SUPPLEMENTARY MATERIAL

Microsatellite analysis of genetic variability in Waler horses from Australia

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Supplementary Table 1. Breeds and sampling background

Breed N Breed sampling background						
Breed	IN	breeu sampring				
Waler (WA)	70	Samples were provided by the Waler Horse Owners and Breeders Association Australia Inc. (WHOBAA). Samples were collected from seven breeding stations as follows: WA1 (29 horses), WA2 (12 horses), WA3 (10 horses), WA4 (one horse), WA5 (7 horses), WA6 (nine horses), WA7 (one horses) and one unknown horse.				
Irish Draught (ID)	46	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Thoroughbred (TB)	70	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Quarter Horse (QH)	66	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Andalusian (AN)	33	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Turkoman (TU)	82	Samples came from the Turkoman Horse breeders in the USA. Samples came to the Animal Genetic lab at Texas A&M University for parentage testing.				
Argentine Criollo (AC)	25	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Syrian Arabian (SY)	100	Samples were collected from Syria where Arabian horse breeders volunteered to give their horse samples.				
Exmoor Pony (EX)	70	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Cleveland Bay (CB)	90	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Welsh Pony (WP)	31	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Timor Pony (TM)	33	Samples provided by owners for study of origins of Timor Pony and for this study				
Belgian Draft (BE)	26	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Dales Pony (DL)	86	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Shire (SH)	32	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				
Highland Pony (HL)	28	Samples contributed by breeders for parentage testing at the Animal Genetic lab at Texas A&M University.				

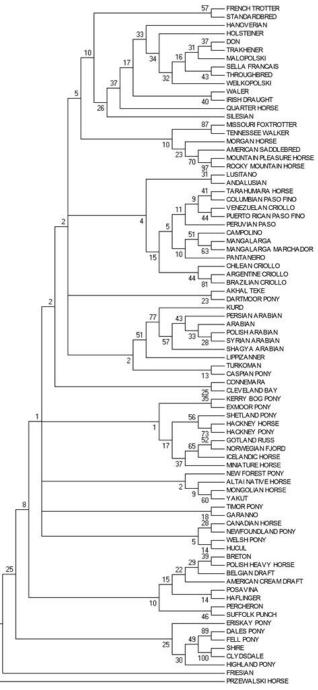
Supplementary Table 2. Information about microsatellite markers used in the study

ECA*	Marker	Primer Sequences 5'to 3'	Allele Size (bp)	Multiplex
4	LEX33	Forward: TTTAATCAAAGGATTCAGTTG	192-222	1
		Reverse: GGGACACTTTCTTTACTTTC		
24	AHT4	Forward: AACCGCCTGAGCAAGGAAGT	142-164	2
		Reverse: CCCAGAGAGTTTACCCT		
2	ASB17	Forward: ACCAGTCAGGATCTCCACCG	81-125	2
		Reverse: GAGGGCGGTACCTTTGTACC		
4	HMS6	Forward: GAAGCTGCCAGTATTCAACCATTG	155-169	2
		Reverse: CTCCATCTTGTGAAGTGTAACTCA		
9	HTG4	Forward: CTATCTCAGTCTTGATTGCAGGAC	126-142	2
		Reverse: CTCCCTCCCTCCTCTGTTCTC		
30	VHL20	Forward: CAAGTCCTCTTACTTGAAGACTAG Reverse: AACTCAGGGAGAATCTTCCTCAG	85-109	2
4	HTG7	Forward: CCTGAAGCAGAACATCCCTCCTTG Reverse: ATAAAGTGTCTGGGCAGAGCTGCT	117-133	3
15	HTG6	Forward: CCTGCTTGGAGGCTGTGATAAGAT	79-105	3
		Reverse: GTTCACTGAATGTCAAATTCTGCT		
3	ASB23	Forward: ACATCCTGGTCAAATCACAGTCC	181-209	2
		Reverse: GAGGGCAGCAGGTTGGGAAGG		
15	ASB2	Forward: CACTAAGTGTCGTTTCAGAAGG	218-256	1
		Reverse: GCACAACTGAGTTCTCTGATAGG		
1	HMS7	Forward: CAGGAAACTCTCATGTTGATACCATC	171-189	2
		Reverse: TGTTGTTGAAACATACCTTGACTGT		
8	ATH5	Forward: ACGGACACATCCCTGCCTGC	126-148	2
		Reverse: GCAGGCTAAGGAGGCTCAGC		
9	HMS3	Forward: CCAACTCTTTGTCACATAACAAGA Reverse: CCATCCTCACTTTTTCACTTTGTT	152-180	1
10	HMS2	Forward: ACGGTGGCAACTGCCAAGGAAG Reverse: CTTGCAGTCGAATGTGTATTAAATG	216-244	3
21	HTG10	Forward: CAATTCCCGCCCCACCCCGGCA Reverse: TTTTTATTCTGATCTGTCACATTT	87-113	2

^{*}Locus locations in the horse genome

Supplementary Table 3. Nei's genetic distances (D) between Waler and other 82 breeds Fifteen Breeds close to Waller are in bold.

Altai Native Horse 0.1469 Hackney Horse 0.3312 Polish Arabian 0.22 American Cream Draft 0.298 Hackney Pony 0.3116 Polish Heavy Horse 0.33 American Saddlebred 0.2318 Haflinger 0.3736 Posavina 0.33 Andalusian 0.2352 Hanoverian 0.0974 Przewalski 1.03 Arabian 0.2853 Highland Pony 0.362 Puerto Rican Paso 0.33 Argentine Criollo 0.232 Holsteiner 0.1656 Quarter Horse 0.07 Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.17 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.12 Brezton 0.1574 Irish Draught 0.0872 Shagya Arabian 0.22 Campolino 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.2 Campolino 0.2626 Kurd 0.2159 Shire 0.33 Canadian Horse 0.2354 <t< th=""><th>Breed</th><th>D</th><th>Breed</th><th>D</th><th>Breed</th><th>D</th></t<>	Breed	D	Breed	D	Breed	D
American Cream Draft 0.298 Hackney Pony 0.3116 Polish Heavy Horse 0.33 American Saddlebred 0.2318 Haflinger 0.3736 Posavina 0.33 Andalusian 0.2352 Hanoverian 0.0974 Przewalski 1.03 Arabian 0.2853 Highland Pony 0.362 Puerto Rican Paso 0.33 Argentine Criollo 0.232 Holsteiner 0.1656 Quarter Horse 0.03 Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.12 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.12 Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.22 Campolino 0.2626 Kurd 0.2159 Shire 0.33 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Caspian Pony 0.2626 Lusitano 0.2357	Akhal Teke	0.2892	Gotland Russ	0.5967	Peruvian Paso	0.2742
American Saddlebred 0.2318 Haflinger 0.3736 Posavina 0.334 Andalusian 0.2352 Hanoverian 0.0974 Przewalski 1.03 Arabian 0.2853 Highland Pony 0.362 Puerto Rican Paso 0.33 Argentine Criollo 0.232 Holsteiner 0.1656 Quarter Horse 0.07 Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.17 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.12 Brezilian Criollo 0.1574 Irish Draught 0.0872 Shagya Arabian 0.27 Breton 0.4558 Kerrybog Pony 0.2402 Sheltand Pony 0.22 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.22 Caspian Pony 0.2669 Lusitano 0.2335 Standardbred 0.22 Cliveland Bay 0.354 Mangalarga	Altai Native Horse	0.1469	Hackney Horse	0.3312	Polish Arabian	0.2906
Andalusian 0.2352 Hanoverian 0.0974 Przewalski 1.03 Arabian 0.2853 Highland Pony 0.362 Puerto Rican Paso 0.33 Argentine Criollo 0.232 Holsteiner 0.1656 Quarter Horse 0.07 Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.15 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.15 Brazilian Criollo 0.1574 Irish Draught 0.0872 Shagya Arabian 0.25 Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.25 Campolino 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.22 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Campolino 0.2626 Lusitano 0.2359 Shire 0.32 Caspian Pony 0.2669 Lusitano 0.2935 <	American Cream Draft	0.298	Hackney Pony	0.3116	Polish Heavy Horse	0.3205
Arabian 0.2853 Highland Pony 0.362 Puerto Rican Paso 0.334 Argentine Criollo 0.232 Holsteiner 0.1656 Quarter Horse 0.07 Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.17 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.17 Brazilian Criollo 0.1574 Irish Draught 0.0872 Shagya Arabian 0.27 Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.2 Campolino 0.2626 Kurd 0.2159 Shire 0.3 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.20 Caspian Pony 0.2669 Lusitano 0.2355 Standardbred 0.2 Chilean Criollo 0.2671 Malopolski 0.0968 Suffolk Punch 0.2 Clydsdale 0.4368 Mangalarga 0.2774 Syrian Arabian 0.2 Clydsdale 0.4368 Mangalarga Marchador <td>American Saddlebred</td> <td>0.2318</td> <td>Haflinger</td> <td>0.3736</td> <td>Posavina</td> <td>0.3016</td>	American Saddlebred	0.2318	Haflinger	0.3736	Posavina	0.3016
Argentine Criollo 0.232 Holsteiner 0.1656 Quarter Horse 0.07 Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.17 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.17 Brazilian Criollo 0.1574 Irish Draught 0.0872 Shagya Arabian 0.27 Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.22 Campolino 0.2626 Kurd 0.2159 Shire 0.3 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.20 Caspian Pony 0.2669 Lusitano 0.2335 Standardbred 0.22 Chilean Criollo 0.2671 Malopolski 0.0968 Suffolk Punch 0.22 Clydsdale 0.4368 Mangalarga 0.2774 Syrian Arabian 0.22 Clydsdale 0.4368 Mangalarga Marchador 0.2828 Tarahumara Horse 0.15 Columbian Paso Fino 0.2299 M	Andalusian	0.2352	Hanoverian	0.0974	Przewalski	1.0365
Belgian Draft 0.4179 Hucul 0.3306 Rocky Mountain Horse 0.17 Boerperd 0.2388 Icelandic Horse 0.4633 Sella Francais 0.12 Brazilian Criollo 0.1574 Irish Draught 0.0872 Shagya Arabian 0.22 Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.2 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.20 Caspian Pony 0.2669 Lusitano 0.2335 Standardbred 0.2 Chilean Criollo 0.2671 Malopolski 0.0968 Suffolk Punch 0.20 Clydsdale 0.4368 Mangalarga 0.2774 Syrian Arabian 0.22 Columbian Paso Fino 0.2299 Miniature Horse 0.2779 Tennessee Walker 0.15 Connemara 0.2081 Missouri Foxtrotter 0.1388 Thoroughbred 0.12 Dales Pony 0.3257 Mongo	Arabian	0.2853	Highland Pony	0.362	Puerto Rican Paso	0.3129
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Brazilian Criollo 0.1574 Irish Draught 0.0872 Shagya Arabian 0.27 Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.22 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.20 Caspian Pony 0.2669 Lusitano 0.2335 Standardbred 0.22 Chilean Criollo 0.2671 Malopolski 0.0968 Suffolk Punch 0.20 Cleveland Bay 0.354 Mangalarga 0.2774 Syrian Arabian 0.22 Clydsdale 0.4368 Mangalarga Marchador 0.2828 Tarahumara Horse 0.12 Columbian Paso Fino 0.2299 Miniature Horse 0.2779 Tennessee Walker 0.15 Connemara 0.2081 Missouri Foxtrotter 0.1388 Thoroughbred 0.12 Dales Pony 0.3257 Mongolian Horse 0.249 Timor Pony 0.36 Dartmoor Pony 0.5307	Belgian Draft	0.4179	Hucul	0.3306	Rocky Mountain Horse	0.1784
Breton 0.4558 Kerrybog Pony 0.2402 Shetland Pony 0.23 Campolino 0.2626 Kurd 0.2159 Shire 0.32 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.20 Caspian Pony 0.2669 Lusitano 0.2335 Standardbred 0.22 Chilean Criollo 0.2671 Malopolski 0.0968 Suffolk Punch 0.26 Cleveland Bay 0.354 Mangalarga 0.2774 Syrian Arabian 0.27 Clydsdale 0.4368 Mangalarga Marchador 0.2828 Tarahumara Horse 0.15 Columbian Paso Fino 0.2299 Miniature Horse 0.2779 Tennessee Walker 0.15 Connemara 0.2081 Missouri Foxtrotter 0.1388 Thoroughbred 0.12 Dales Pony 0.3257 Mongolian Horse 0.249 Timor Pony 0.38 Dartmoor Pony 0.23 Morgan Horse 0.1741 Trakhener 0.17 Don 0.1826 Mountain Pleasu	Boerperd	0.2388	Icelandic Horse	0.4633	Sella Français	0.1231
Campolino 0.2626 Kurd 0.2159 Shire 0.33 Canadian Horse 0.2358 Lippizanner 0.2567 Silesian 0.20 Caspian Pony 0.2669 Lusitano 0.2335 Standardbred 0.23 Chilean Criollo 0.2671 Malopolski 0.0968 Suffolk Punch 0.26 Cleveland Bay 0.354 Mangalarga 0.2774 Syrian Arabian 0.27 Clydsdale 0.4368 Mangalarga Marchador 0.2828 Tarahumara Horse 0.15 Columbian Paso Fino 0.2299 Miniature Horse 0.2779 Tennessee Walker 0.15 Connemara 0.2081 Missouri Foxtrotter 0.1388 Thoroughbred 0.12 Dales Pony 0.3257 Mongolian Horse 0.249 Timor Pony 0.33 Dartmoor Pony 0.23 Morgan Horse 0.1741 Trakhener 0.17 Don 0.1826 Mountain Pleasur_Horse 0.1134 Turkoman 0.12 Exmoor Pony 0.4373 Newfou	Brazilian Criollo	0.1574	Irish Draught	0.0872	Shagya Arabian	0.2733
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Chilean Criollo0.2671Malopolski0.0968Suffolk Punch0.2671Cleveland Bay0.354Mangalarga0.2774Syrian Arabian0.2772Clydsdale0.4368Mangalarga Marchador0.2828Tarahumara Horse0.15Columbian Paso Fino0.2299Miniature Horse0.2779Tennessee Walker0.15Connemara0.2081Missouri Foxtrotter0.1388Thoroughbred0.12Dales Pony0.3257Mongolian Horse0.249Timor Pony0.38Dartmoor Pony0.23Morgan Horse0.1741Trakhener0.17Don0.1826Mountain Pleasur_Horse0.1134Turkoman0.13Eriskay Pony0.5307Newforest Pony0.159Venezuelan Criollo0.13Exmoor Pony0.4373Newfoundland Pony0.2723Weilkopolski0.07Fell Pony0.3273Norwegian Fjord0.3843Welsh Pony0.24French Trotter0.4405Pantaneiro0.2504Yakut0.22Friesian0.7894Percheron0.3267	Canadian Horse	0.2358	Lippizanner	0.2567	Silesian	0.206
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Columbian Paso Fino 0.2299 Miniature Horse 0.2779 Tennessee Walker 0.19 Connemara 0.2081 Missouri Foxtrotter 0.1388 Thoroughbred 0.12 Dales Pony 0.3257 Mongolian Horse 0.249 Timor Pony 0.38 Dartmoor Pony 0.23 Morgan Horse 0.1741 Trakhener 0.17 Don 0.1826 Mountain Pleasur_Horse 0.1134 Turkoman 0.15 Eriskay Pony 0.5307 Newforest Pony 0.159 Venezuelan Criollo 0.13 Exmoor Pony 0.4373 Newfoundland Pony 0.2723 Weilkopolski 0.07 Fell Pony 0.3273 Norwegian Fjord 0.3843 Welsh Pony 0.24 Friesian 0.7894 Percheron 0.3267 Yakut 0.22	Cleveland Bay	0.354	Mangalarga	0.2774	Syrian Arabian	0.2283
Connemara 0.2081 Missouri Foxtrotter 0.1388 Thoroughbred 0.12 Dales Pony 0.3257 Mongolian Horse 0.249 Timor Pony 0.38 Dartmoor Pony 0.23 Morgan Horse 0.1741 Trakhener 0.17 Don 0.1826 Mountain Pleasur_Horse 0.1134 Turkoman 0.15 Eriskay Pony 0.5307 Newforest Pony 0.159 Venezuelan Criollo 0.13 Exmoor Pony 0.4373 Newfoundland Pony 0.2723 Weilkopolski 0.07 Fell Pony 0.3273 Norwegian Fjord 0.3843 Welsh Pony 0.24 Friesian 0.7894 Percheron 0.3267 Yakut 0.25	Clydsdale	0.4368	Mangalarga Marchador	0.2828	Tarahumara Horse	0.1531
Dales Pony 0.3257 Mongolian Horse 0.249 Timor Pony 0.38 Dartmoor Pony 0.23 Morgan Horse 0.1741 Trakhener 0.17 Don 0.1826 Mountain Pleasur_Horse 0.1134 Turkoman 0.15 Eriskay Pony 0.5307 Newforest Pony 0.159 Venezuelan Criollo 0.13 Exmoor Pony 0.4373 Newfoundland Pony 0.2723 Weilkopolski 0.02 Fell Pony 0.3273 Norwegian Fjord 0.3843 Welsh Pony 0.24 French Trotter 0.4405 Pantaneiro 0.2504 Yakut 0.22 Friesian 0.7894 Percheron 0.3267	Columbian Paso Fino	0.2299	Miniature Horse	0.2779	Tennessee Walker	0.1988
Dartmoor Pony 0.23 Morgan Horse 0.1741 Trakhener 0.17 Don 0.1826 Mountain Pleasur_Horse 0.1134 Turkoman 0.13 Eriskay Pony 0.5307 Newforest Pony 0.159 Venezuelan Criollo 0.13 Exmoor Pony 0.4373 Newfoundland Pony 0.2723 Weilkopolski 0.07 Fell Pony 0.3273 Norwegian Fjord 0.3843 Welsh Pony 0.24 French Trotter 0.4405 Pantaneiro 0.2504 Yakut 0.22 Friesian 0.7894 Percheron 0.3267	Connemara	0.2081	Missouri Foxtrotter	0.1388	Thoroughbred	0.1297
Don0.1826Mountain Pleasur_Horse0.1134Turkoman0.15Eriskay Pony0.5307Newforest Pony0.159Venezuelan Criollo0.13Exmoor Pony0.4373Newfoundland Pony0.2723Weilkopolski0.07Fell Pony0.3273Norwegian Fjord0.3843Welsh Pony0.24French Trotter0.4405Pantaneiro0.2504Yakut0.22Friesian0.7894Percheron0.3267	Dales Pony	0.3257	Mongolian Horse	0.249	Timor Pony	0.3899
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Exmoor Pony0.4373Newfoundland Pony0.2723Weilkopolski0.073Fell Pony0.3273Norwegian Fjord0.3843Welsh Pony0.243French Trotter0.4405Pantaneiro0.2504Yakut0.223Friesian0.7894Percheron0.3267	Don	0.1826	Mountain Pleasur_Horse	0.1134	Turkoman	0.1557
Fell Pony0.3273Norwegian Fjord0.3843Welsh Pony0.24French Trotter0.4405Pantaneiro0.2504Yakut0.22Friesian0.7894Percheron0.3267	Eriskay Pony	0.5307	Newforest Pony	0.159	Venezuelan Criollo	0.1324
French Trotter 0.4405 Pantaneiro 0.2504 Yakut 0.22 Friesian 0.7894 Percheron 0.3267	Exmoor Pony	0.4373	Newfoundland Pony	0.2723	Weilkopolski	0.0729
Friesian 0.7894 Percheron 0.3267	Fell Pony	0.3273	Norwegian Fjord	0.3843	Welsh Pony	0.2448
	French Trotter	0.4405	Pantaneiro	0.2504	Yakut	0.2239
Garanno 0.1359 Persian Arabian 0.2804	Friesian	0.7894	Percheron	0.3267		
	Garanno	0.1359	Persian Arabian	0.2804		



Supplementary Figure: Dendogram of the genetic relationship among 83 horse breeds.

Supplementary Text 1. Amplification conditions used in the study

We genotyped 2705 horses by using 15 microsatellite markers. The amplification of these microsatellites was done in multiplex PCR reactions and performed in a total volume 20μl reactions containing 30 ng of genomic DNA, 0.8 μM of primers, 1xPCR buffer, 2.5mM of MgCl2, 0.2 mM of dNTPs and l unit of AmpliTaq (PE Applied Biosystems, MA). A hot start procedure was used in which DNA template and primers were combined and heated at 95°C for 10 min. The temperature was then lowered and held at 85°C for 10 minutes for the addition of the remaining reagents. Thirty two cycles of 1 minute at 95°C, annealing 56°C for multiplex 1 and 60, for multiplex 2 and 3 for 30 seconds and 72°C for 45 seconds, then cycling was completed with a final extension at 72°C for 10 minutes. The PCR products were separated by electrophoresis on a 6% polyacrylamide gel using the ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Fragment sizes of microsatellite alleles were determined using the STRand computer software (www.vgl.ucdaviess.edu/STRand). Alphanumerical nomenclature was used for allele size

designation in accordance with the International Society for Animal Genetics.