CONCLUSIONES

1. Es posible obtener ADN genómico en suficiente cantidad y calidad para numerosos tipos de análisis, a partir de las plumas de los avestruces, y, en general, de cualquier ave.

2. La técnica de RAPDs es útil para la identificación de marcadores de sexo, especialmente cuando no se tiene ninguna información previa sobre el genoma de la especie.

3. El sexo de los avestruces puede ser determinado de forma fácil, rápida y segura mediante el análisis por PCR del marcador molecular de sexado descrito en este trabajo.

4. Los 8 marcadores microsatélites estudiados presentan un elevado polimorfismo, equivalente al que encontramos en especies de aves silvestres, y son útiles para la identificación individual y la determinación de relaciones de parentesco en los avestruces.

5. Con los datos generados por el análisis de los alelos de microsatélites y sus frecuencias se ha comprobado que las subespecies *S. c. australis* y *S. c. massaicus* son, evolutivamente, las más cercanas. El grado de divergencia entre las subespecies *S. c. camelus y S. c. molybodophanes* debe ser estudiado con más profundidad, incrementando el número de ejemplares analizados.

6. La falta de homología de secuencia de regiones de genes muy conservados evolutivamente confirma la gran divergencia del avestruz de las demás especies, y, en concreto del resto de las aves.

7. La región entre el Exón 2 y Exón 3 del gen de la Hormona de crecimiento en el avestruz presenta una elevada homología en la región codificante con la secuencia del pollo. En el Intrón 2 presenta dos SNPs, candidatos a ser marcadores de la subespecie *S. c. molybdophanes*.

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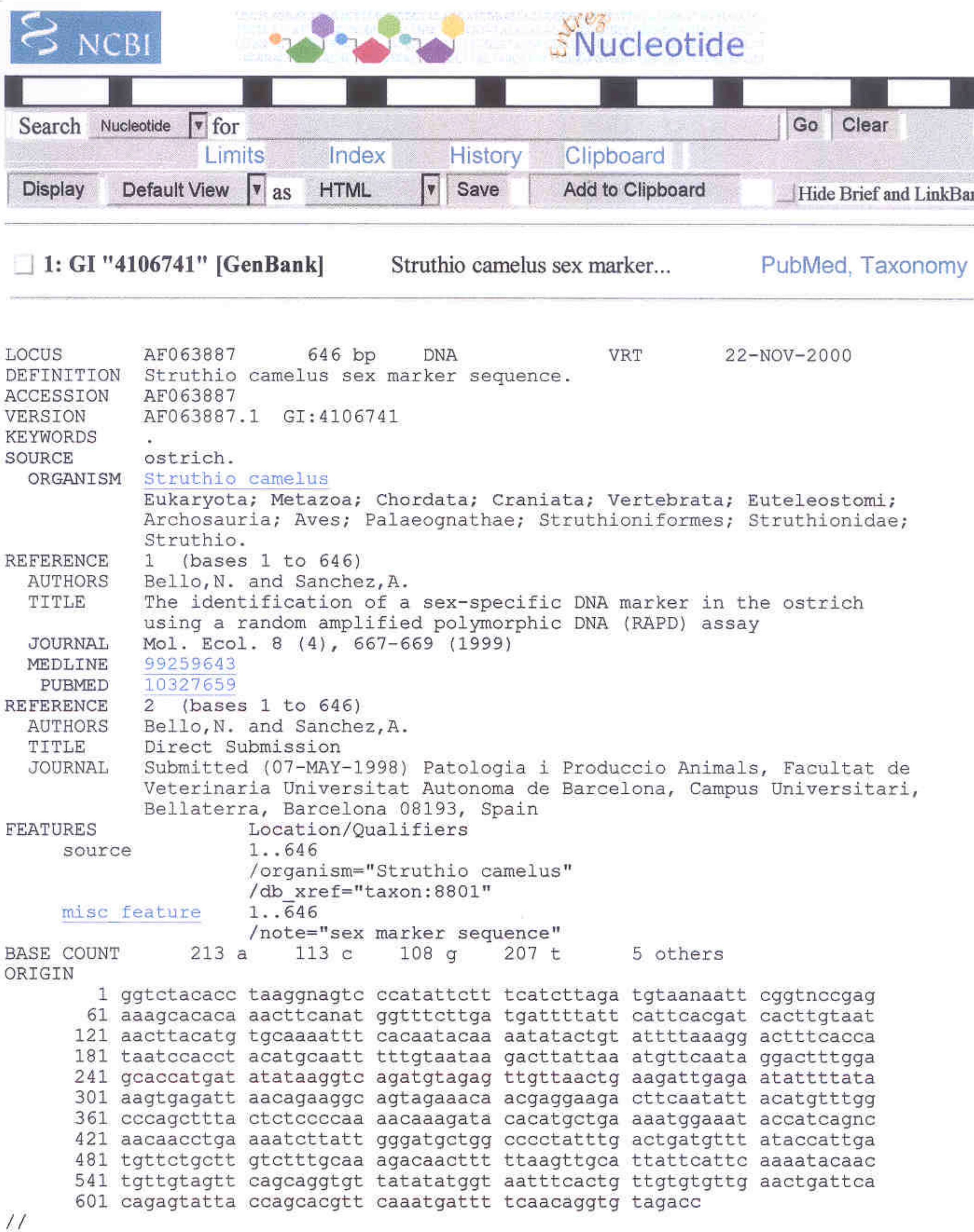
ANEXO I. SECUENCIA DEL MARCADOR DE SEXO (GenBank)

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LOCUS       AF063887      646 bp    DNA             VRT       22-NOV-2000
DEFINITION  Struthio camelus sex marker sequence.
ACCESSION   AF063887
VERSION     AF063887.1  GI:4106741
KEYWORDS    .
SOURCE      ostrich.
  ORGANISM  Struthio camelus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Archosauria; Aves; Palaeognathae; Struthioniformes; Struthionidae;
            Struthio.
REFERENCE   1  (bases 1 to 646)
  AUTHORS   Bello,N. and Sanchez,A.
  TITLE     The identification of a sex-specific DNA marker in the ostrich
            using a random amplified polymorphic DNA (RAPD) assay
  JOURNAL   Mol. Ecol. 8 (4), 667-669 (1999)
  MEDLINE   99259643
   PUBMED   10327659
REFERENCE   2  (bases 1 to 646)
  AUTHORS   Bello,N. and Sanchez,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAY-1998) Patologia i Produccio Animals, Facultat de
            Veterinaria Universitat Autonoma de Barcelona, Campus Universitari,
            Bellaterra, Barcelona 08193, Spain
FEATURES             Location/Qualifiers
     source          1..646
                     /organism="Struthio camelus"
                     /db_xref="taxon:8801"
     misc_feature    1..646
                     /note="sex marker sequence"
BASE COUNT      213 a    113 c    108 g    207 t      5 others
ORIGIN
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       61 aaagcacaca aacttcanat ggtttcttga tgattttatt cattcacgat cacttgtaat
      121 aacttacatg tgcaaaattt cacaatacaa aatatactgt attttaaagg actttcacca
      181 taatccacct acatgcaatt tttgtaataa gacttattaa atgttcaata ggactttgga
      241 gcaccatgat atataaggtc agatgtagag ttgttaactg aagattgaga atattttata
      301 aagtgagatt aacagaaggc agtagaaaca acgaggaaga cttcaatatt acatgtttgg
      361 cccagcttta ctctccccaa aacaaagata cacatgctga aaatggaaat accatcagnc
      421 aacaacctga aaatcttatt gggatgctgg cccctatttg actgatgttt ataccattga
      481 tgttctgctt gtctttgcaa agacaacttt ttaagttgca ttattcattc aaaatacaac
      541 tgttgtagtt cagcaggtgt tatatatggt aatttcactg ttgtgtgttg aactgattca
      601 cagagtatta ccagcacgtt caaatgattt tcaacaggtg tagacc
//
```

ANEXO II. CONDICIONES DE AMPLIFICACIÓN Y SECUENCIA DE CEBADORES DE LOS MICROSATÉLITES

Se optimizaron las amplificaciones de los microsatélites en dos PCR múltiplex, que únicamente diferían en al temperatura de unión del cebador: Para los microsatélites L001, L012, L011 y Osm1 dicha temperatura fue de 58ºC, mientras que para L009, L014, Osm2 y Osm6 la temperatura fue de 60ºC. En ambos casos el ciclo térmico de las PCR consistió en una desnaturalización inicial a 94ºC durante 120seg., seguida de 27 ciclos de 30 seg. a 94ºC, 30 seg. a 58/60ºC y 30 seg. a 72ºC, y una extensión final de 15 minutos a 72ºC.

Las reacciones de PCR se realizaron en un volumen total de 15 µl, que contenían 1,5 µl de 10XPCR buffer, 0,15 µl de dNTP 5 mM, 0,15 µl de cada uno de los 8 cebadores (Cebador a y b de cada uno de los 4 microsatélites) a concentración 10 µM, 0,45 µl de $MgCl_2$ 50 mM, 1,6 U de Taq Polimerasa (GibcoBRL®) y 3 µl de la solución de ADN genómico. El resultado de la PCR se comprobó mediante electroforesis capilar con marcaje fluorescente en un equipo ABI Prism 310 Genetic Analyzer® (Perkin Elmer).

Cebador	**Secuencia 3´- 5´**
L001a	GAACCACATGACCTCAATCAG
L001b	GCAGAGCGCTTTGCAATATTC
L009a	ACCAAAGCGAGAGAGGCATTG
L009b	TCAAGCTGTCAGGGCAGCTC
L011a	ACTGAAGTTTCCTTCTCCCC
L011b	TTCCTGAAGCAACCACAC
L012a	AAAGGAGCAGTGGCTCTAGG
L012b	GAGCTCCACAAGAACAGGAC
L014a	CCAGTCAGGAGCACCTGTTC
L014b	AGAGCAGGGATGACTGTGGC
Osm1a	AATCTGCCTGCAAAGACCAG
Osm1b	TCCCAGTCTTGAAGTCAGCA
Osm2a	AAGCCACGGCAATGAATAAG
Osm2b	CCTCAACCATTCTGTGATTCTG
Osm6a	TTTGACCATTCAGCATGCAT
Osm6b	AGAACTGCTGCCTTTCCTCA