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Genetic barcoding confirms first breeding record of the Yellow Bittern, *Ixobrychus sinensis*, (Aves: Pelecaniformes, Ardeidae) in the Western Palearctic

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Abstract

We confirmed the first breeding record of the native Asian yellow bittern (*Ixobrychus sinensis*) in the Western Palearctic by genetic barcoding and morphometric analysis. In 2012 three small herons had been captured in Wadi Lahami at the western Red Sea coast of Egypt and these individuals had been tentatively determined as yellow bitterns. From blood samples of these birds we amplified and sequenced the barcoding marker (cytochrome-oxidase) and cytochrome-*b* and used sequences from own samples and further data inferred from GenBank for comparison. All three Egyptian specimens in question formed a monophyletic clade with sequences of *I. sinensis* and these were well separated from the sister species *I. minutus* in both phylogenetic reconstructions (p-distance, cytochrome-*b*: 9.6 %). A discriminant analysis based on six body-size parameters confirmed the assignment of these Egyptian specimens to *I. sinensis*. Along with this documentation we discuss phylogenetic relationships within Botaurinae and biogeographic aspects.

Key words

Yellow bittern, *Ixobrychus sinensis*, genetic barcoding, Botaurinae, North Africa.

Introduction

A network of national rarities committees in Europe steadily ensures the documentation and verification of rare bird records closely following the guidelines recommended by the Association of European Records and Rarities Committees (Barthel *et al.*, 1993a, 1993b). Painstaking efforts are made in order to verify field records documented by photographs or voice recordings and to distinguish vagrant individuals from those who have eventually escaped from captivity on the one hand and from those individuals for which breeding was suspected or even proven on the other hand. First breeding records of rare species outside their known breeding range might hint to recent establishment of new breeding populations due to range expansions and should therefore be subjected to thorough examination.

Recent avian faunal interchange among continental Europe and North Africa occurred bidirectionally in multiple colonization events during the Pleistocene and the Holocene until today. In the 1960ies and 1970ies, historical invasions from North Africa to the Iberian Peninsula have been documented based on first breeding records of previously non-native species, such as trumpeter finch,

Bucanetes githagineus (García, 1972), little swift and white-rumped swift, *Apus affinis* and *A. caffer* (Allen & Brudenell-Bruce, 1967; Del Junco & Gonzalez, 1969), black-winged kite, *Elanus caeruleus* (England, 1963) and as one very recent example the long-legged buzzard, *Buteo rufinus cirtensis* (Elorriaga & Muñoz, 2010). On the other hand there are also several examples of ongoing range expansions of Eurasian species to or within North Africa, such as the Eurasian collared dove, *Streptopelia decaocto* (Yahia & Hamza, 2011) and the little bittern, *Ixobrychus minutus* (Hering & Fuchs, 2011).

Occasional records of East Asian species in Europe are even more rare events, and one of the very rare Asian bird records in North Africa was documented from Egypt in 2012: In the mangroves of Wadi Lahami at the western Red Sea coast two bitterns had been captured on 26.04.2012 and based on differences in size and plumage colour pattern from the Western Palaearctic little bittern *Ixobrychus minutus* these birds had been tentatively determined as yellow bitterns *I. sinensis* (Hering *et al.*, 2013). However, the collection site was located several thousand kilometres West from the westernmost limits of the Asian breeding range of the yellow bittern which, except for a few scattered Arabian and island populations, extends from Japan and the Korean Peninsula in the North across large parts of China far into the Indomalayan Realm in the South (del Hoyo *et al.*, 1992; BirdLife International, 2013; Barthel & Hering, 2013). The bitterns were ringed and measured for a number of morphological parameters, blood samples were taken for genetic analyses and all individuals were released. About one month later (29.05.2012) one of the ringed birds was recaptured along with a further unringed bird. By chance, two of the ringed individuals from 2012 were recaptured one year later at the same site in Wadi Lahami (08.07.2013). Sound recordings were analysed and identified as yellow bittern songs and three nests with egg remains were found in the mangroves near a lagoon (Hering *et al.*, 2013).

In order to confirm morphological and bioacoustic species determination in the field we performed a molecular genetic analysis with the samples taken from all Egyptian specimens including sequencing of the standard barcoding marker cytochrome-oxidase I. We furthermore took measurements from museum specimens of little bittern and yellow bittern and compared them with the measurements taken from the respective individuals in the field.

Material and methods

Molecular genetics

We extracted DNA from blood samples of six birds from North Africa (Egypt: three specimens from Wadi Lahami, two specimens from Abu Simbel; Libya: one specimen North of Benghazi; Table 1) in a chloroform-isoamylalcohol extraction. For intra- and intergeneric comparison fresh tissue samples were available for *I. minutus*, *I. sinensis* and *I. eurhythmus*. Further tissue samples were taken from whole skins of the Dresden collection, the oldest being collected in 1937 (Table 1). In order to avoid contamination, skin samples taken from museum specimen were processed in a separate clean lab. There, each step of analysis (sampling, extraction, PCR) was performed under separate working benches. DNA was extracted using the sbeadex® forensic kit (LGC Genomics) according to the manufacturer's instructions with small modifications, e.g. overnight incubation of tissue with proteinase K (instead of one hour) and elution with 50 µL of sterile water (instead of 100 µL elution volume).

For genetic species identification we chose two mitochondrial genes: the standard barcoding marker cytochrome-oxidase I (COI) and cytochrome-*b* (cyt-*b*). Standard PCR with universal bird primers for COI (Hebert *et al.*, 2004; Kerr *et al.*, 2007, 2009a) did not yield any satisfying results. We therefore designed specific primers using two alignments including GenBank sequences of several heron species; COI: (BirdF1-Ixmod = 5′-GGA ACC GCC CTA AGC CTA C-3′ and BirdR1-Ixmod = 5′-AGG ATR TAG ACT TCT GGG TG-3′); cyt-*b*: (Ixo-cyt*b*F = 5′-ACA CAA ATC CTA ACC GG-3′; Ixo-cyt*b*R = 5′-AGG ATT AGG AGR ATT GTG-3′). DNA extracted from museum specimen samples was amplified in two shorter fragments for COI (primer combinations: fragment 1: BirdF1_Ixmod = 5'-GGA ACC GCC CTA AGC CTA C-3' and BirdR2_Ixmod = 5'-CGT GGG AAT GCT ATG TCG G-3'; fragment 2: BirdF2_Ixmod = 5'-TYG GAG GAT TYG GAA AC-3' and BirdR3_Ixmod = 5'-ATG AAG TTG ATT GCC CC-3'). Optimization of lab protocols was achieved via gradient PCRs in order to estimate the optimal annealing temperature for every primer combination, PCR protocols were as follows: for cytochrome-*b* denaturation at 94 °C for 5 min followed by 35 repeats of denaturation at 94 °C for 45 sec, annealing at 53 °C for 45 sec, and elongation at 72 °C for 60 sec and terminated by a final elongation phase at 72 °C for 5 min. This standard protocol was slightly modified only for the whole fragment of COI (annealing temperature: 55 °C) and the two smaller fragments of COI (annealing temperatures: 51 °C and 50 °C for fragments 1 and 2, respectively). PCR products were purified using the ExoSAP-IT enzymatic cleanup (USB Europe GmbH, Staufen, Germany; modified protocol: 30 min at 37 °C, 15 min at 80 °C). Subsequent sequencing reactions were performed using the primers indicated above, and nucleotide sequences were resolved on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were included in the according GenBank alignments of 611 bp for COI and 898 bp for cyt-*b*.

Phylogenetic reconstructions were carried out for each of the two mitochondrial markers separately using Bayesian inference of phylogeny with MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001). The GTR + I model

Table 1. Samples and sequences of bitterns and herons used for phylogenetic reconstruction (including accession numbers for cytb and COI; own sequences in bold); *= sequence inferred from GenBank; **= sequence shorter than 200bp (provided in Appendix); ***= mitochondrial genome, same GenBank number for cytb and COI; Collection acronyms: MTD_C= Senckenberg Natural History Collections, Dresden (SNSD); tissue samples at SNSD: MAR= J. Martens, TC= tissue catalogue; USNM= Smithsonian National Museum of Natural History, USA; UWBM= Burke Museum of Natural History and Culture, USA; ROM= Royal Ontario Museum, Canada; YIO= Yamashina Institute for Ornithology, Japan; NSMTA= National Museum of Nature and Science Tokyo, Japan; MIY= Miyakojima City Museum, Japan; MACN= Museo Argentino de Ciencias Naturales, Argentina.

sample Id	species	country	locality	cytb	COI
MTD_C34678**	*Ixobrychus minutus*	Germany	Saxony, Dresden-Löbtau	—	—
MTD_C40774**	*Ixobrychus minutus*	Germany	Saxony, Zabelitz	—	—
MTD_C44007	*Ixobrychus minutus*	Germany	Saxony, Waldteiche Moritzburg	—	KJ941181
MTD_51194	*Ixobrychus minutus*	Germany	captivity, Tierpark Berlin	—	KJ941180
MTD_ZooDD2012	*Ixobrychus minutus*	Germany	captivity, Zoo Dresden	KJ941169	KJ941175
MAR8954	*Ixobrychus minutus*	Germany	Hesse, Gießen	KJ941162	KJ941177
MAR7430	*Ixobrychus minutus*	Libya	Cyrenaica, N of Benghazi	KJ941170	KJ941176
MAR8623	*Ixobrychus minutus*	Egypt	Abu Simbel	KJ941167	KJ941179
MAR8629	*Ixobrychus minutus*	Egypt	Abu Simbel	KJ941168	KJ941178
UWBM 56888*	*Ixobrychus minutus*	Russia	Astrakhanskaya Oblast, Astrakhan	—	GQ481994
PB53/3	*Ixobrychus minutus*	Afghanistan	Khoster Basin, 230 km SE of Kabul	—	KJ941174
MAR8394	*Ixobrychus sinensis*	Egypt	Wadi Lahami	KJ941163	KJ941172
MAR8601	*Ixobrychus sinensis*	Egypt	Wadi Lahami	KJ941165	KJ941173
MAR8648	*Ixobrychus sinensis*	Egypt	Wadi Lahami	KJ941164	KJ941171
AY465750*	*Ixobrychus sinensis*	—	unknown	AY465750	—
USNM 641867*	*Ixobrychus sinensis*	Japan	Chugoku, Iwakuni	—	JF499138
YIO—20676*	*Ixobrychus sinensis*	Japan	Honshu, Aichi, Chita-gun, Mihama-cho	—	AB843552
YIO—64002*	*Ixobrychus sinensis*	Japan	Nansei Isl., Okinawa, Shimajiri—gun	—	AB843553
YIO—62832*	*Ixobrychus sinensis*	Japan	Izu Isl., Tokyo, Hachijo-machi	—	AB843554
Mur17561	*Ixobrychus eurhythmus*	Russia	Oblast Amur, Muraviovka Nature Reserve	KJ941166	KJ941182
UWBM 71976*	*Ixobrychus eurhythmus*	Russia	Primorsky Kraj, Gayvoron	—	GQ481989
UWBM 47046*	*Ixobrychus eurhythmus*	Russia	Khabarovski Kraj, Khurmuli	—	GQ481990
UWBM 47126*	*Ixobrychus eurhythmus*	Russia	Khabarovski Kraj, Khurmuli	—	GQ481991
UWBM 71975*	*Ixobrychus eurhythmus*	Russia	Primorsky Kraj, Gayvoron	—	GQ481992
UWBM 71990*	*Ixobrychus eurhythmus*	Russia	Primorsky Kraj, Gayvoron	—	GQ481993
HQ690247***	*Ixobrychus cinnamomeus*	—	—	HQ690247	HQ690247
YIO—65127*	*Ixobrychus cinnamomeus*	Japan	Okinawa	—	AB843549
MIY3*	*Ixobrychus cinnamomeus*	Japan	Okinawa, Sakishima Isls, Miyakojima	—	AB842852
NSMTA—17743*	*Ixobrychus cinnamomeus*	Japan	Okinawa, Kunigashira	—	AB842853
AY465749*	*Ixobrychus flavicollis*	China	—	AY465749	—
ROM 1B 3954*	*Ixobrychus exilis*	Canada	Ontario	AF193832	DQ433699
ROM 1B 3514*	*Ixobrychus exilis*	Canada	Ontario	—	DQ433698
MACN—Or—cp—5*	*Ixobrychus involucris*	Argentinia	Buenos Aires	—	FJ027686
RBINS 12306*, **	*Ixobrychus sturmii*	DR of Kongo	—	—	HQ997924
MTD_TC410	*Ardea cinerea*	Germany	Saxony, Obertriebel	KJ941159	KJ941183
MTD_TC752	*Ardea cinerea*	Germany	Saxony, Bärnsdorf bei Moritzburg	KJ941158	KJ941185
MTD_TC824	*Ardea cinerea*	Germany	Saxony, Radeburg—Bärnsdorf	KJ941161	KJ941184
Ardcin10*	*Ardea cinerea*	India	Andhra Pradesh	—	HM804908
MAR5955	*Ardea purpurea*	Republic of Cape Verde	Santiago	—	KJ941186
MAR4050	*Ardea purpurea*	Republic of Cape Verde	Santiago	KJ941160	KJ941187
Ardpur4*	*Ardea purpurea*	India	Andhra Pradesh	—	HM804909
Ardpur6*	*Ardea purpurea*	India	Andhra Pradesh	—	HM804868

was applied as the best-fit substitution model to both data sets (according to estimates with MrModeltest 2; Nylander, 2004) and sequence data were additionally partitioned by codon position. Bayesian inference of phylogeny was carried out using the Metropolis-coupled Markov chain Monte Carlo algorithm with two parallel runs, each with one cold and three heated chains. Convergence of the two runs was confirmed by average standard deviations of split frequencies approaching zero. The chains ran for 10^6 generations with every 100^{th} generation sam-

Fig. 1. Yellow bittern (*Ixobrychus sinensis*); Egypt, Wadi Lahami, July 2013; picture: J. Hering.

pled (burn-in: 3000). The remaining trees were used for generating a 50% majority rule consensus tree. For one missing African species, *I. sturmii,* only a short barcode sequence was available, that was shown to differ considerably from the same COI fragment of *I. minutus* (Sonet *et al.*, 2011). We therefore performed an independent run with MrBayes including the short *I. sturmii* sequence in order to roughly infer its phylogenetic relationships.

In addition we carried out a genetic sex determination for the Egyptian birds according to the heron-specific protocols given in Wang *et al.*, (2011).

Morphology

Six morphological parameters of feather proportions, bill and tarsus were measured according to the standardized methods given in Eck *et al.*, (2011): wmax, bsk, bp, bwp, tar, tdiad. A digital calliper was used to take measurements from 61 specimens of three bittern species housed at Senckenberg Naturhistorische Sammlungen Dresden (SNSD): *I. minutus* (n=30), *I. sinensis* (n=21), *I. eurhythmus* (n=10). The morphological data set was analyzed in a discriminant analysis and results were corroborated by further statistical tests with SPSS 11.5.1.

Results

Molecular genetics

According to molecular sexing the two specimens from Abu Simbel turned out as females (two PCR bands) while the three specimens from Wadi Lahami were identified as males (one single PCR band; not shown). Based on the mitochondrial sequence data the five birds from Egypt could be assigned to two different bittern species. As expected, the two mitochondrial genes yielded the same groupings. The phylogeny based on the barcoding marker COI is shown in Fig. 2. Samples MAR8623 and MAR8629 from Abu Simbel (both females) clustered with all samples of *I. minutus* in all reconstructions. Pairwise differences among cyt-*b* sequences of *I. minutus* corresponded to a maximum of four substitutions (863 bp; uncorrected p-distance of 0.5 %). COI sequences of the three samples from Wadi Lahami clustered with three *I. sinensis* sequences from Japan and one further sequence of unknown origin. Sequences from Japan and from Egypt clustered in two separate clades, however none of the two clades received reliable support from Bayesian posterior probabilities (Fig. 2). Analogously cyt-*b* sequences of the Egyptian individuals determined as yellow bitterns according to their phenotype clustered with one *I. sinensis* sequence of unknown origin. Pairwise differences among all *I. sinensis* cyt-*b* sequences corresponded to a maximum of five substitutions (three of them separating the Egyptian sequences from the GenBank sequence) and an uncorrected p-distance of 0.6 %. In all phylogenetic reconstructions *I. minutus* and *I. sinensis* were sister taxa and differed from each other by a maximum of 83 substitutions which corresponded to 9.6 % p-distance (cyt-*b*).

The mitochondrial phylogeny based on 1512 bp of concatenated cyt-*b* and COI sequences shows Botaurinae as a well supported group with South American *Zebrilus undulatus* as a basal offshoot from the remaining taxa (Fig. 3). The clade uniting all study species of *Ixobrychus* and *Botaurus* received strong support, however only the latter genus appeared as monophyletic. Three major clades could be distinguished, two of them representing

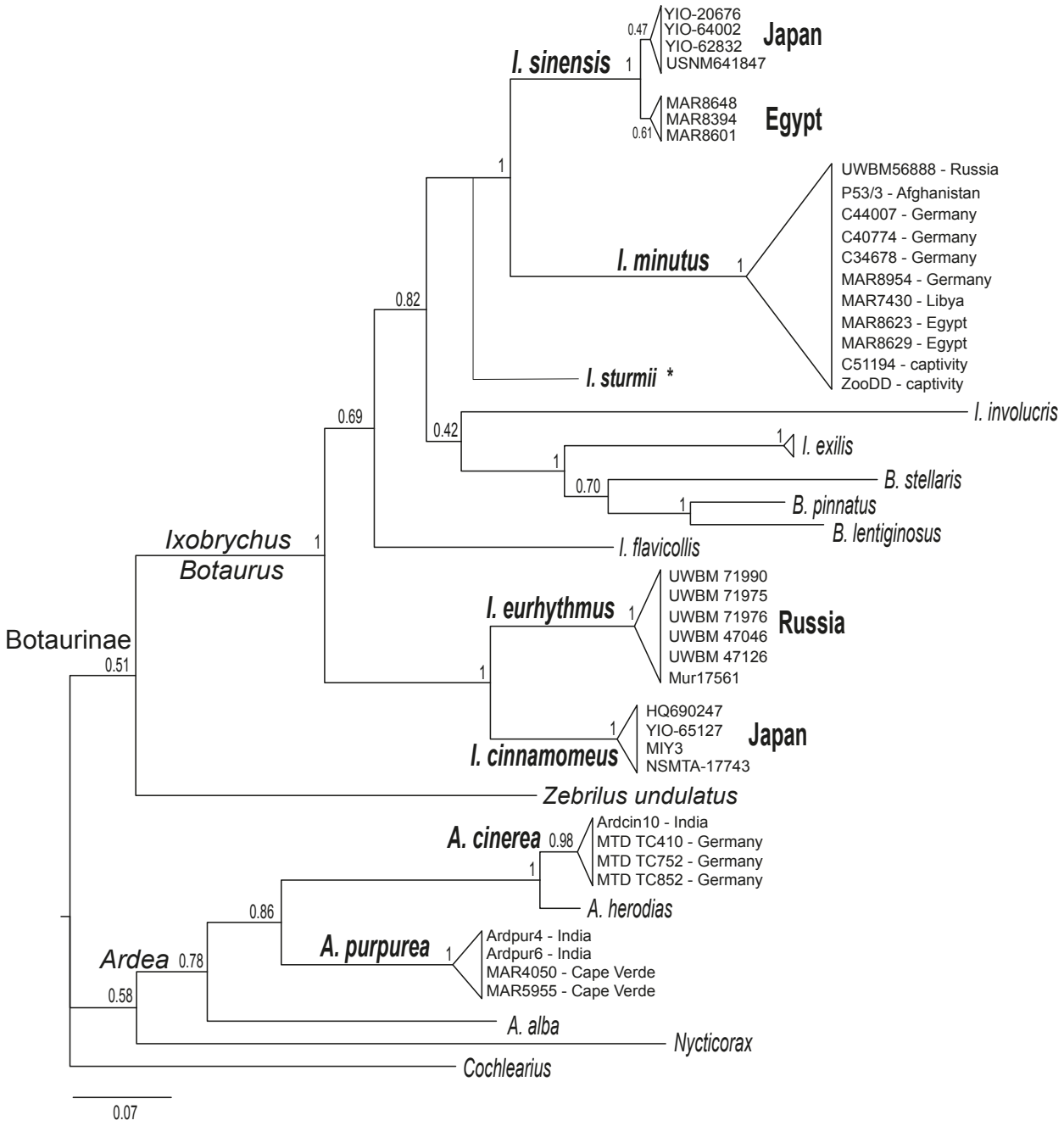

Fig. 2. Bayesian mitochondrial phylogeny of Botaurinae and allied heron species based on a 611-bp alignment of the standard barcoding marker COI, 611 bp, partitioned by codon; sample/specimen numbers and sampling sites shown at respective clades; *= position of a short COI sequence of African *I. sturmii* as inferred from an independent run with MrBayes indicated by thin grey line.

Old World radiations (Fig. 3, clade 1a, 1b) and one rather representing a New World radiation (Fig. 3, clade 2). *I. minutus* and *I. sinensis* appeared as well-supported sister species (Fig. 3, clade 1a) and are opposed to another well supported Australasian clade uniting *I. eurhythmus*, *I. cinnamomeus* and *I. flavicollis* (Fig. 3, clade 1b). However, the sister group relationship of these two clades was poorly supported. Clade 2 included a monophyletic subclade of three *Botaurus* species (*B. stellaris*, *B. lentiginosus*, *B. pinnatus*) which turned out as clear sister of *I. exilis* (Fig. 3). The position of Neotropic *I. involucris* as sister to clades 1a, 1b was only poorly supported.

Morphology

Discriminant analysis of morphological parameters was based on two discriminant functions: Function 1 had an Eigenvalue of 5.68 and explained 82.6 % of the total variance. Function 2 had an Eigenvalue of 1.2 and of all variables only wing length (wmax) correlated most strongly with function 2 (all other variables correlated most strongly with function 1). The scatterplot of the two discriminant functions shows three separate clusters that correspond to the three target species: *I. minutus*, *I. sinensis* and *I. eurhythmus* (Fig. 4). All specimens of

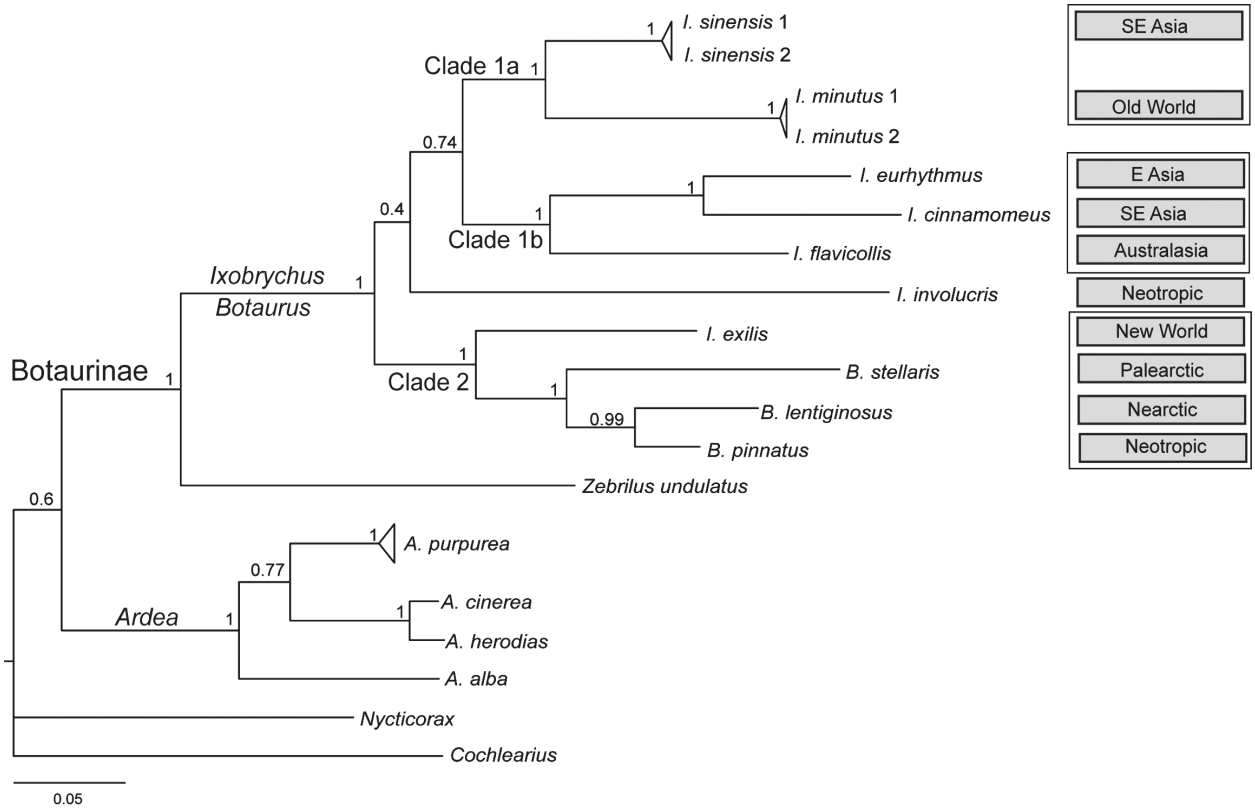

Fig. 3. Bayesian mitochondrial phylogeny of Botaurinae; 1512 bp of partial cytochrome-*b* and cytochrome oxidase I, partitioned by gene and codon; continental breeding distribution of species indicated in boxes right of the respective branches.

the latter two species were correctly assigned to the respective group, however only 92.3 % of all *I. minutus* specimens were correctly assigned by the discriminant analysis. Two outliers in the scatterplot were falsely assigned to *I. sinensis*: two female birds, one captive from the Zoological Garden Dresden (MTD C42222) and one from Valencia, Spain (MTD C51688; Fig. 4). The two species were even quite well separated in a scatterplot of wing length against beak length (Fig. 5). The specimens from Egypt fell into the correct clusters according to the species determination by phenotype and genetic barcoding.

Intraspecific variation in *I. sinensis* is best explained by wing length (strongest impact on disciminant function 2; Fig. 4), in a way that birds from Japan had significantly longer wings than those from Sulawesi (135.7 ± 3.5 mm *vs*. 128.4 ± 4.9 mm; Mann-Whitney-U-test, $p < 0.01$). All other parameters did not differ significantly between the two series of specimens.

The sister species *I. minutus* and *I. sinensis* differed significantly in wing and bill dimensions from each other but not in tarsus dimensions (Mann-Whitney-U-test, $p < 0.001$; Table 2).

Discussion

Breeding range

Based on the unmistakable results of genetic barcoding, morphometrics, nest records and recapture data, breeding of the yellow bittern, *I. sinensis*, in Egypt can be reliably confirmed (Hering *et al.*, 2013). Though, there are no estimates of population size and density available for the Egyptian breeding population, it has to be considered that the breeding range of *I. sinensis* is actually much wider than previously thought. Though these westernmost populations might be scattered and small, there is possibly no large distribution gap between them and the core breeding area in Southeast Asia (for range-wide breeding and wintering occurrences see map in Barthel & Hering, 2013). It is known that the yellow bittern has recently colonized Oman with first records in 1984 and breeding was confirmed in 2002 (Eriksen & Eriksen, 1999; Eriksen *et al.*, 2003). Other records from the Middle East were reported from the island of Socotra, off the South coast of Yemen (Aspinall *et al.*, 2004). Supposedly, breeding habitat of the yellow bittern might be relatively scarce on the Arabian Peninsula but very likely this secretive species might be a native breeder in several other marginal regions of its core Southeast Asian breeding area where it has escaped from

Table 2. Feather and body dimensions of little bittern (*I. minutus*), yellow bittern (*I. sinensis*) and Schrenck's bittern (*I. eurhythmus*); mean values ± standard deviation (n individuals) given for wing length (maximum chord, Wmax), bill to skull (BSk), bill depth (Bp), bill width (BWp), tarsus length (Tar1), tarsus diameter (Tdiad) all in mm and weight (if indicated on labels, in g); ** = significant metric differences among *I. minutus* and *I. sinensis* (Mann-Whitney U test, $p < 0.001$).

	Wmax**	BSk**	Bp**	BWp**	Tar1	Tdiad
I. eurhythmus	137.3 ± 4.6 (10)	49.6 ± 3.5 (9)	12.0 ± 0.7 (9)	8.4 ± 0.5 (9)	47.1 ± 2.6 (10)	3.2 ± 0.4 (10)
I. sinensis	130.7 ± 5.8 (21)	54.4 ± 1.8 (20)	9.7 ± 0.6 (21)	7.5 ± 0.3 (21)	44.8 ± 1.7 (16)	4.1 ± 0.4 (20)
I. minutus	144.9 ± 5.6 (30)	51.3 ± 2.7 (28)	10.4 ± 0.7 (29)	7.9 ± 0.4 (29)	45.5 ± 1.8 (29)	4.0 ± 0.5 (30)

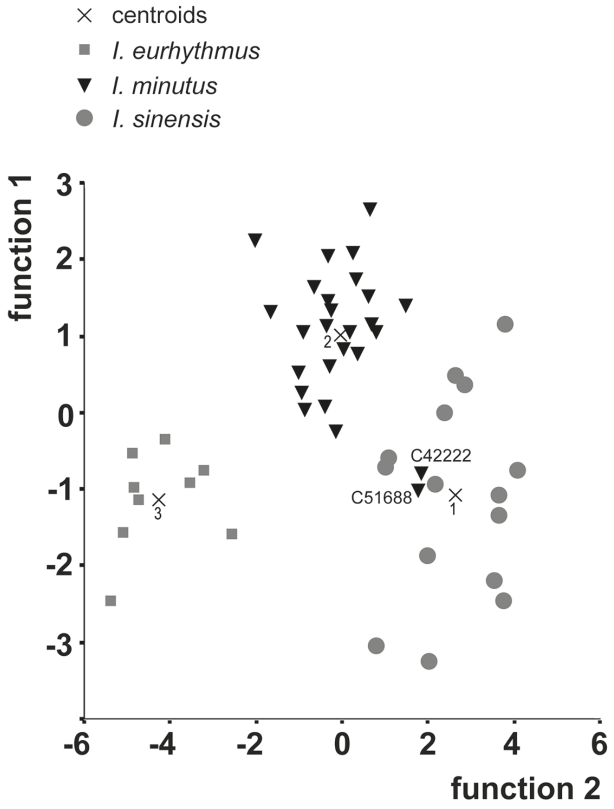

Fig. 4. Discriminant analysis of five morphological parameters for three bittern species; two outlier specimens of *I. minutus* marked by their respective specimen numbers.

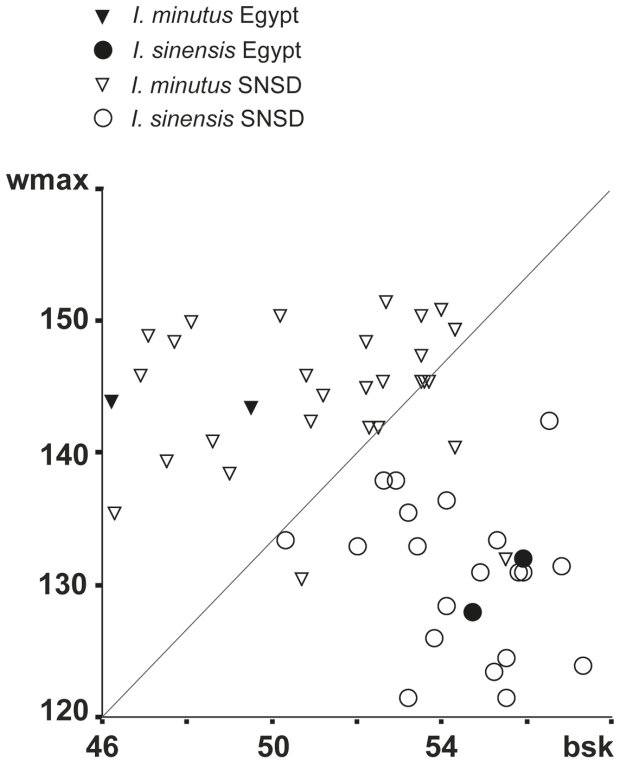

Fig 5. Scatterplot wing length (wmax) vs. bill length (bsk); the specimens captured in Egypt, 2012, highlighted by filled black symbols.

the notice of field ornithologists so far (see Barthel & Hering, 2013). For example, beyond the species' continental range, breeding of the yellow bittern has been confirmed for a few more remote island populations such as on the Seychelles (Gerlach & Skerret, 2002), and on Guam where it is considered a year-round resident native bird (Vice & Pitzler, 1999).

There are a number of records of vagrant birds beyond the Asian breeding range northward from Attu Island in the Aleutian Archipelago (Gibson & Kessel, 1992), southward from Australia and from the Christmas Islands (Davies *et al.*, 1991), and from the Maldives (Rasmussen & Anderton, 2005) (for further listings see Barthel & Hering, 2013). Previous records from the Western Palearctic such as documented from the British Isles by Melling *et al.* (2008) have been taken into serious doubt and do not originate from cases of natural dispersal or long-distance vagrancy (British Ornithologists Union Records Committee, 2006; Barthel & Hering, 2013). Therefore, the Egyptian yellow bitterns from the years 2012 and 2013 documented in Hering *et al.* (2013) are in fact the first breeding records confirmed for the Western Palearctic of this species.

Though ringing data and recoveries are scare for Asian bird species in general, and despite a particular data deficiency for the yellow bittern, the northernmost populations of that species from Japan, Korea and northern and central China are considered to migrate to the Philippines and to large parts of Indonesia (del Hoyo *et al.*, 1992). In contrast, throughout most of continental Southeast Asia populations are considered to be sedentary, while the vast part of the Greater Sundas (except Sumatra) and other

parts of Indonesia is considered to harbour exclusively wintering grounds including the island of Sulawesi. Despite known records from the latter island (large wintering populations in S Sulawesi), it appeared as the only uninhabited island across the large Indonesian wintering area on published distribution maps (e.g. in del Hoyo *et al*., 1992). There are further records of the yellow bittern on Sulawesi listed by Salvadori (1875; two females from Menado, July 1872; documented in Walden, 1872) and by Brüggemann (1878; the same two females plus two further specimens revisited). Yellow bitterns were also observed on the offshore island Talise, N of Minahassa headland of Sulawesi (Lee & Kussoi, 1999). BirdLife International (2013) ranks the population of the island of Sulawesi as "native non breeding". In that context, it is noteworthy, that the Sulawesi specimens from the SNSD collection examined by us were collected from June to September in the years 1877–1892 (all from the very northern headland, near Menado; compare the July specimens listed in Salvadori, 1875). With one record from June and one from July each, at least these two individuals were collected during the breeding season before migrant birds from the northernmost breeding range could have reached Sulawesi. According to collection dates it is not unlikely that these specimens represent a sedentary population from northern Sulawesi, which would offer an explanation for the significantly shorter wing length of Sulawesi birds compared to the specimens from Japan (in relation to all other body size parameters). Also in comparison to body size dimensions reviewed in Barthel & Hering (2013) wing length of SNSD specimens from Sulawesi ranged at shortest values while other parameters ranged at comparable dimensions. However, our results are based on a limited sampling size for yellow bitterns only (locally and range-wide) and any conclusions drawn must be considered as tentative. In contrast, Melling *et al.* (2008) rejected the general expectation of a North-South gradient in body size dimensions and stated that "there is no evidence that migratory northern birds tend to have longer wings" without providing particular details on what data this lack of evidence had been based and which populations had been compared. However, the absence of notable metric differentiation across the so far studied breeding range was also explicitly supported by Barthel & Hering (2013). Nevertheless, the summer records from Sulawesi indicate that the yellow bittern is more widely distributed and possibly even a resident breeder even in those parts of its Indonesian distribution range, where it is still being considered a non-breeding winter visitor so far (e.g. on Sumbava Island where a nest with nestlings was found in May 1988; Johnstone *et al.*, 1996).

Phylogenetic relationships

In first phylogenetic studies of herons Botaurinae had been largely underrepresented with only one *Ixobrychus* species included that unambiguously resulted as sister to one *Botaurus* species (McCracken & Sheldon, 1998; Sheldon *et al.*, 2000). A sampling approach with three Asian and one Palearctic *Ixobrychus* species included suggested the monophyly of the latter two genera (Chang *et al.*, 2003; the phylogeny by Zhang *et al.* 2004 comprised the same species set plus the debated Asian *I. flavicollis* and corroborated the results from the previous analysis). Reciprocal monophyly of the two genera in question was not reflected by our phylogeny including seven *Ixobrychus* species and three *Botaurus* species. Despite poor node support for many within-clade relationships of Botaurinae we can infer two hypotheses particularly from the close and well-supported sister-group relationship of *Botaurus* with *I. exilis*: i) paraphyly of *Ixobrychus* and ii) a plausible phylogeographic scenario of one Old World radiation with a diversification centre in East Asia (Clades 1a, 1b) and a mainly New World radiation including a single colonization event into the Palearctic by ancestors of *B. stellaris* (Clade 2). Moreover, it has been repeatedly demonstrated that the completeness of taxon sampling might strongly affect inference of phylogeny (Albert *et al.*, 2009; Braun & Kimball, 2002; Zwickl & Hillis, 2002), and therefore our results based on mitochondrial DNA only must be regarded as preliminary. Yet, a complete phylogenetic approach for Botaurinae would still be lacking a few species mainly from Australia, such as *Botaurus poiciloptilus* and *I. novaezelandiae*. The latter taxon had been previously included as a subspecies in *I. minutus* (del Hoyo *et al.*, 1992) and was documented by a few specimens only. Today *I. novaezelandiae* is considered extinct and often treated as a species of its own (Dickinson & Remsen, 2013; Clements *et al.*, 2013). Similarly Australian *I. dubius* was separated from *I. minutus* by Christidis & Bowles (2008) because it had been previously suggested to be a genetically distinct lineage and more closely related to *I. sinensis*. More data on these missing taxa including further sequences from nuclear markers for all Botaurinae species are needed to complete the phylogeographic picture of bitterns and allies.

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Appendix

Cytochrome-oxidase (COI) sequences shorter than 200 bp (not accepted by GenBank) used for phylogenetic reconstructions:

MTD_C34678

TCATCCGAGCCGAACTTGGCCAACCAGGAACACTTCTAGGAGATGACCAAATTTACAACGTTATTGTCACTGCT-
CATGCCTTCGTAATAATTTTCTTCATAGTAATACCAATTATAATCGGCGGATTCGGAAACTGATTAGTCCCCCTCA-
TAATTGGTGCCCCCGACATAGCATTCCCACG

MTD_C40774

TCATCCGAGCCGAACTTGGCCAACCAGGAACACTTCTAGGAGATGACCAAATTTACAACGTTATTGTCACTGCT-
CATGCCTTCGTAATAATTTTCTTCATAGTAATACCAATTATAATCGGCGGATTCGGAAACTGATTAGTCCCCCTCA-
TAATTGGTGCCCCCGACATAGCATTCCCAC