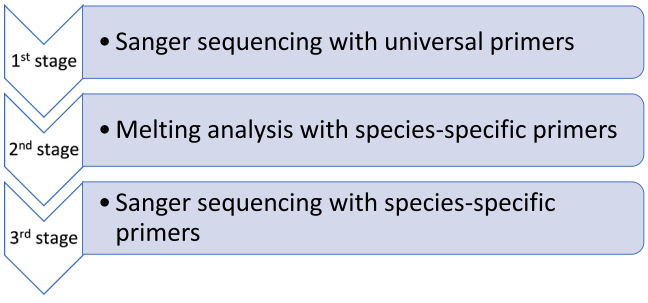

Fig. 1. Staged experimental design overview.

Cervus elaphus DNA and water were used as a reference standard and a non-template control (NTC), respectively.

For the second stage of analysis, species-specific touchdown qPCR reactions were performed as described in Table 3A, with the following changes: final polymerase concentration 0.05 U/µL, final primer concentration 58 nM, DNA input of 1 ng, and reaction volume of 15 µL. The PCR protocol is described in Table 4. The full set of seven reference samples (see Table 1) and water were used as controls. The entire detection panel was repeated (n = 5) to ensure consistent amplification patterns for species differentiation. Melting analysis with pill DNA was performed in duplicate in two separate runs.

LightCycler 480 software 1.5.1.62 SP3 was used to evaluate qPCR assays using Tm-calling analysis with melting temperature peaks and their respective -(d/dT) Fluorescence 465–510 values as primary endpoints.

2.6. In silico PCR

Primer binding and potential amplification were evaluated *in silico* (by computer modelling) using BLASTN (NCBI) against a reference genomes (see Supplementary Table 1) in FASTA format. Short-sequence alignment was performed with parameters optimised for primer matching (word size 7, reward 1, penalty -1, gap open 2, gap extend 1, e-value 1000). The resulting hits were processed in a custom Python pipeline (pandas), which simulated PCR conditions by filtering for minimum binding length, limiting total mismatches, and explicitly enforcing 3′ end integrity by requiring near-complete coverage of the primer terminus (allowing up to two uncovered bases) and perfect matching within the last three nucleotides. Only correctly oriented forward (plus strand) and reverse (minus strand) combinations producing amplicons (specific products of PCR amplification) within a predefined size ranges (50–400 bp and 1000–1600 bp) were retained. Filtered binding events were subsequently used to extract predicted amplicon sequences directly from the reference genome using Biopython.

To further assess potential structural constraints on amplification, predicted amplicons were screened for inverted repeats indicative of hairpin-forming secondary structures using a custom sliding-window algorithm. The analysis evaluated stem lengths of 6–12 bp and loop sizes of 3–20 bp, allowing up to 1 mismatch, and calculated a composite stability score that incorporated stem length, mismatch proportion, and GC content as a proxy for thermodynamic stability.

As a benchmark for this custom pipeline, Sequence Extractor 1.1 (www.bioninformatics.org/seqext/index.html) was tested using the default settings for the advanced options, except that the number of matching bases was set to 17.

2.7. Sanger sequencing

Sanger sequencing of selected amplicons (first and third stages of analysis) was outsourced to SEQme (https://www.seqme.eu/en/) and performed in Standard sequencing mode using the corresponding forward primer for each amplicon. Samples were purified using ExoSAP according to the manufacturer's protocol before the sequencing. For the first stage of the analysis, amplicons of the *Cervus elaphus* reference sample were sequenced together with pill DNA. The reference-sample amplicons sequenced in the third stage of analysis together with pill DNA are listed in Table 5.

Sanger sequencing data (AB1 files) were manually quality-trimmed based on chromatogram evaluation. Mixed base positions were manually identified from overlapping peak signals. Major and minor profiles

Table 2
Primer sequences, A: Universal primers, B: Species-specific primers.

A

Name	Product length	Sequence	Source
12S rRNA_F	443 bp	AAACTGGGATTAGATACCCCACTAT	Li, 2024 [10]
12S rRNA_R		GAGGGTGACGGGCGGTGTGT	
16S rRNA_F	236 bp	AYAAGACGAGAAGACCC	Sarri, 2014 [22]
16S rRNA_R		GATTGCGCTGTTATTCC	
*COX1*_ F	221 bp	AAGCTGGCGCAGGAACAGGC	*in-house*
*COX1*_R		AGCAGTGACTAATACGGATCACA	
*Cytb*_A_F	472 bp	TACCATGAGGACAAATATCATTCTG	Kocher, 1989 [23]
*Cytb*_A_R		CCTCCTAGTTTGTTAGGGATTGATCG	
*Cytb*_B_F	386 bp	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	Filip, 2023 [24]
*Cytb*_B_R		AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	

B

Name	Target	Product length	Sequence	Source
A_F	*Rusa timorensis,* mtDNA 15334/16434	~1100 bp	AAACCAGAAAAGGAGAGCAAC	Zein, 2007 [25]
A_R			TCATCTAGGCATTTTCAGTGCC	
B_F	*Cervus elaphus, ND6*	~190 bp	TGATATATACACTCAGACCCCA	Yang, 2019 [26]
B_R			GCTGTATTTGCGTCTGCTC	
C_F	*Rangifer tarandus, NDP*	~210 bp	CGCTAACACGTCATATACCAA	Yang, 2019 [26]
C_R			CTGCGAATAGACTTCCGAT	
D_F	*Rusa unicolor, Cytb*	~104 bp	CATTTATCATCGCGGCACT	Yang, 2019 [26]
D_R			AGGAATTTTATCTGCGTCTGA	
E_F	*Dama dama,* mtDNA control region	~94 bp	AAATCAATTCTCGTCAACATGCA	Powell, 2019 [27]
E_R			TCCCTGTCTAGCGGGTTGTT	

Table 3
PCR conditions for qPCR assay with universal primers, A: Composition of PCR mix, B: Cycling conditions.

A

	Final concentration	Final volume (1 reaction)
H_2O		4.0 µL
5 × PCR buffer	1 ×	2.0 µL
$MgCl_2$	1.5 mM	0.6 µL
EvaGreen	0.5 ×	0.3 µL
dNTPs	0.2 mM	0.1 µL
GoTaq G2 Flexi polymerase	0.1 U/ µL	0.2 µL
Primer mix	0.5 µM	2.0 µL
DNA/NTC		0.9 µL

B

95 °C	2 min	
95 °C	15 s	10 ×
60 °C touchdown*	50 s	
72 °C	20 s	
95 °C	15 s	25 ×
50 °C	50 s	
72 °C	20 s	
Fluorescence reading		
72 °C	3 min	
95 °C	1 min	
50 °C	45 s	
Melting from 50 °C to 90 °C		
Fluorescence reading		
37 °C	1 s	
95 °C	2 min	
95 °C	15 s	7 ×
72 °C	50 s	
95 °C	15 s	7 ×
72 °C touchdown*	50 s	
72 °C	20 s	
95 °C	15 s	20 ×
65 °C	50 s	
72 °C	20 s	
Fluorescence reading		
72 °C	3 min	
95 °C	1 min	
50 °C	45 s	
Melting from 50 °C to 90 °C		
Fluorescence reading		
95 °C	15 s	10 ×
65 °C	50 s	
72 °C	20 s	
Melting from 50 °C to 90 °C		
Fluorescence reading		
54 °C	1 s	

* = temperature decreases by 1 °C for each cycle.

correspond to the higher- and lower-intensity sequence signals at mixed-base positions. These sequences (FASTA format) were analysed using the Standard Nucleotide BLAST tool (NCBI) and visualised using Jalview 2.11.5.1.

3. Results

3.1. First stage analysis

In the first stage of the experiment, five amplification products generated with species-universal primers were sequenced and used for species identification. All amplicons of the *Cervus elaphus* reference sample were correctly identified as *Cervus elaphus* with 100% query cover and 100% identity.

In the pill DNA (see Table 6), the major profiles of all genes except *COX1* were predominantly identified as *Rusa unicolor,* with identity percentages of 98.8–100%. The *COX1* amplicon was identified as *Cervus nippon* or *Rangifer tarandus* with the same identity score. Most of the gene's minor profiles (see Table 6) indicated the presence of *Dama dama,* except for the *Cytb*_A amplicon, which indicated *Cervus elaphus*, and the *Cytb*_B amplicon, which indicated *Alces alces* and *Axis axis* together with *Dama dama*.

Table 5
Reference samples' amplicons sequenced in the third stage of analysis.

Primer Mix	Expected target	Sequenced reference samples
B	*Cervus elaphus*	*Cervus elaphus*
		Dama dama
		Dama mesopotamica
		Cervus nippon
		Rusa timorensis
D	*Rusa unicolor*	*Cervus elaphus*
		Rusa timorensis
E	*Dama dama*	*Dama dama*

Table 6
Alignment results of species-universal pill DNA amplicons. The top hit for each sequenced region is listed with percentage identity. For the complete alignment table, see Supplementary Table 2. Major and minor profiles represent signals with higher and lower intensities at mixed-base positions.

	Pill major profile	Pill minor profile
12S rRNA	*Rusa unicolor* (100%)	*Dama dama* (95.3%)
16S rRNA	*Rusa unicolor* (98.8%)	*Dama dama* (98.1%)
COX1	*Cervus nippon / Rangifer tarandus* (98.2%)	*Dama dama* (94.7%)
***Cytb*_A**	*Rusa unicolor* (99.5%)	*Cervus elaphus* (92.6%)
***Cytb*_B**	*Rusa unicolor* (100%)	*Alces alces* (92.6%) / *Dama dama* (92.0%)/*Axis axis* (92.0%)

To formulate a working hypothesis, top hits in major profiles and hits with at least 95% identity in minor profiles were used. After the first stage of analysis, we hypothesised that the pill DNA was a mixture, consisting mainly of *Rusa unicolor* with additional *Dama dama, Cervus nippon,* and/or *Rangifer tarandus* DNA.

3.2. Second stage analysis

The results from both *in silico* PCR methods predicting amplification of the reference species with species-specific primers were inconclusive and did not correspond to the *in vitro* results (see Supplementary Table 1). None of the tested features (minimal binding length, total number of mismatches, position of potential mismatches relative to the 3´ end, GC content, and presence of hairpin secondary structures) explained the discrepancies between predicted and observed amplification. Although predicted amplicons with the lowest mismatch count in the annealing regions are generally more likely to be amplified *in vitro*, this did not apply universally across all reference species. The hairpin analysis score did not seem to influence the amplification success. These findings suggest that the discrepancies are more likely to be rooted in limitations of the reference sequences than in the experimental design.

The qPCR results were evaluated based on presence or absence of amplification and the melting point value of amplicons (the temperature at which the amplified DNA strands separate). Primer mix A could be evaluated only by amplification presence/absence, because its melting curve contained multiple peaks, suggesting gradual melting of the long DNA molecule in regions with variable GC content. As we were unable to obtain a reference sample of *Rusa unicolor*, it was substituted in the experiment by a *Rusa timorensis* sample. Both species share 98–99% of the mitogenome, and they can share haplotypes due to intergression [28]. Species-specific qPCR and melting analysis would most likely not distinguish these two species reliably.

The second stage qPCR analysis provided consistent, reproducible results for reference samples, and distinguished individual species based on their specific amplification patterns after the first 34 amplification cycles (see Table 7A).

After the first 34 cycles, pill DNA showed amplification only in primer mix E. This corresponded to the *Dama dama* reference; however, the lack of amplification in primer mix B prevented a reliable species-level conclusion. To reduce the risk of overlooking minor contributions, the next amplification run was extended by an additional 10 cycles (see Table 4 for the complete program and Table 7B for results).

During extended amplification, pill DNA also produced amplicons also in primer mixes B and D. Based on primer mix B, the pill DNA may contain *Cervus elaphus* or *Cervus nippon* DNA. Although both species also amplified in primer mix A, the absence of amplification in pill DNA is likely due to the larger amplicon size and a high fragmentation of DNA extracted from the pill. It was therefore not considered an exclusion criterion for *Cervus elaphus/nippon* identification.

The primer mix D clearly indicated the presence of *Rusa timorensis* DNA.

Table 7
Melting points of individual amplicons. A: Melting after 34 cycles, B: Melting after an extended program (44 cycles). ✓ = amplification present, melting point not available, - = amplification absent, N/A = sample not tested.

	PM A	PM B	PM C	PM D	PM E
A					
Cervus elaphus	-	76.9	-	-	-
Dama dama	-	77.5	-	-	80.9
Dama mesopotamica	✓	-	-	-	-
Cervus nippon pseudoaxis	-	77.2	-	-	-
Rangifer tarandus fennicus	-	-	80.3	-	-
Rusa timorensis	✓	-	-	80.0	-
Rusa alfredi	-	-	-	-	-
Pill DNA	-	-	-	-	80.9
B					
Cervus elaphus	✓	76.6	-	78.4	80.9
Dama dama	-	77.2	-	-	80.9
Dama mesopotamica	✓	77.5	-	-	-
Cervus nippon pseudoaxis	✓	76.9	-	-	-
Rangifer tarandus fennicus	-	-	80.0	-	-
Rusa timorensis	✓	77.2	-	79.7	-
Rusa alfredi	N/A	N/A	N/A	N/A	N/A
Pill DNA	-	76.9		79.7	80.9

The melting point in primer mix E was the same for *Dama dama* and *Cervus elaphus* under extended qPCR conditions. However, early amplification of pill DNA supported *Dama dama* as the more probably contributor.

The melting analysis supported the hypothesis that *Rusa unicolor* and *Dama dama* DNA were present the pill. The presence of *Cervus elaphus/nippon* remained possible, but identification at this stage remained unsatisfactory. By contrast, the presence of *Rangifer tarandus* was clearly disproved, because the *Rangifer tarandus* reference sample was the only sample amplifying in the *Rangifer tarandus*-specific primer mix C.

3.3. Third stage analysis

Selected amplicons (see Table 5) were submitted for Sanger sequencing. Some results may have been affected by partial sample loss during transportation; however, sufficient data were obtained for species identification.

In primer mix B (see Table 8A), all reference samples were correctly identified, supporting the high species specificity of this primer mix. In this primer mix, pill DNA was identified as *Cervus elaphus*, consistent with the melting analysis results.

Some inconsistencies were observed in the species identification of the *Cervus elaphus* reference sample in primer mix D (see Table 8B). Nevertheless, the pill DNA and *Rusa timorensis* reference alignments were practically identical, consistent with previous results.

Sequences obtained for the *Dama dama* reference and pill DNA were identical, supporting the *Dama dama* identification despite unsuccessful BLAST-based species alignment (see Table 8C). Because GenBank records for ungulates may not meet forensic quality criteria and often contain erroneous or mislabeled sequences [29–32], greater weight was given to observed sequence concordance with the reference sample than to BLAST species-identification result. For complete alignment tables, see Supplementary Table 3.

Combining the results of all three genetic identification stages, DNA extracted from the Purtier Placenta 6th Edition pill was identified as a mixture of *Rusa timorensis/unicolor*, *Dama dama*, and *Cervus elaphus*.

4. Discussion

In this project, we attempted to verify whether the declared main component of the Purtier Placenta 6th Edition was of deer origin. Using a three-stage analysis, we obtained sufficient evidence that the capsule contained a DNA mixture from at least three *Cervidae* species: *Rusa*

Table 8
Alignment results for species-specific primers. Hits with identical scores were combined to one entry, number of combined hits listed as number of accessions. Hits with query cover > 90% are listed. A: Primer mix B, BLAST hits with percent identity > 98% are listed, B: Primer mix D, BLAST hits with percent identity > 97% are listed, C: Primer mix E, all BLAST hits are listed.

Sample	Species identified	Number of accessions	Total score	Query cover	Percent identity
A					
Cervus elaphus	*Cervus elaphus*	11	257	100%	100.00%
	Cervus elaphus	1	254	100%	99.28%
	Cervus elaphus	7	246	100%	98.56%
Dama dama	*Dama dama*	14	257	100%	100.00%
Dama mesopotamica	*Dama mesopotamica*	1	246	100%	99.26%
Cervus nippon	*Cervus nippon*	5	254	100%	99.29%
	Cervus elaphus	3	248	100%	98.57%
	Cervus canadensis	5	248	100%	98.57%
	Cervus hanglu	1	248	100%	98.57%
	Cervus canadensis	1	246	99%	98.56%
Rusa timorensis	*Rusa unicolor*	10	246	100%	99.26%
	Rusa unicolor	17	241	100%	98.53%
	Rusa unicolor	1	239	99%	98.52%
Pill DNA	*Cervus elaphus*	12	228	100%	99.21%
	Cervus elaphus	1	224	100%	98.43%
B					
Cervus elaphus	*Odocoileus virgianus*	27	87.9	96%	98.00%
	Subulo gouazoubira	1	87.9	90%	100.00%
	Bisbalus citus	1	84.2	92%	97.92%
	Cervus elaphus	3	82.4	90%	97.87%
	Pudu pudu	1	82.4	90%	97.87%
Rusa timorensis	*Rusa unicolor*	66	95.3	100%	98.15%
	Axis porcinus	1	95.3	100%	98.15%
	Cervus albirostris	1	95.3	100%	98.15%
	Rusa timorensis	4	95.3	100%	98.15%
	Cervus eldii	1	93.5	98%	98.11%
	Rusa unicolor	3	93.5	98%	98.11%
Pill DNA	*Rusa unicolor*	67	100.0	96%	100.00%
	Axis porcinus	1	100.0	96%	100.00%
	Cervus albirostris	1	100.0	96%	100.00%
	Rusa timorensis	4	100.0	96%	100.00%
	Cervus eldii	1	99.0	95%	100.00%
	Rusa unicolor	3	99.0	95%	100.00%
	Rusa unicolor	8	95.3	96%	98.15%
C					
Dama dama	*Ovis ammon*	2	63.9	98%	91.49%
Pill DNA	*Ovis ammon*	2	63.9	98%	91.49%

timorensis/unicolor as the major component, with minor components of *Dama dama* and *Cervus elaphus*.

The first stage of analysis indicated the presence of *Rusa unicolor*, *Dama dama, Cervus nippon* and/or *Rangifer tarandus*. The presence of *Rangifer tarandus* was effectively excluded during the second stage of analysis, because primer mix C amplified only the *Rangifer tarandus* reference sample.

The melting analysis was based on the comparison of the reference and sample profiles, evaluating the presence or absence of amplification in species-specific primer mixes. The lack of amplification in primer mix A was not considered an exclusion criterion for identification, even though both *Rusa timorensis* and *Cervus elaphus* amplified in this primer mix. We hypothesize that the lack of amplification of the pill DNA is the consequence of DNA fragmentation. However, this hypothesis could not be verified by electrophoresis, because DNA extracted from the pill represents a mixture of all 12 ingredients described in the Introduction.

During the second stage of analysis, we encountered inconsistencies between the *in silico* and *in vitro* PCR results. The primers were checked bioinformatically for various possible PCR limitations, and all primers predicted to work with species that did not amplify *in vitro* work amplified successfully with at least one other species sample. This suggests that the issue is unlikely to lie within the primer mixes themselves but rather in the reference sequences used. Haplotype mismatch between the tested sample and the reference sequence, undescribed interindividual variation, interspecific hybridisation or introgression, or simple database sequence error are the most probable explanations for the observed inconsistencies.

The same issues may also explain problematic species identification for several reference samples during the third stage of analysis, namely identification of the *Cervus elaphus* reference sample primarily as *Odocoileus virgianus* in primer mix D and identification of *Dama dama* reference sample as *Ovis ammon* in primer mix E. Considering the known limitations and possible mislabelling of public database sequences [29–32], greater weight was given to concordance between the reference sample and the test material PCR and sequencing results than to the BLAST species identification results.

The qPCR and Sanger sequencing analysis face some technical limitations when analysing mixtures. In species-universal PCR reactions, preferential amplification can occur, and minor fractions can be suppressed due to primer bias, different annealing specificities, and related factors. Melting analysis with species-specific primers partly overcomes this obstacle, but cannot be considered quantitative, as better annealing of individual primer mixtures can influence reaction yield in favour of one fraction. Species-specific primers are prone to false negatives when mutations occur at the primer annealing site and may fail to detect unexpected species. We mitigated these risks through a staged experimental design, in which primers were selected based on universal Sanger sequencing results and sequences from closely related species.

Species-universal Sanger sequencing provides consensus sequences, in which minor fractions can be hidden in the background. These methodological limitations could be addressed in future work by targeted amplicon-based massively parallel sequencing.

The technological procedure for placenta processing is described in patent application EP4452411A1, assigned to EHJ IP Ltd. CerviCenta™

is listed as one embodiment, but the originating deer species is not specified. Riway holds US patent US11071766B2 for strawberry plant extract and its use; however, this patent does not mention Purtier Placenta or the NucleiClavem trademark name.

No other patents directly attributable to Riway, Purtier Placenta, or its main ingredients were identified in the patent databases searched (Google Patents, EPO, WIPO, USPTO, the Intellectual Property Office of Singapore, and China National Intellectual Property Administration records), despite the company's claims of using proprietary patented technologies. Only trademark protection for the brand and ingredient names was found, with no additional information about the composition, species origin, or manufacturing process.

According to the manufacturer's website, Purtier Placenta is sourced and produced in New Zealand, one of the world's leading deer-farming countries [33]. *Cervus elaphus* is among the most widespread and economically significant deer species in New Zealand. It is therefore notable that most of the DNA detected in the Purtier Placenta capsule belonged to the *Rusa* deer. No unambiguous clinical evidence was identified showing superior health benefits of either species in this context; however, *Cervus elaphus* products are widely used in TCM, which make them economically more valuable than products derived from *Rusa* deer.

The claim of deer origin was ultimately supported, although the exclusive use of *Cervus elaphus* imagery may be seen as misleading, as it was detected only as a minor component. The language barrier may add to the confusion for Czech customers: the English word “deer” usually describes all members of the *Cervidae* family, whereas its direct Czech translation, "jelen", has a narrower meaning and is used only for the red deer (*Cervus elaphus*).

Future work could use amplicon-based massively parallel sequencing or other methods capable of quantifying individual mixture components. Tissue of origin identification, including verification of placenta as the source tissue, could be addressed using genetic (RT-qPCR, RNA-seq, etc.) and, in particular, epigenetic approaches in the future.

5. Conclusion

The mammalian DNA present in the Purtier Placenta Live Stem Cell Therapy 6th Edition capsule was a mixture of several species from the *Cervidae* family. The main component of the mixture was identified as a *Rusa timorensis* or *R. unicolor*, with minor admixtures of *Dama dama* and *Cervus elaphus*. Our results support the claim that the capsule contains material of deer origin, although more specific information about the capsule's species composition is lacking and the marketing presentation may be misleading.

CRediT authorship contribution statement

Ondřej Fischer: Writing – review & editing, Supervision, Resources, Investigation, Formal analysis, Conceptualization. **Martin Loskot:** Validation, Resources, Methodology, Investigation, Data curation. **Shira Vinitzky:** Validation, Methodology, Investigation, Data curation. **Lucie Kotková:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Data curation. **Drabek Jiri:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT-5.2 and NotebookLM to assist with literary screening, generating summaries, highlighting key passages in regulatory documents, checking grammar, and developing a custom BLASTN pipeline. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2026.103594.

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