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ORIGINAL ARTICLE

A proteomic analysis of *Nipponia nippon* (ID#162)

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ABSTRACT

We investigated the proteome of a female Crested Ibis (*Nipponia nippon,* ID#162) that died on March 10, 2010 at the Sado Japanese Crested Ibis Conservation Center. Protein preparations from the brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum, ovary and neck muscle were subjected to in-solution shotgun mass spectrometry (MS)/MS analyses using an LTQ Orbitrap XL mass spectrometer. A search of the National Center for Biotechnology Information *Gallus gallus* databases revealed 4253 GI (GenInfo Identifier) numbers with the sum of the same 11 tissues examined in the Crested Ibis. To interpret the obtained proteomics data, it was verified in detail with the data obtained from the brain of the Crested Ibis. It has been reported that drebrin A is specifically expressed in adult chicken brain. In the shotgun proteomic analyses of the Crested Ibis, we identified drebrin A as a brain-specific protein. Furthermore, Western blotting analysis of the protein preparations from 10 tissues of the Crested Ibis and 150-day-old hens using anti-drebrin antibodies showed intensive expression of approximately 110 kDa polypeptides of drebrin in both brains. We believe firmly that the present data will contribute to initial and fundamental steps toward understanding the Crested Ibis proteome.

Key words: *Crested Ibis, drebrin A, Nipponia nippon, shotgun proteomic and LC-MS/MS.*

INTRODUCTION

The Crested Ibis (Aves, Pelecaniformes, Threskiornithidae, *Nipponia nippon*) is a critically threatened wild bird worldwide. The last wild Japanese Crested Ibis died in Japan in 2003. In 1999, Japan's Ministry of the Environment commenced a breeding program of Crested Ibises at the Sado Japanese Crested Ibis Conservation Center on Sado Island, in the Sea of Japan, using five birds which came as gifts from the Chinese government. The number of Crested Ibises in captivity has so far increased from five to 200.

Recently, the Crested Ibis has been genetically analyzed by examining its mitochondrial DNA. Yamagishi and his colleagues determined the mitochondrial DNA sequences of the Crested Ibis, and found a difference of only 11 base pairs (0.065%) between two wild Japanese Crested Ibises and a Chinese Ibis of a total 16 782 base pairs (Yamagishi *et al*. 2009). These differences indicate only minor differences between them, and hence these values are sufficiently trivial to regard them as belonging to the same species. The mitochondrial DNA of 17 stuffed Crested Ibis specimens, including one preserved at the Yamashina Institute for Ornithology, was also examined. The mitochondrial D-loop region is commonly used for the genetic classification of domestic fowl (Fumihito *et al*. 1996). Haplotype analysis was carried out of the Crested Ibis by D-loop region, resulting in it being classified it into four haplotypes. The results of this analysis indicate that the DNA sequence of a stuffed Crested Ibis found dead in Sado City in 1926 and preserved at the Niibo History and Folklore Museum on Sado, is the same as that of the Crested

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Ibis which has been bred at the Sado Japanese Crested Ibis Conservation Center (Yamagishi *et al*. 2009).

To conduct an effective breeding program (Urano *et al*. 2011; Kasuga *et al*. 2012; Kaneko *et al*. 2013), it is necessary to identify the global protein expression within each tissue in a Crested Ibis' body and to provide a biochemical, molecular biological and physiological baseline for the Crested Ibis. The genome analysis for Crested Ibis is reported to be complete (http://news.xinhuanet.com/english2010/sci/2011-04/15/c_13830955.htm); however, it cannot be used for proteomic analysis, as it has not been placed in the public domain. The genomes of three species of birds – chicken (Hillier *et al*. 2004; Wallis *et al*. 2004; Rubin *et al*. 2010), zebra finch, (Warren *et al*. 2010) and domestic turkey (Dalloul *et al*. 2010) – have so far been analyzed, and whole-genome analysis has been conducted. The chicken protein database provided by NCBI is available (Ramaroson *et al*. 2008; Sokale *et al*. 2011). It will be directly available for proteomic analysis with the further annotations of the zebra finchj and turkey genome. In this article, we focus and report on proteomic analysis conducted on 11 Crested Ibis tissues employing the shotgun method, using an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

MATERIALS AND METHODS

Tissues of Crested Ibis and chicken

Eleven tissues – brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum, ovary and neck muscle – were extracted from the dead female Japanese Crested Ibis (ID#162). These tissues were kept at −80°C until analysis. A 150-day-old hen (Boris Brown, layer chicken) was provided by a nearby poultry farm. Ten tissues – brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum and ovary – were extracted from the hen.

Protein preparation, electrophoresis and Western blotting

The organs were separately minced, lyophilized and powdered with a pestle and mortar in the presence of liquid nitrogen (Awang *et al*. 2010), and then water-soluble proteins were prepared. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), gels were stained with Coomassie Brilliant Blue R250 (CBB) (Umeki *et al*. 2006).

To perform Western blotting analysis, 10 μg of each protein extract was subjected to SDS-PAGE, followed by electroblotting to polyvinylidene fluoride (PVDF) membrane (Hybond-P; GE Healthcare, Tokyo, Japan). The blotted membrane was rinsed with phosphate buffered saline and 0.1% Tween 20 (PBST) containing 5% skim milk (Nanjo *et al*. 2006) and then incubated with primary monoclonal antibody against drebrin (mAbcam60932; Abcam, Tokyo, Japan) at 1/2000 dilution for 1 h at room temperature. It was then incubated with horseradish peroxidase-conjugated secondary antibody (goat polyclonal to mouse immunoglobulin G heavy and light chain (IgG-H&L); Cappel, Santa Ana, CA, USA) at 1/3000 dilution. The membrane treated with ECL™ (Amersham Pharmacia Biotech, Buckinghamshire, UK) was visualized at an exposure time of 6 min using a luminoimage analyzer LAS-3000 (Fuji Film, Tokyo, Japan).

LC-MS/MS analysis

Liquid chromatography – mass spectrometry/mass spectrometry (LC-MS/MS) analysis, which is a system that combines the KYA DiNA-A (KYA, Tokyo, Japan) and LTQ-Orbitrap XL (Thermo Fisher Scientific) was connected to an ion source which ionizes under the condition of ionization voltage 1.7–2.5 kV and a capillary transfer temperature of 200°C at the ESI nano stage. The analysis was then carried out. Two micrograms of the extracted protein was digested with trypsin, and desalted using a StageTip (Thermo) before it was injected into the nano-HPLC (high-performance LC). After the desalting process, the sample was subjected to re-solubilization in 2% acetonitrile (ACN)/0.1% formic acid (FA). It was applied to a trap column into which HiQ sil C18, 0.5 mm internal diameter (i.d.) × 1 mmL 3 μm particle size had been packed under conditions of 2% ACN/0.1% FA and a flow rate of 10 μL/min. Five minutes later, peptides were eluted from the separation column (HiQ silL C18W, 75 μm i.d. × 50 mmL 3 μm particle size) at a flow rate of 300 nL/min. Peptides were separated using a mobile phase gradient of solvent A and B: 4–33% of solvent B in 120 min, 33–100% in 125 min, 100% solvent B during 135 min. Solvent A was 2% ACN/0.1% FA; solvent B was 80% ACN/0.1% FA. LC-MS/MS data was acquired in data-dependent acquisition (DDA) mode controlled by Xcalibur 2.0 software (Thermo Fisher Scientific). A typical DDA cycle consisted of an MS scan within m/z 350–1600 performed under a target mass resolution of 60 000 followed by MS/MS fragmentation of the five most intense precursor ions under a normalized collision energy of 35% in the linear trap. Singly charged ions were dynamically excluded from the MS/MS experiments, and the m/z of fragmented precursor ions were dynamically excluded for a further 60 s. Protein identification was carried out using the Proteome Discoverer Version 1.3 and the SEQUEST search engine (Thermo Fisher Scientific) employing the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) *Gallus gallus* protein database (37 490 entries). The identification of peptides was performed using the following parameters: enzyme, trypsin; missed cleavages, 2; MS tolerance, 10 ppm; MS/MS tolerance, 0.8 Da; static modification, carbamidomethylation; dynamic modification, oxidation (H, M, W). False discovery rates for peptide identification were under 5.0%.

RESULTS

Tissue extraction and electrophoresis from the autopsy

An autopsy of the dead Japanese Crested Ibis (ID#162) was conducted by the Sado Japanese Crested Ibis Conservation Center. As can be seen from the autopsy pictures, it appears as fresh tissue without tissue damage or discoloration (Fig. 1). To consider whether proteomes can be examined using these tissues, water-soluble proteins were extracted from each of the 10 tissues and subjected to SDS-PAGE analysis. Fresh tissues from a 150-day-old hen were used as a control.

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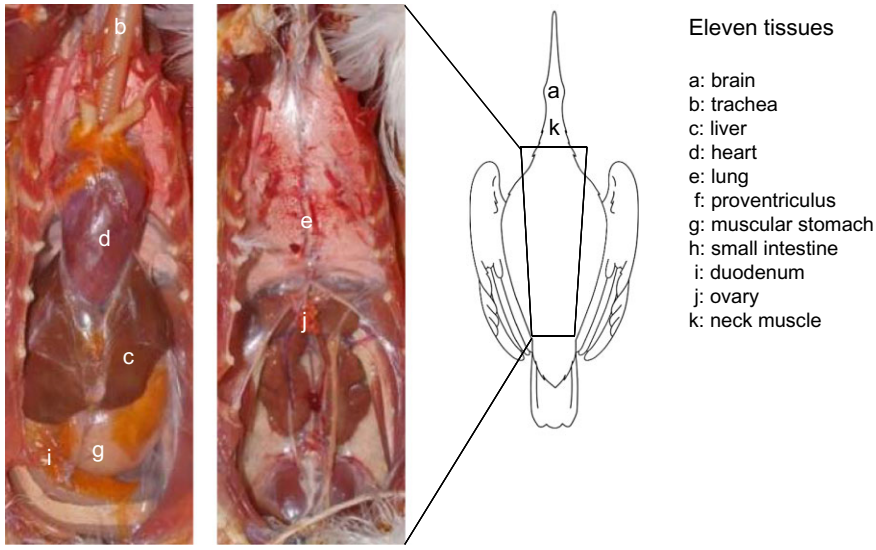

Figure 1 Dissection of the female Crested Ibis (*Nipponia nippon,* ID#162) that died on March 10, 2010 at the Sado Japanese Crested Ibis Conservation Center.

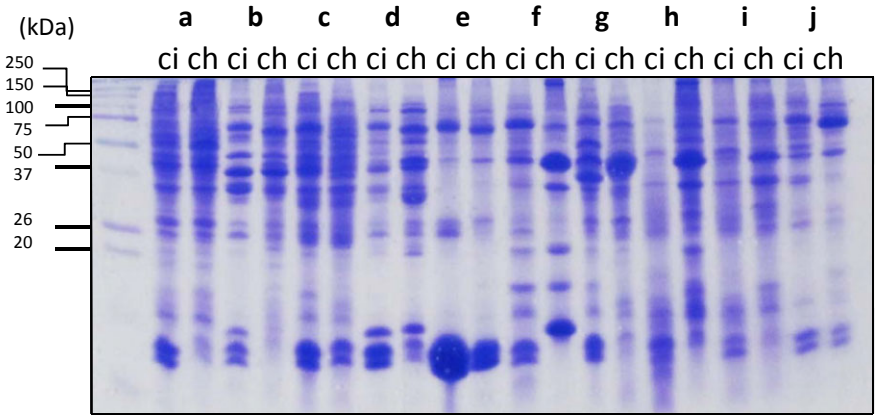

Figure 2 Quality identification of the proteins of 10 tissues of the Crested Ibis before proteome examination. The soluble proteins, prepared from 10 tissues of the Crested Ibis and of chicken, were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Coomassie Brilliant Blue staining. ci, Crested Ibis; ch, chicken: a, brain; b, trachea; c, liver; d, heart; e, lung; f, proventriculus; g, muscular stomach; h, small intestine; i, duodenum; j, ovary.

The separation profiles of tissue proteins from the Crested Ibis on SDS-gels indicated that most of the proteins had not broken down (Fig. 2). Furthermore, when comparing proteins from 10 tissues of the Crested Ibis and those of the chicken, although they were not exactly the same, they looked similar (Fig. 2). Judging from these results, we decided to use the proteins extracted from the tissues of dead Crested Ibises to characterize the proteome.

Shotgun proteomic analysis by LC-MS/MS

The protein preparations from the brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum, ovary and neck muscle of the Crested Ibis were digested with trypsin and subjected to LC-MS/MS analysis. Since the protein profiles of the Crested Ibis tissues on SDS-gel were closely similar to those of chicken, a database homology search was carried out using the SEQUEST search engine and the NCBI *Gallus gallus* protein databases: 4253 GenInfo identifier (GI) numbers were obtained as the sum of 11 tissues (Table 1): brain, 707; trachea, 235; liver, 134; heart, 251; lung, 75; proventriculus, 254; muscular stomach, 564; small intestine, 429; duodenum, 660; ovary, 654; neck muscle, 290 cases. A summary of protein lists identified in the Crested Ibis is shown in Tables 2–12, with more details in Tables S1–S11.

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Table 1 The total number of GenInfo identifier numbers of tissues of the Crested Ibis

707 of the brain
235 of the trachea
134 of the liver
251 of the heat
75 of the lung
254 of the proventriculus
564 of the muscular stomach
429 of the small intestine
660 of the duodenum
654 of the ovary
290 of the neck muscle

Western blotting analysis with anti-drebrin antibody

Shotgun proteomic analysis revealed that polypeptides of drebrin A (GI no. 410607), E2 (GI no. 410592) and E1 (*DBN1*, GI no. 45382803) were expressed in the brain tissues of the Crested Ibis in Table S1; on the other hand, these proteins were not detectable in the other tissues (Tables S2–S11). It has been reported that drebrin A is specifically expressed in the adult chicken brain (Kojima *et al*. 1993). The DNA sequence of *DBN1* is conserved in humans, chimpanzees, rhesus monkeys, dogs, cows, mice, rats and zebra fish (NCBI database, http://www.ncbi.nlm.nih.gov), indicating that the *drebrin* gene is conserved beyond species. To interpret the obtained proteomics data, the tissue-specific expression of drebrin in the Crested Ibis was examined by employing Western blotting analysis with a mouse monoclonal anti-human drebrin antibody (mAbcam60932). The commercially available monoclonal antibody exhibited cross-reactivity to mouse, chicken and quail in addition to human drebrin. The protein extracts of brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum and ovary from the Crested Ibis and chicken were applied to SDS-PAGE, followed by Western blotting. The expression of drebrin polypeptide (approximately 110 kDa) was found specifically in the brain tissues of both the Crested Ibis and chicken, whereas it was not or hardly detected in the other tissues of the Crested Ibis and chicken (Fig. 3C). The *Nipponia nippon* genome has not been placed in the public domain; however, the present results clearly indicate that part of the proteome of *Nipponia nippon* can be elucidated by using the *Gallus gallus* databases.

DISCUSSION

The proteome of brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum, ovary and neck muscle of a female Crested Ibis (*Nipponia nippon,* ID#162) was investigated employing in-solution shotgun MS/MS analysis. A homology search with the NCBI *Gallus gallus* protein database revealed 4253 GI numbers with the sum of 11 tissues examined in the Crested Ibis. Many interesting and suggestive proteins were identified in each tissue dissected from the body of the bird.

Interpretation of the proteomics data

Three well-known drebrin isoforms, drebrin A (110 kDa), E2 and E1 (Kojima *et al*. 1988, 1993) were detected in the brain proteomic analysis of the Crested Ibis as shown in Table S1. Although two protein isoforms (drebrin E2 and E1) are expressed in the embryo, drebrin A is the protein that is specifically expressed in the adult brain of the chicken (Kojima *et al*. 1988, 1993). It is a consequence of alternative splicing at the levels of messenger RNA (mRNA) from a single gene in a stage-dependent manner (Fig. 3A,B), (Kojima *et al*. 1988, 1993). Although the Western blot analysis clearly showed 110 kDa drebrin A to be exclusively expressed in the brain (Fig. 3C), the present proteomic analysis could not distinguish the three isoforms from drebrin A. Knowing this it is very important to read the obtained data in Tables S1 to S11.

Brain-specific proteins of Crested Ibis

The brain proteome of the Crested Ibis is summarized in Table 2. Drebrin was first isolated and identified as one of the intracellular regulators of the neuronal morphogenesis in the chicken brain (Kojima *et al*. 1988, 1993; Shirao *et al*. 1988), and the *DBN1* gene is conserved from humans to zebra fish through species. In the chicken embryo brain, three isoforms, two embryonic types (E1 and E2), and an adult type (A) were transcribed from a single gene through alternative RNA splicing mechanisms (Kojima *et al*. 1988, 1993; Shirao *et al*. 1988). In human drebrins, two isoforms 1(A) and 2(E) have been found in complementary DNA (cDNA) libraries (Toda *et al*. 1993; Fisher *et al*. 1994; Ota *et al*. 2004), while two isoforms of drebrin A/E were isolated in the rat (Shirao *et al*. 1989). Drebrin containing a homology domain of actin depolymerizing factor (ADF) (Lappalainen *et al*. 1998) has a side-binding protein of actin filament and developmental stages of neuronal cells in the brain (Shirao *et al*. 1989, 1990, 1992, 1994; Imamura *et al*. 1992; Asada *et al*. 1994; Ishikawa *et al*. 1994; Suda *et al*. 1994; Shirao 1995). Drebrin A and E in the adult human brain may be co-localized in postsynaptic terminals, and the disappearance of drebrin may contribute to the pathogenesis of memory disturbance in Alzheimer's disease (AD) (Harigaya *et al*. 1996). Decreased levels of drebrin have also been reported in the brains of patients with AD and Down syndrome (Shim & Lubec 2002). Hippocampal drebrin loss causes mild cognitive impairment in humans (Counts *et al*. 2012). In drebrin A-specific knockout

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Table 2 Protein identification of brain of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
45382393	Gamma-enolase	88.93	37.56	7	10	434	47.3	4.97	Metabolic process
45384340	Creatine kinase B-type	63.69	24.41	0	7	381	42.8	6.37	Metabolic process
363742094	**V-type proton atpase subunit B, brain isoform**[*1]	39.96	21.15	0	8	506	55.8	5.69	Metabolic process; transport
56606150	Amphiphysin	35.68	16.42	6	6	682	75.2	4.70	Cell organization and biogenesis; response to stimulus; transport
363745438	**Puromycin-sensitive aminopeptidase**[*1]	32.86	18.48	6	11	844	95.2	5.17	
45382773	Beta-synuclein	30.89	31.58	5	5	133	14.1	4.35	Cell death; metabolic process; regulation of biological process
45382765	Alpha-synuclein	28.01	26.57	3	3	143	15.0	4.70	Cell communication; cell death; cell organization and biogenesis; cellular homeostasis; defense response; metabolic process; regulation of biological process; response to stimulus; transport
46048768	Alpha-enolase	25.99	19.59	4	8	434	47.3	6.58	Cell growth; cell organization and biogenesis; metabolic process; regulation of biological process; response to stimulus
363737914	**Talin-2**[*1]	23.30	4.68	6	6	2542	271.6	5.55	Cell organization and biogenesis
363742430	**Adenylyl cyclase-associated protein 1**[*1]	17.15	6.70	2	2	537	57.6	7.99	Cell organization and biogenesis
12230748	WD repeat-containing protein 1	16.99	15.93	5	5	609	66.5	6.67	
363740107	**AP-1 complex subunit beta-1**[*1]	15.94	9.18	4	6	948	104.2	5.06	Transport
50731811	**V-type proton atpase subunit C 1**[*1]	15.13	10.73	3	3	382	44.1	7.37	Metabolic process; transport
363738596	**Synapsin-2**[*1]	7.86	8.32	3	3	637	69.6	8.54	Cell communication; transport
71895253	ATP-dependent RNA helicase DDX3X	6.56	1.54	1	1	651	72.0	6.99	
410607	Drebrin A	4.49	5.36	0	2	653	71.6	4.51	
462065	Fatty acid-binding protein, brain	2.81	16.67	1	1	132	14.9	5.91	Transport

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 3 Protein identification of proventriculus of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological Process
401094	Transgelin	13.12	9.50	3	3	200	22.3	8.81	Development
45383035	Filamin	8.69	1.38	1	3	2610	272.8	6.35	
363740308	**Glutamine synthetase-like**[*1]	8.26	8.56	2	2	374	42.0	6.42	Metabolic process
45383307	Acidic chitinase precursor	5.60	2.90	1	1	482	52.2	5.57	Metabolic process
118102574	**Prolargin**[*1]	4.71	5.61	2	2	374	42.9	8.95	
50732569	**Anterior gradient protein 2 homolog isoform 2**[*1]	4.69	6.40	1	1	172	19.8	8.85	Transport
50732203	**Serine/threonine-protein kinase OSR1**[*1]	4.36	5.07	2	2	533	58.1	6.34	Metabolic process; regulation of biological process; response to stimulus
118086389	**Adenylyl cyclase-associated protein 2**[*1]	3.62	3.13	1	1	480	53.2	6.80	Cell organization and biogenesis
118092977	**Xaa-Pro aminopeptidase 1**[*1]	3.23	1.44	1	1	623	70.0	6.15	Metabolic process
52345956	Aquaporin-4	2.58	6.57	1	1	335	36.2	8.63	Defense response; response to stimulus; transport
363728309	**Leukocyte cysteine proteinase inhibitor 1**[*1]	2.57	12.37	1	1	97	11.1	6.95	
363735661	**PDZ and LIM domain protein 1 isoform 4**[*1]	2.35	2.34	1	1	428	46.4	7.53	
363730896	**Ribonuclease UK114 isoform 1**[*1]	2.23	11.51	1	1	139	14.8	8.43	
50729726	**Transgelin-3**[*1]	2.10	6.03	1	1	199	22.5	7.87	Development
118103033	**Synaptic vesicle membrane protein VAT-1 homolog**[*1]	1.98	3.14	1	1	382	41.7	6.62	Metabolic process
363744123	**PI-PLC X domain-containing protein 3-like**[*1]	1.86	4.97	1	1	322	36.3	6.13	Metabolic process; regulation of biological process

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 4 Protein identification of lung of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
50755288	**Betaine–homocysteine S-methyltransferase 1**[*1]	10.25	8.64	2	2	405	45.0	7.64	Metabolic process
71894965	Catalase	7.11	8.09	0	1	235	26.2	8.62	Cell communication; cell death; cell organization and biogenesis; metabolic process; regulation of biological process; response to stimulus
50752703	**Sorbitol dehydrogenase**[*1]	6.93	5.07	1	1	355	38.1	7.39	Metabolic process
50767570	**Sepiapterin reductase**[*1]	2.69	4.48	1	1	268	28.8	6.09	Metabolic process
113351	Alcohol dehydrogenase 1	2.46	9.57	0	2	376	39.8	8.37	Metabolic process
169640761	Cationic amino acid transporter-2B	2.41	2.90	0	1	655	71.2	5.53	Defense response; metabolic process; regulation of biological process; response to stimulus; transport
169640763	Cationic amino acid transporter-2C	2.41	5.31	0	1	358	38.5	5.69	Defense response; metabolic process; regulation of biological process; response to stimulus; transport
238055157	Cationic amino acid transporter 2	2.41	2.91	0	1	654	71.2	5.53	Defense response; metabolic process; regulation of biological process; response to stimulus; transport
363734592	**Histone-lysine N-methyltransferase SUV420H1**[*1]	2.28	1.18	1	1	848	95.6	8.82	
363730614	**Gamma-aminobutyric acid type B receptor subunit 2**[*1]	2.18	0.90	1	1	890	101.0	8.44	

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 5 Protein identification of ovary of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
363744118	**Disabled homolog 2-like**[*1]	17.88	8.96	5	5	692	75.3	6.92	
118095485	**Sorting nexin-1**[*1]	16.39	10.89	3	5	514	58.1	5.41	Cell communication; transport
363741484	**Myelin transcription factor 1**[*1]	15.83	0.89	1	1	1118	123.5	4.93	Metabolic process; regulation of biological process
363747068	**Transgelin-2-like**[*1]	15.56	26.13	4	4	199	22.2	8.87	
118094103	**4-trimethylaminobutyraldehyde dehydrogenase**[*1]	14.28	8.56	4	4	514	56.1	7.64	Metabolic process
57525441	Peptidyl-prolyl cis-trans isomerase FKBP4	13.61	12.44	4	4	442	50.4	5.68	Metabolic process
363738196	**Synaptic vesicle membrane protein VAT-1 homolog-like**[*1]	13.38	9.57	3	3	418	45.8	5.10	Metabolic process
363728304	**Alpha-2-macroglobulin**[*1]	12.86	4.39	5	6	1479	164.5	6.52	
363745440	**Importin subunit beta-1-like**[*1]	11.82	3.59	2	2	863	95.7	4.82	Transport
50732309	**Kinesin-1 heavy chain**[*1]	11.37	3.52	2	3	966	110.1	6.44	Cell differentiation; cell organization and biogenesis; metabolic process
1730110	Very low-density lipoprotein receptor	5.86	2.32	2	2	863	94.8	5.01	Cell organization and biogenesis; development; metabolic process; regulation of biological process; transport
45383996	Nucleophosmin	4.77	3.06	0	1	294	32.6	4.78	Cell communication; cell growth; cell organization and biogenesis; cell proliferation; cellular homeostasis; metabolic process; regulation of biological process; response to stimulus; transport

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 6 Protein identification of liver of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
363742669	**Hepatoma-derived growth factor**[*1]	6.92	9.48	2	2	232	26.2	5.16	
118093509	**Isocitrate dehydrogenase [NADP] cytoplasmic**[*1]	6.41	5.30	2	2	415	46.6	7.94	Metabolic process
50750794	**Kynureninase**[*1]	6.08	2.74	1	1	474	53.2	6.48	Metabolic process
113351	Alcohol dehydrogenase 1	5.10	4.26	0	2	376	39.8	8.37	Metabolic process
284795259	Hepatitis B virus x interacting protein	4.84	21.98	1	1	91	9.5	4.87	Cell death; cell differentiation; metabolic process; regulation of biological process
50729254	**2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial**[*1]	4.00	3.34	1	1	419	45.2	8.22	Metabolic process
57530465	**Tyrosine–trna ligase, cytoplasmic**[*1]	3.97	2.28	1	1	527	59.2	6.65	Metabolic process
363739376	**Serine hydroxymethyltransferase, cytosolic**[*1]	3.85	5.57	1	1	485	53.4	8.00	Metabolic process
118083958	**Cystathionine beta-synthase**[*1]	3.63	5.20	1	1	558	61.7	7.59	Metabolic process
363743615	**Mitochondrial import inner membrane translocase subunit Tim13-A-like**[*1]	2.79	13.86	1	1	101	11.0	8.18	Cell organization and biogenesis; transport
118098116	**4-aminobutyrate aminotransferase, mitochondrial**[*1]	2.16	2.40	1	1	500	56.5	8.37	Metabolic process; response to stimulus

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 7 Protein identification of trachea of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
46048771	Adenylate kinase isoenzyme 1	20.74	29.90	5	5	194	21.7	8.59	Metabolic process
310772215	Phosphatidylethanolamine-binding protein 1	16.61	18.72	3	3	187	20.9	7.44	
363735918	**Titin**[*1]	14.16	0.19	0	5	34487	3831.6	6.39	Cell differentiation; cell organization and biogenesis; development; metabolic process
363734519	**Methylmalonate-semiald ehyde dehydrogenase [acylating], mitochondrialisoforme X2**[*1]	13.48	9.70	3	3	567	61.6	6.80	Metabolic process
363728304	**Alpha-2-macroglobulin**[*1]	11.59	4.80	5	6	1479	164.5	6.52	
45384504	Sarcalumenin precursor	9.93	8.07	3	3	471	54.1	7.03	Metabolic process
84619526	Phosphoglucomutase-1	5.20	3.15	2	2	603	66.6	8.82	Cellular homeostasis; metabolic process
82233792	Adenylosuccinate synthetase isozyme 2	3.04	3.10	1	1	451	49.4	6.32	Metabolic process
118102025	**Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial**[*1]	2.49	4.85	1	1	681	72.0	8.60	Metabolic process
118101125	**Aflatoxin B1 aldehyde reductase member 4**[*1]	2.05	4.60	1	1	326	36.6	7.23	Metabolic process
363747177	**Non-receptor tyrosine-protein kinase TYK2-like, partial**[*1]	1.68	1.78	1	1	673	75.9	7.12	Metabolic process
363729110	**Transmembrane and TPR repeat-containing protein 4**[*1]	1.64	1.64	1	1	791	89.2	9.09	

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 8 Protein identification of heart of the Crested Ibis

GI no.	Prorein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
45382651	Pyruvate kinase muscle isozyme	35.44	14.34	6	6	530	58.0	7.61	Metabolic process
118595754	Myoglobin	26.72	11.04	2	2	154	17.4	8.32	Cell differentiation; development; response to stimulus; transport
71896123	NADH dehydrogenase ubiquinone flavoprotein 1	13.44	13.29	4	4	459	50.1	8.31	Metabolic process
136463	Transthyretin	12.12	38.00	0	3	150	16.3	5.24	Cell differentiation; cell growth; cell organization and biogenesis; metabolic process; reproduction; response to stimulus; transport
54020693	ADP/ATP translocase 3	11.23	11.41	0	3	298	32.7	9.72	Transport
127137	Myosin light chain 1, cardiac muscle	9.77	18.04	1	3	194	22.0	5.29	Development; regulation of biological process
5509946	P32 subunit of splicing factor SF2	7.25	16.91	0	2	207	23.7	4.53	
71895985	Phosphoglycerate mutase 1	6.79	5.51	1	1	254	28.9	7.49	Metabolic process
45384002	Cathepsin D precursor	6.03	2.26	1	1	398	43.3	6.32	Cell communication; cell organization and biogenesis; metabolic process; response to stimulus
71894843	Fatty acid-binding protein, heart	5.51	17.29	0	2	133	14.8	6.33	Metabolic process; transport
46048795	Vitronectin precursor	5.14	3.97	1	1	453	51.6	5.35	Response to stimulus
50751398	Camp-dependent protein kinase catalytic subunit beta	4.82	6.03	1	1	398	46.0	8.40	Metabolic process
118102025	**Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial**[*1]	3.66	4.85	1	1	681	72.0	8.60	Metabolic process
118101780	**Adenylate kinase 2, mitochondrial**[*1]	3.23	6.67	1	1	240	26.1	8.27	Metabolic process

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 9 Protein identification of muscular stomach of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
401094	Transgelin	245.58	26.00	7	7	200	22.3	8.81	Development
57529439	6-phosphogluconate dehydrogenase, decarboxylating	49.17	16.36	5	5	483	53.3	6.98	Metabolic process
363741727	**Adenosylhomocysteinase A**[*1]	34.54	7.31	5	5	862	91.2	9.66	Metabolic process; transport
363734519	**Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial**[*1]	24.61	15.70	6	6	567	61.6	6.80	Metabolic process
310772215	Phosphatidylethanolamine-binding protein 1	24.30	19.25	4	4	187	20.9	7.44	
45383183	Dihydropyrimidinase-related protein 3	23.90	15.26	4	6	570	62.0	6.73	Development; metabolic process
2493432	Alpha-actinin-4	23.81	10.51	2	7	904	104.1	5.26	Transport
118086389	Adenylyl cyclase-associated protein 2	19.05	11.04	4	4	480	53.2	6.80	Cell organization and biogenesis
50755288	**Betaine–homocysteine S-methyltransferase 1**[*1]	18.15	13.83	4	4	405	45.0	7.64	Metabolic process
119331154	Profilin-2	17.95	20.71	2	2	140	15.0	6.99	Cell organization and biogenesis; regulation of biological process
118094103	**4-trimethylaminobutyraldehyde dehydrogenase**[*1]	14.40	8.56	4	4	514	56.1	7.64	Metabolic process
57530409	Cytosolic non-specific dipeptidase	13.34	11.16	3	3	475	53.0	6.05	Metabolic process
2842685	Myotrophin	12.89	14.41	1	1	118	12.9	5.29	Cell growth; cell organization and biogenesis; metabolic process; regulation of biological process
124249432	Rho GDP-dissociation inhibitor 1	12.39	17.65	3	3	204	23.3	5.29	Regulation of biological process
61098378	Aspartyl aminopeptidase	12.36	8.25	3	3	473	51.8	6.99	Metabolic process
57530288	Prolyl endopeptidase	10.80	8.73	4	4	710	80.7	6.06	Metabolic process
71896651	Alcohol dehydrogenase class-3	7.60	4.01	2	2	374	39.6	7.83	Metabolic process; response to stimulus
313760671	Ras suppressor protein 1	6.82	24.55	4	4	277	31.5	8.48	Regulation of biological process

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 10 Protein identification of small intestine of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
50732569	**Anterior gradient protein 2 homolog isoform 2**[*1]	18.33	24.42	3	3	172	19.8	8.85	Transport
118102948	**Proteasome subunit beta type-3**[*1]	11.39	8.78	1	1	205	23.1	5.41	Metabolic process
71896753	Heterogeneous nuclear ribonucleoproteins A2/B1	10.58	4.58	1	1	349	37.0	8.65	Metabolic process; transport
45382993	Collagen alpha-3(VI) chain precursor	9.89	1.88	4	4	3137	339.4	6.71	Metabolic process; regulation of biological process
1708877	Lumican	9.21	5.25	1	1	343	38.6	6.52	
226874900	Proteasome subunit alpha type-2	8.96	17.09	1	2	234	25.9	7.49	Metabolic process
118092623	**Inorganic pyrophosphatase**[*1]	7.61	5.52	1	1	290	32.6	5.76	Metabolic process
363744275	**Phosphoglucomutase 5**[*1]	6.69	5.64	2	2	567	62.7	7.14	Metabolic process
363742447	**Histone H2A.x-like**[*1]	6.56	22.38	1	2	143	15.0	10.87	
118101780	**Adenylate kinase 2, mitochondrial**[*1]	6.33	7.50	2	2	240	26.1	8.27	Metabolic process
261490822	Coactosin-like protein	6.30	11.27	1	1	142	16.1	5.44	
363735594	**Thioredoxin-dependent peroxide reductase, mitochondrial**[*1]	6.21	5.98	1	1	234	25.7	8.34	Metabolic process
71895985	Phosphoglycerate mutase 1	6.09	5.51	1	1	254	28.9	7.49	Metabolic process
71897287	Phosphoglucomutase 2	4.89	1.97	1	1	609	67.9	7.01	Metabolic process
118094989	**Coatomer subunit beta'**[*1]	4.76	1.20	1	1	913	103.1	5.24	Transport
57529515	Ornithine aminotransferase, mitochondrial	4.69	3.64	1	1	439	48.3	7.44	Metabolic process
45383183	Axin-2	4.53	3.16	1	1	570	62.0	6.73	Development; metabolic process
118094764	**Cystathionine gamma-lyase**[*1]	4.30	5.01	1	1	399	43.9	7.25	Cell organization and biogenesis; metabolic process

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 11 Protein identification of duodenum of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
45383890	Protein disulfide-isomerase A3 precursor	40.98	19.21	9	9	505	56.1	6.02	Cell death; cellular homeostasis; metabolic process; regulation of biological process
118092623	**Inorganic pyrophosphatase**[*1]	23.71	17.59	3	4	290	32.6	5.76	Metabolic process
57530409	Cytosolic non-specific dipeptidase	23.11	11.16	4	4	475	53.0	6.05	Metabolic process
71896431	Filamin-B	22.16	2.26	2	4	2567	275.7	5.92	Development
72535134	Cytoplasmic aconitate hydratase	17.42	5.96	1	3	889	98.0	7.33	Cellular homeostasis; development; metabolic process; regulation of biological process; response to stimulus
57530180	Plastin-3	14.43	8.92	2	5	628	70.8	5.74	
61098378	Aspartyl aminopeptidase	13.81	8.25	3	3	473	51.8	6.99	Metabolic process
118096822	**Transketolase**[*1]	13.52	6.06	2	2	627	68.4	7.52	Metabolic process
261490822	Coactosin-like protein	13.49	33.10	3	3	142	16.1	5.44	
45382931	Proteasome subunit alpha type-7	13.22	20.48	3	3	249	28.1	8.84	Metabolic process; regulation of biological process
363741621	Exportin-2	12.98	6.80	4	4	971	110.4	5.62	Transport
118083958	**Cystathionine beta-synthase**[*1]	11.81	5.20	1	1	558	61.7	7.59	Metabolic process
148277073	Thioredoxin reductase 1	10.45	7.82	2	3	499	55.0	6.27	Cell proliferation; cellular homeostasis; development; metabolic process; regulation of biological process
118102948	**Proteasome subunit beta type-3**[*1]	10.26	8.78	1	1	205	23.1	5.41	Metabolic process
363741727	**Adenosylhomocysteinase A**[*1]	9.65	5.22	4	4	862	91.2	9.66	Metabolic process; transport
56118984	Alpha-centractin	9.64	4.26	1	1	376	42.6	6.64	
71894765	Transaldolase	9.53	3.26	1	1	337	37.6	6.95	Metabolic process
363728070	**Tetratricopeptide repeat protein 38-like**[*1]	7.97	6.65	1	2	466	52.4	6.28	
363743351	**26S protease regulatory subunit 8**[*1]	6.03	2.68	1	1	411	46.1	7.55	Metabolic process; regulation of biological process; reproduction; response to stimulus

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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Table 12 Protein identification of neck muscle of the Crested Ibis

GI no.	Protein/description	Score	Coverage	# Unique peptides	# Peptides	# AAs	MW [kDa]	Calc. pI	Biological process
363734519	**Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial*1**	28.37	15.70	6	6	567	61.6	6.80	Metabolic process
363734951	**Adenylosuccinate synthetase isozyme 1 A*1**	14.91	11.83	4	4	507	56.8	7.08	Metabolic process
46048771	Adenylate kinase isoenzyme 1	14.82	29.90	5	5	194	21.7	8.59	Metabolic process
363735512	**Ankyrin repeat domain-containing protein 2-like*1**	9.65	7.49	2	2	334	37.8	5.44	
46048903	Voltage-dependent anion-selective channel protein 2	9.08	12.01	2	3	283	30.2	8.53	Regulation of biological process; transport
310772215	Phosphatidylethanolamine-binding protein 1	8.01	13.37	2	2	187	20.9	7.44	
118085838	**Probable acyl coa dehydrogenase 6-like*1**	5.74	6.10	2	2	410	44.9	8.15	Metabolic process
363735594	**Thioredoxin-dependent peroxide reductase, mitochondrial*1**	5.49	10.68	2	2	234	25.7	8.34	Metabolic process
118098350	**Cytochrome b-c1 complex subunit 2, mitochondrial-like*1**	5.07	3.94	0	1	457	48.5	8.94	Metabolic process
118094283	**Glycogen debranching enzyme*1**	4.38	0.52	1	1	1532	174.6	7.03	Metabolic process
313661454	Chaperone activity of bc1 complex-like, mitochondrial	2.84	3.55	1	1	648	72.4	6.81	
118087111	**Inositol monophosphatase 1*1**	2.68	3.60	1	1	278	30.1	5.72	Metabolic process; regulation of biological process
82075377	26S proteasome non-atpase regulatory subunit 1	2.53	1.26	1	1	955	106.0	5.36	Metabolic process; regulation of biological process
118101125	**Aflatoxin B1 aldehyde reductase member 4*1**	2.53	4.91	1	1	326	36.6	7.23	Metabolic process
363744671	**Zinc finger protein 462*1**	2.49	0.57	1	1	2452	277.8	7.55	

Protein names indicated in bold (Tables 2 to 12) refer to proteins that were never characterized before in chicken. The proteins listed in each table were sorted by tissue specificity and reliability. GI, GenInfo identifier; AA, amino acids; MW, molecular weight; Calc. pI, calculated isoelectric point.

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A

GI no.	Protein/Description	Score	Coverage	# Unique Peptides	# Peptides	# AAs	MW [kDa]	calc. pI	Molecular Function	Cellular Component	Biological Process
410592	drebrin E2, partial	4.49	26.52	0	2	132	14.1	4.58	protein binding	cytoplasm	cell differentiation; development
410607	drebrin A	4.49	5.36	0	2	653	71.6	4.51	protein binding		
45382803	drebrin	4.49	5.77	0	2	607	66.6	4.44	protein binding	cytoplasm	cell differentiation; development

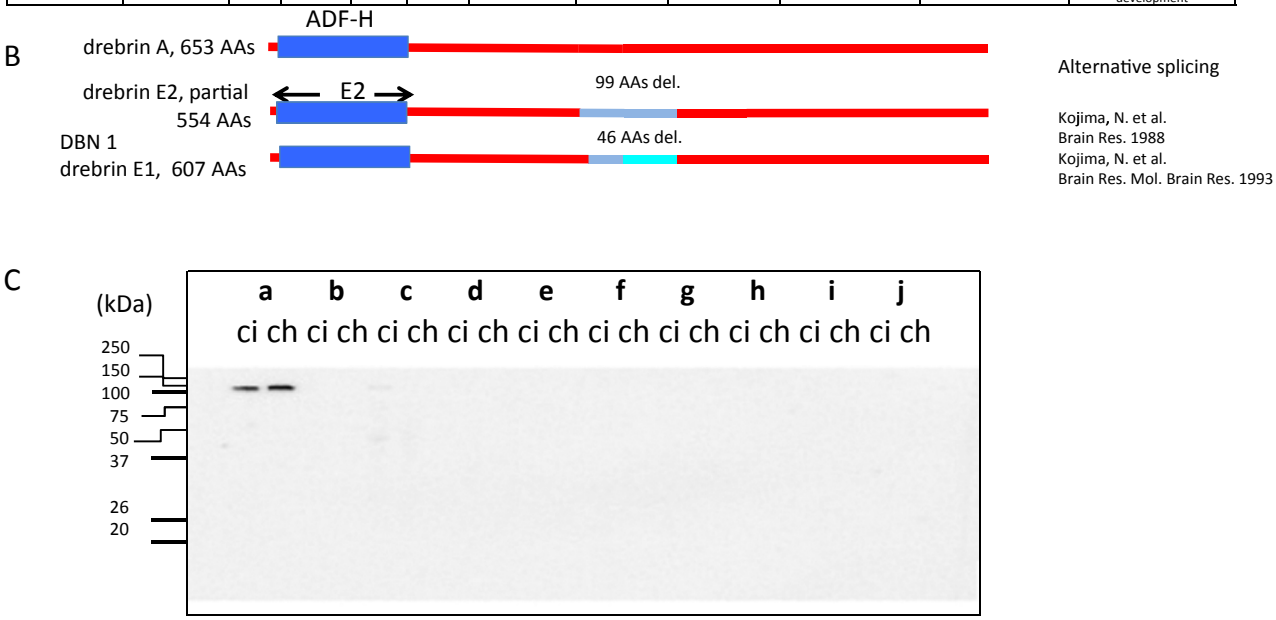

Figure 3 A case study of drebrin protein. (A) Selection of drebrin information in Table S1 (Crested Ibis brain). (B) Schematic illustration of protein structure of drebrin isoforms. (C) Western blotting (WB) analysis. The soluble proteins prepared from 10 tissues of the Crested Ibis and chicken were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Western blotting with anti-drebrin antibody. ci, Crested Ibis; ch, chicken: a, brain; b, trachea; c, liver; d, heart; e, lung; f, proventriculus; g, muscular stomach; h, small intestine; i, duodenum; j, ovary.

mice, drebrin A is involved in hippocampal synaptic plasticity and hippocampus-dependent learning during ageing (Kojima *et al*. 2010a,b). Drebrin A may thus have a critical function in the brain in Crested Ibises as well as in humans and other animals.

We also detected (alpha/beta)-synuclein (GI no. 45382765)/(GI no. 45382773) in the brain proteins. The genes for chicken alpha/beta-synucleins were cloned and characterized as being expressed in the developing embryo (Tiunova *et al*. 2000). Alpha-synuclein was detected in the zebra finch optic tectum by Western blot analysis (Sloley *et al*. 2007). This protein has been linked with schizophrenia-related proteins in humans (Ahmad *et al*. 2012; Boassa *et al*. 2013; Lashuel *et al*. 2013; Sanchez-Guajardo *et al*. 2013; Singh *et al*. 2013). The Crested Ibis may thus, like humans, have a tendency to schizophrenia with ageing.

Proventriculus protein

A water channel family protein, aquaporin-4 (AQP4) was found in the soluble fraction of the Crested Ibis proventriculus (Table 3), although AQP4 was an integral membrane protein. AQP4 was not detected in the other tissues. It is possible that the proventriculus AQP4 is more easily released from the membrane than the other AQP4. The *Gallus gallus AQP4* gene (GI no. 52345956) located on chromosome 2 has been cloned (Saito *et al*. 2005). It encodes a protein with 335 amino acid residues. The *AQP4* gene and its product are highly conserved in humans, chimpanzees, rhesus monkeys, dogs, cows, mice, rats, zebra fish, fruit flies, mosquitos, *Magnaporthe oryzae*, *Arabidopsis thaliana*, rice and chickens (http://www.ncbi.nlm.nih.gov).

Lung protein

The detected lung proteins of the Crested Ibis, including the cationic amino acid transporter-2 (CAT-2) are summarized in Table 4. The *CAT-2* gene has been cloned and characterized in chicken (Humphrey *et al*. 2008), as the gene is located on chromosome 4, having a span of over 76 kb with 16 exons. It has been shown that the chicken *CAT-2* gene is alternatively spliced to produce three isoforms (CAT-2A/B/C) (GI no. 238055157) (GI no. 312283680) (GI no. 169640763), and an abundant expression of

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chicken CAT-2C isoform mRNA is observed in the pectoralis of 2-week old chicks. The pectoralis cells actively absorb arginine, which is utilized to produce nitric oxide (NO). Analysis of cytokine-activated macrophages of CAT-2 knockout mice revealed a reduction in arginine uptake and in NO production (Nicholson *et al*. 2001). Furthermore, CAT-2 has a critical role in regulating inflammatory responses in the lung (Rothenberg *et al*. 2006), and is an important regulator of fibrotic responses in the lung and an allergen-induced gene in experimental asthma (Niese *et al*. 2010). The CAT-2 (*SLC7A2*) gene and its product are conserved in humans, chimpanzees, rhesus monkeys, dogs, cows, mice, rats, zebra fish, fruit flies, *Caenorhabditis elegans*, *A. thaliana* and chickens (http://www.ncbi.nlm.nih.gov). We thus infer that the *CAT-2* gene plays a role in regulating inflammatory responses in the lung of the Crested Ibis.

Ovary proteins

The detected ovary proteins of the Crested Ibis, including nucleophosmin (NPM) (GI no. 45383996) and very low-density lipoprotein receptor (VLDL-R) (GI no. 1730110), are summarized in Table 5. The *NPM* gene mapped on chromosome 13 was isolated and characterized in chicken embryos (Maridor & Nigg 1990). NPM is involved in post-transcriptional regulation of chicken connective tissue growth factor (ccn2) mRNA during chondrocyte differentiation (Mukudai *et al*. 2008). Chicken NPM resembles human NPM, which participates in a continuous shuttle between the nucleus and cytoplasm (Borer *et al*. 1989). Analysis of NPM knockout mice revealed that NPM is essential for embryonic development and the maintenance of genomic stability (Grisendi *et al*. 2005). In human cancer, a growing body of evidence shows that NPM is directly implicated in the pathogenesis of cancer, for example NPM binding to the alternative reading frame (ARF) tumor suppressor gene (Moulin *et al*. 2008). NPM is therefore likely to have a critical function in the Crested Ibis.

The *VLDL-R* gene and its product are conserved in humans, chimpanzees, rhesus monkeys, dogs, cows, mice, rats, zebra fish, fruit flies, mosquitos, *C. elegans* and chickens (http://www.ncbi.nlm.nih.gov). In chickens, VLDL-R has been identified in the cDNA library of the adult ovary, and Northern blot analysis has shown that exclusive expression of VLDL-R occurs in the ovary (Bujo *et al*. 1994). The *VLDLR* gene is mapped on the sex chromosome Z: the male sex chromosomes are composed of two Z chromosomes. (In bird species, the female sex chromosomes are composed of Z and W chromosomes.) Immunoaffinity chromatographic analysis of VLDL-R showed its role in receptor-mediated endocytosis (Barber *et al*. 1991). The VLDL-R is an essential receptor in avian species: receptor-deficient mutant hens are sterile and exhibit severe hyperlipidemia with aortic atherosclerosis (Bujo & Yamamoto 1996). It is likely that the Crested Ibis VLDL-R also serves a critical function in conception.

Other proteins

Alcohol dehydrogenase 1 (ADH-1) (GI no. 113351) was detected in the liver (Table 6) and lung (Table 4) of the Crested Ibis. The *ADH-1* gene and its product are conserved in humans, chimpanzees, rhesus monkeys, dogs, cows, mice, rats and chickens (http://www.ncbi.nlm.nih.gov). The chicken *ADH-1* gene was isolated from the chicken liver cDNA library (Estonius *et al*. 1990) and mapped on chromosome 4. The Crested Ibis would thus have a tolerance to alcohol.

Catalase (GI no. 71894965), gamma-aminobutyric acid type B receptor subunit 2 (GABA(B)R2) (GI no. 363730614) and histone-lysine N-methyltransferase SUV420H1 (GI no. 363734592) were detected in the lung proteome of Crested Ibis (Table 4). Catalase (*CAT*), GABA(B)R2(*GABBR2*) and *SUV420H1* genes are conserved in a wide range of species, including chicken. In vertebrates, GABA(B)R2 is the major inhibitory neurotransmitter in the central nervous system. The expression of GABA(B)R2 was shown to be present throughout the rat's auditory system using Western blot and immunohistochemical analyses (Jamal *et al*. 2011). It has also been reported that GABA(B)R2 is present in rat testis and sperm, but not in the lung (Jamal *et al*. 2011).

We characterized the partial proteome of the Crested Ibis (*Nipponia nippon*) using the *Gallus gallus* databases in the present study. Needless to say, *Nipponia nippon* is bound to have a unique proteome. *Nipponia nippon* genome DNA sequencing has recently been performed (http://news.xinhuanet.com/english2010/sci/2011-2004/15/c_13830955.htm) and its completion will open a new era in *Nipponia nippon* molecular studies, including proteomics.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Protein identification of the brain of the Crested Ibis.
Table S2 Protein identification of the proventriculus of the Crested Ibis.
Table S3 Protein identification of the lung of the Crested Ibis.
Table S4 Protein identification of the ovary of the Crested Ibis.
Table S5 Protein identification of the liver of the Crested Ibis.
Table S6 Protein identification of the trachea of the Crested Ibis.
Table S7 Protein identification of the heart of the Crested Ibis.
Table S8 Protein identification of the muscular stomach of the Crested Ibis.
Table S9 Protein identification of the small intestine of the Crested Ibis.
Table S10 Protein identification of the duodenum of the Crested Ibis.
Table S11 Protein identification of the neck muscle of the Crested Ibis.

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