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## The importance of genetic research in zoo breeding programmes for threatened species: the African dwarf crocodiles (genus *Osteolaemus*) as a case study

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The threatened African dwarf crocodiles (genus *Osteolaemus*) are distributed throughout West and Central Africa. Traditionally two subspecies were described (*Osteolaemus tetraspis tetraspis* and *Osteolaemus tetraspis osborni*), although recent molecular studies demonstrate the presence of three allopatric lineages that should be recognized as full species. These highly divergent taxa are distributed in the three major forested biogeographic zones of western Africa: Congolian (*Osteolaemus osborni*), Lower Guinean (*Osteolaemus tetraspis*) and Upper Guinean (*Osteolaemus sp. nov. cf. tetraspis*). Largely because of their diminutive size, dwarf crocodiles are regularly kept in zoos and aquariums worldwide. In Europe, the collection is managed by a European studbook coordinated by Leipzig Zoo, Germany, since 2006, while American zoological institutions do not yet manage these species as part of a studbook programme. To facilitate *ex situ* conservation efforts, it is important to identify accurately each individual to the appropriate species following the latest systematic understanding of the genus. Population aggregation analysis with mitochondrial and nuclear gene sequences was used for both species identification and detection of interspecific hybridization. The results of our study show that only European collections house all three *Osteolaemus* taxa, although only a single individual *O. osborni* was confirmed. The most prevalent species present in both European and North American institutions was *O. tetraspis*. Additionally, several *O. sp. nov. cf. tetraspis* were identified, likely originating from the Senegambia region, especially in the North American col-

lections. This will represent an important resource for future conservation efforts as *Osteolaemus* are highly threatened in this region of West Africa. Unfortunately, both zoo populations showed relatively high frequencies (c. 25–28%) of hybridization between *O. tetraspis* and *O. sp. nov. cf. tetraspis* bred in captivity. We highly recommend that zoological institutions ensure they know the species identity of the *Osteolaemus* they maintain and work together to transfer individuals into single-species colonies to avoid further hybridization. In the USA, this may necessitate the creation of a studbook programme. It may also prove valuable to consider a cooperative programme between the European Association of Zoos and Aquaria and the Association of Zoos & Aquariums, wherein each Association focuses its resources largely on a single *Osteolaemus* species. This would, however, require trans-Atlantic transfer of individuals. The case study of dwarf crocodiles in zoological institutions reinforces the importance of genetic research in conservation-breeding programmes, highlights the potential for collaboration between European and American zoological institutions for the *ex situ* conservation of threatened wildlife, and foreshadows some of the regulatory challenges in managing captive populations internationally.

**Key-words:** captive breeding; *ex situ* conservation; genetic barcoding; hybridization; *osborni*; *Osteolaemus*; population aggregation analysis; *tetraspis*.

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## INTRODUCTION

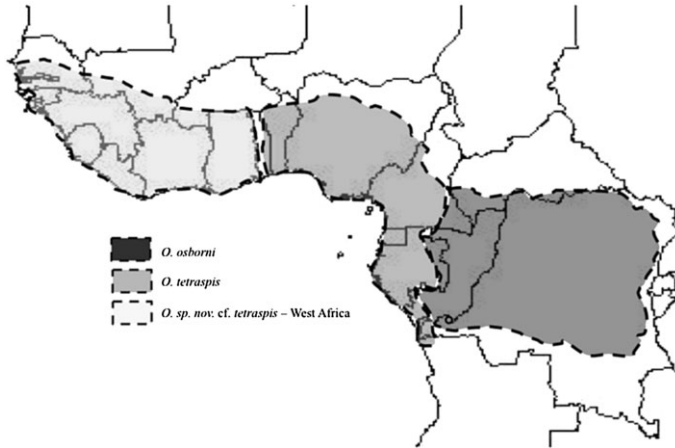
African dwarf crocodiles of the genus *Osteolaemus* have had a muddled taxonomic history. The genus was originally described with a single content species, *Osteolaemus tetraspis*, by Cope (1861) from a specimen collected in the 'Ogobai' (= Ogooué) River Basin of Gabon. Later, Murray (1862) described *Crocodylus frontatus* from a dwarf crocodile collected in the Benué River of Nigeria, and Lilljeborg (1867) described *Halcrosia afzelii* from a dwarf crocodile collected in Sierra Leone. Interestingly, neither of these latter authors referenced Cope's work on *O. tetraspis*, suggesting they simply were not aware of it, and both taxa were synonymized with the nominate species (Boulenger, 1889).

Schmidt, K. P. (1919) described a fourth dwarf crocodile species from the Ituri forest of the Congo Basin, even placing it in a monotypic genus (*Osteoblepharon*) to account for what he felt were considerable morphological differences from *Osteolaemus*. In reality, it amounted to only four cranial characters and the genus *Osteoblepharon* was finally refuted by Wermuth (1953) who reduced the genus *Osteolaemus* to a single species with two allopatric subspecies: *Osteolaemus tetraspis osborni* from the Congo Basin and *Osteolaemus tetraspis tetraspis* from everywhere else.

This taxonomy stood until 2009 when a series of largely genetic studies were implemented to test the taxonomic hypothesis of two species within *O. tetraspis*. Eaton *et al.* (2009) sampled individuals wild caught in the Congo Basin (Republic of Congo), the Ogooué Basin (Gabon), and the Upper Guinea biome (Ghana and Cote d'Ivoire). Their results supported the previously recognized Congo Basin (*O. osborni*) and Ogooué Basin (*O. tetraspis*) forms, but also revealed that West African individuals were a third, equally divergent form. Eaton *et al.* (2009) proposed that the three lineages should be regarded as full species corresponding to the three major biogeographic zones of western Africa: Congolian (the Congo Basin:

*Osteolaemus osborni*), Lower Guinean (Ogooué River Basin of Gabon, as well as Equatorial Guinea, portions of Cameroon and coastal Congo: *O. tetraspis*), and Upper Guinean (from Ghana and Cote d'Ivoire westwards: *Osteolaemus sp. nov. cf. tetraspis*). The authors proposed possible biogeographic barriers, such as the Dahomey Gap, the Cameroon Volcanic Line and the Batéké Plateau, keeping these species in allopatry (Fig. 1), but recommended that further sampling be undertaken before the exact distributional limits would be known. All three taxa were depicted side by side for the first time in Shirley & Eaton (2010), which clearly demonstrated their cryptic morphology, although also hinted at the presence of distinguishing morphological characters awaiting in-depth study (Plate 1).

Following Eaton *et al.*'s groundbreaking work, Shirley, Vliet *et al.* (2014) included a more geographically comprehensive *Osteolaemus* sample set in a species delimitation and phylogenetic analysis focused on the systematics of the genus *Mecistops* (slender-snouted crocodiles). Not only did they support the results of Eaton *et al.* (2009) using robust Bayesian species delimitation methods but also provided the first estimate of divergence timing for dwarf crocodiles using fossil-calibrated phylogenetic reconstruction. Shirley, Vliet *et al.* (2014) showed that the three *Osteolaemus* last shared a common ancestor  $\pm 11$  Mya (9–14 Mya) when the two western species diverged from *O. osborni*, and that *O. tetraspis* and *O. sp. nov. cf. tetraspis* have been isolated for  $\pm 8$  Mya (5.5–10.5 Mya). Also, since the work carried out by Eaton *et al.* (2009), both Franke *et al.* (2013) and Smolensky *et al.* (2014) found significant evidence that the Cameroon Volcanic Line is not the biogeographic barrier between *O. tetraspis* and *O. sp. nov. cf. tetraspis*. Instead, they found that the former taxon might be distributed all the way to Togo, indicating that the Dahomey Gap, an extensive upland savannah extending from western Ghana, through Benin and Togo (see Fig. 1 in Smolensky *et al.*, 2014), is the next most likely biogeographic-barrier



**Fig. 1.** Updated distribution of the genus *Osteolaemus* following the results of the genetic analysis carried out with wild-caught captive animals of known provenance for this study and the results of Smolensky *et al.* (2014), which provided evidence that *O. tetraspis* extends as far west as Togo.

candidate. Additionally, Franke *et al.* (2013) and Shirley, Villanova *et al.* (2014) detected significant subspecific phylogeographic structure within *O. sp. nov. cf. tetraspis* identifying two evolutionary significant units: one in the Upper Guinea forests of Ghana, Cote d'Ivoire and Liberia (haplogroup I), and the other restricted to the Senegambian forest block of Senegal, Gambia and Guinea (haplogroup II).

Morphological differentiation between any *Osteolaemus* taxa has only been described for cranial characters, preventing reliable diagnosis of living specimens unless their geographic origin is known. This has considerably hindered efforts to manage specimens in European Association of Zoos and Aquaria (EAZA) and Association of Zoos & Aquariums (AZA) institutions, as only about ten individuals have confident collection-locality data. Dwarf crocodiles are among the most represented crocodylians in EAZA and AZA collections largely because of their diminutive size, tractability and relative ease with which they breed in captivity. These zoo populations represent a potentially significant conservation resource as wild *Osteolaemus* populations continue to decline. *Osteolaemus tetraspis*, the sole

dwarf crocodile recognized by the International Union for Conservation of Nature (IUCN), has been listed as Vulnerable on the IUCN Red List since 1996 (IUCN, 2014). Across its distribution, the major threats to the species are habitat alteration and hunting for the bushmeat trade. However, the impacts of these threats vary throughout the ranges of dwarf crocodiles. For example, the soon to be recognized West African *Osteolaemus* species faces far more critical threats to its conservation, such as the bushmeat trade, habitat destruction by commercial logging and degradation of wetland habitats (Shirley *et al.*, 2009; Eaton, 2010).

Conservation breeding in zoos often faces the classical dilemma of whether to manage individuals from naturally isolated populations separately, or should they be combined to reduce the risk of inbreeding depression and ensure sufficient gene diversity and the adaptive potential of the captive population (Edmands, 2007; Senn *et al.*, 2014). While these two issues are often the focus of conservation-breeding discussion, the lack of taxonomic resolution leading to inadvertent hybridization and the loss of unique populations should be of equal concern in the



**Plate 1.** The three different *Osteolaemus* taxa are very difficult to distinguish morphologically, making *Osteolaemus* a cryptic species complex. Top image: *Osteolaemus tetraspis* (*below*) and *Osteolaemus* sp. nov. cf. *tetraspis* (*above*). Zoo Leipzig, Germany. Bottom image: *Osteolaemus osborni*. Zoologico Santillana del Mar, Spain.

modern era of taxonomic discovery (Kitchener, 2010; Shirley, Villanova *et al.*, 2014). Aside from introgression and simply muddling species boundaries, the risks of mixing different species are outbreeding depression and hybrid sterility (Edmands, 2007; Senn *et al.*, 2014). Ultimately, hybridization may eliminate the conservation value of such populations and, therefore, solutions must be considered carefully. Clarification of taxonomic issues using molecular techniques has significant potential to inform

such conservation-breeding decision making (Kitchener, 2010).

In 2006, EAZA established a European studbook (ESB), maintained at Leipzig Zoo, Germany, in order to manage captive *Osteolaemus* populations better. No studbook or coordinated breeding programme has yet been established by the AZA. Underscoring the link to *in situ* conservation efforts for these crocodiles, the ESB has been approached by African reptile parks seeking founder stock for future breeding and reintroduction

purposes. To support this link to grass roots conservation initiatives in Africa, we undertook to identify *Osteolaemus* maintained in EAZA and AZA institutions utilizing the latest systematic and taxonomic research described here. Individuals were also screened for evidence of hybridization to prevent the exportation of hybrid individuals for *in situ* reintroduction efforts.

## MATERIALS AND METHODS

Appropriate institutional veterinary or curatorial staff were asked to take samples and send blood cards from all known *Osteolaemus* in the EAZA and AZA populations. Samples for a total of 129 non-related individual animals were received from zoos (91), private collections (17) and museums (21) in the EAZA region. Including the siblings/offspring of these non-related individuals (also maintained within the population), the total number of samples received was 172, *c.* 92.5% of the EAZA captive population and *c.* 82.5% of all founders/potential founders in this population (Schmidt, F., 2013a). Thirty-two samples were received from nine AZA collections (accession numbers and analyses published in Shirley, Villanova *et al.*, 2014). There is no studbook for *Osteolaemus* in AZA institutions, so it is not possible to know precisely the total size of the AZA *Osteolaemus* population or the proportion of the total population these 32 samples represent. Based on recent International Species Information System (ISIS) Zoological Information Management System (ZIMS) data (October 2013), combined with information from an older (2011) AZA Crocodylian Advisory Group (CAG) space survey for AZA institutions that do not report to ISIS (Crocodylian Advisory Group, 2012), the AZA population is believed to be roughly 71 crocodiles at 24 institutions. As such, the 32 AZA samples received would represent *c.* 45% of the AZA captive population. For the *Osteolaemus* in EAZA, 34 of the 129 samples were newly sequenced as part of this study and 75 samples had been sequenced as part of the Franke *et al.* (2013) study. No new individuals

were sequenced from the AZA for the study reported here (but see Shirley, Villanova *et al.*, 2014).

A 565 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) and a 642 bp fragment of the nuclear lactate dehydrogenase A (LDH-A) were sequenced. Extraction of genomic DNA, polymerase chain reaction and sequencing protocols, as well as sequence manipulation and alignment, were described in Franke *et al.* (2013) and Shirley, Villanova *et al.* (2014). These genes were selected to enable comparison with the extensive published *Osteolaemus* data from Eaton *et al.* (2009). The GenBank accession numbers for the 27 non-hybrid individuals, of the 34 newly sequenced EAZA individuals, are KM406124–KM406150 (COI) and KM406151–KM406177 (LDH-A) (Appendix S1).

Species identification was carried out using both genes in a population aggregation analysis (PAA) framework (Davis & Nixon, 1992). Unique haplotypes (PAA1) and fixed nucleotide positions (PAA2) that segregated the three *Osteolaemus* species within a reference data set of wild-caught, known-locality individuals were searched for [96 reference sequences published by Eaton *et al.*, (2009), Shirley, Villanova *et al.* (2014) and Shirley, Vliet *et al.* (2014): these studies found a total of 83 variable bases for COI segregating the three *Osteolaemus* species, although the number of fixed sites segregating any two species ranged from 44 to 57, and five variable LDH-A sites; three sites segregated *O. osborni* from both other species and two sites segregated *O. sp. nov.* cf. *tetraspis* from both other species]. Zoo specimens were assigned to species by aligning their sequences with the wild references and matching unique haplotypes and fixed nucleotide positions in both COI and LDH-A. Hybrids were detected by examining the LDH-A lineage-specific diagnostic sites in the post-sequencing chromatograms for heterozygosity, which manifest as double peaks, whereas non-hybrid individuals will show only a single peak indicating homozygosity. Where available, both the COI and LDH-A parental haplotypes were used to confirm detected hybrids.

## RESULTS

All three *Osteolaemus* species were found in collections within EAZA and AZA, although the taxa were disproportionately represented (Table 1). Only a single *O. osborni* has been found in captivity to date, at Zoo de Santillana del Mar, Cantabria, Spain (an EAZA facility). No *O. osborni* appear to exist in the AZA. *Osteolaemus tetraspis* was the most abundant dwarf crocodile species in both populations, representing 60.5% and 59% of the tested individuals in the EAZA and AZA populations, respectively. *Osteolaemus sp. nov. cf. tetraspis* represented only 11% and 16% of EAZA and AZA populations, respectively. Both *O. sp. nov. cf. tetraspis* mitochondrial haplogroups described by Franke *et al.* (2013) and Shirley, Villanova *et al.* (2014) were present in the EAZA population, although Upper Guinea animals were somewhat more numerous. In contrast, all West African individuals analysed in the AZA were from haplogroup II. Detailed data sets for the results of the PAA analysis and GenBank accession data for all reference sequences have been previously published in Eaton *et al.* (2009), Franke *et al.* (2013) and Shirley, Villanova *et al.* (2014).

Hybrid individuals were detected at an overall rate of 25–28% in the two populations (Table 1). Discordance between the maternal and paternal haplotypes for both gene regions supported the hybrid identifications in three and six cases in the EAZA and AZA populations, respectively. Only *O. tetraspis* × *O. sp. nov. cf. tetraspis* hybrids were detected, and hybridization occurred in both directions

(i.e. ♂♂ and ♀♀ of both species will reproduce with the opposite sex of the opposing species). Comparison of our genetic results with genealogical accounts for the identified hybrid individuals (i.e. in the ESB or in ZIMS) confirmed that hybrids were F<sub>1</sub>, F<sub>2</sub> and backcrosses in both EAZA and AZA collections. No known wild-caught individuals (i.e. F<sub>0</sub>) were identified as hybrids.

An error was identified in the records for *Osteolaemus* in at least one case from the AZA population. Five offspring purportedly from a pair of wild-caught *Osteolaemus* at the Memphis Zoo, TN, USA (0564 + 0565, now deceased and not sequenced as part of this study) were sequenced. One of the recorded offspring possessed *O. tetraspis* mtDNA and was homozygous for *O. tetraspis* LDH-A, while the remaining four offspring possessed both *O. sp. nov. cf. tetraspis* mtDNA and homozygous LDH-A alleles. This individual was unmarked and no records were found to indicate its origin. Following confiscation by police authorities, this individual was thought to originate from Memphis Zoo and was ‘returned’ there. However, our analyses clearly indicate that this individual was not from the Memphis breeding group.

While *O. osborni* is reasonably distinct physically and can be identified by external features, *O. tetraspis* and *O. sp. nov. cf. tetraspis* truly represent cryptic species and, at the time of writing, it is still not possible to distinguish them by external morphology (Plate 1). There are anecdotal reports of noticeable differences among hatchlings and juveniles of these taxa, including snout shape, eye colour, and the pigmented bands

TAXON	EAZA		AZA	
	NO.	FREQUENCY	NO.	FREQUENCY
<i>Osteolaemus tetraspis</i>	104	60.5%	19	59%
<i>Osteolaemus osborni</i>	1	0.5%	0	0%
<i>Osteolaemus sp. nov. cf. tetraspis</i>	19	11%	5	16%
Hybrids	48	28%	8	25%

**Table 1.** Proportional occurrence of *Osteolaemus* taxa in the European Association of Zoos and Aquaria (EAZA) ( $n = 172$  animals) and the Association of Zoos & Aquariums (AZA) ( $n = 32$  animals) collections in 2012.



**Plate 2. Juveniles.** *Top image:* Animals with copper, brown and golden bands, upturned snouts and dark eyes. In this case the animals originate from Ogooué Basin parents. Zoo Leipzig, Germany. *Bottom image:* Animal with black and yellow bands, wide snouts and light greenish-yellowish eyes. In this case the animal originates from West African parents. Walter Zoo, Gossau, Switzerland.

on the torso and tail (Plate 2). Our results tentatively suggest that these external features may be used to distinguish dissimilar looking juveniles. For example, sequenced juveniles that had light greenish–yellowish eyes all had *O. sp. nov. cf. tetraspis* haplotypes compared with those with dark brown eyes, which all had *O. tetraspis* haplotypes. This result is very preliminary owing to the small sample size, and further work is needed to confirm these assumptions.

## DISCUSSION

The 34 specimens analysed as part of this study were all readily identifiable to one of the three species proposed by previous

authors (e.g. Eaton *et al.*, 2009; Franke *et al.*, 2013; Shirley, Villanova *et al.*, 2014; Shirley, Vliet *et al.*, 2014), which continues to support the hypothesis of *Osteolaemus* as a cryptic species complex (Appendix S1). Additionally, our results, revealing both the taxonomic identities of individuals and the presence of a significant number of hybrids, require decisions to be made as to how EAZA and AZA institutions should manage dwarf crocodiles in the future.

In some managed breeding programmes, the decision was made to continue breeding hybrids, even after genetic analysis. For example, ESB programmes for Beaded lizards *Heloderma horridum* and Galapagos giant tortoises *Geochelone nigra* justified

continuation of subspecific hybridization for reasons of small captive population size, the importance of keeping the species as a model for research or education purposes in EAZA collections (Furrer *et al.*, 2006) and to ensure a genetically healthy population (Draeby, 2008). In the last-mentioned situation, EAZA believed that the removal of hybrids and separation of subspecies into separate breeding populations would have left too few individuals to retain adequate gene diversity. Similarly, a recent study on the Critically Endangered Dama gazelle *Nanger dama* provided evidence that, despite phenotypic differences among populations, it should be regarded as single species without a subspecific division. In future, captive breeding should involve mixing the three currently separated populations in zoos to maximize gene diversity and minimize inbreeding (Senn *et al.*, 2014). Another justification for maintaining ongoing hybridization comes from the captive Amur leopard *Panthera pardus orientalis* population, which is strongly influenced by a founder animal from another subspecies, whose genetic introgression simply cannot be bred out. As a result, however, the captive population shows much higher gene diversity than the remnant wild population, which suffers from a remarkably reduced genetic diversity (Uphyrkina *et al.*, 2002).

In other examples, where hybrids formed a relatively minor proportion of the population and could be excluded from further breeding without significantly decreasing the gene diversity in the studbook, the decision was taken to form several taxonomically pure subpopulations in the breeding programme. EAZA established a European Endangered Species Programme (EEP) for the West African chimpanzee *Pan troglodytes verus* (Carlsen & de Jongh, 2006). A strategy was developed to ensure that breeding only occurred in genetically pure chimpanzees to preserve a healthy and self-sustaining population in EAZA, whereas hybrid animals are being phased out (Hvilsom *et al.*, 2013). However, decisions may differ from region to region; in contrast to EAZA, AZA decided to

manage their chimpanzees at the species level (Carlsen & de Jongh, 2006).

All of the above discussion, however, deals with the much more common issue of inter-subspecific hybridization in captivity. The *Osteolaemus* issue could be argued to be biologically more significant at the species level and, as such, the *Osteolaemus* ESB decided to divide the population of dwarf crocodiles into three separate species following our genetic results. The decision is justified by the fact that 72% of tested individuals in the EAZA population were assignable to a pure taxonomic unit [11% to *O. sp. nov.* cf. *tetraspis* and 60.5% to *O. tetraspis* (and 0.5% for the single *O. osborni*)] and managing to prevent further hybridization only meant that c. 28% of individuals would have to be excluded because of their hybrid status (Table 1).

The impact on gene diversity of different management scenarios was modelled using the population management software PMx (Lacy *et al.*, 2012), and we found that managing the species separately could still result in the long-term maintenance of gene diversity for each species (> 90% for 100 years) (Schmidt, F., 2013a). Because crocodiles are long-lived animals, the ESB still includes many founder and potential founder ( $F_0$ ) specimens that contribute to maintaining high gene diversity within the subpopulations. For example, the *O. tetraspis* in EAZA collections descended from 14 founder animals, of which 11 are still alive. Gene diversity is 93.6% and would be increased to 98.5% by including 20 additional founders (Schmidt, F., 2013a). There are at least 20 potential founders in the EAZA population –  $F_0$  specimens living in the population that have not yet reproduced – making this a feasible management objective. The smaller *O. sp. nov.* cf. *tetraspis* population in EAZA descends from only five founder animals, of which four are still living. The resulting relatively low gene diversity of 85.9% could still be increased to 95.8% by including seven additional potential founders (Schmidt, F., 2013a), which are also available and living in the population.



The comparative effects of 'removing' (i.e. by managing species separately) three founder and three potential founder individuals from the generalized *Osteolaemus* population do not result in significantly lower gene diversity. Rather, gene diversity of each individual species increases by now striving to breed the potential founder individuals that were previously not given breeding opportunities. In light of this, the ESB decided to exclude all hybrids from further breeding, separate several mixed founder pairs, recombine individuals into taxonomically pure breeding pairs, and continue the studbook with two taxonomically pure breeding subpopulations representing *O. tetraspis* and *O. sp. nov. cf. tetraspis*.

Additionally, specific recommendations were made for the *O. osborni*, *O. sp. nov. cf. tetraspis* and hybrid populations (Schmidt, F., 2013a). It was recommended that *O. osborni* should no longer be maintained in EAZA institutions. A population cannot comprise a single individual and the threatened status of this species in the wild will likely prevent future importation of founder stock. The ESB recommended including *O. sp. nov. cf. tetraspis* haplogroup II individuals (all ♂♂) originating from Senegambia in breeding groups for the time being but pairing them only with F<sub>1</sub> ♀♀ (i.e. they were not to be paired with founder or potential founder ♀♀) pending genetic results for the AZA population. If haplogroup II crocodiles were found in abundance in the AZA, then the decision was to send the EAZA ♂♂ to AZA collections. Similarly, the ESB recommended phasing out hybrid individuals in the ESB population by discontinuing any breeding and placing them outside the ESB at facilities that would not breed them, in order to free space for taxonomically pure individuals. The ESB further recommended that untested animals be treated as hybrids pending future evaluation of their taxonomic status and inclusion in the ESB. These recommendations led to 29 transfers of adult *Osteolaemus* between European zoological institutions (Schmidt, F., 2013a,b), resulting in 19 potential breeding pairs for pure *O. tetraspis* and

six potential breeding pairs for pure *O. sp. nov. cf. tetraspis*. Through this specifically focused management action, the proportion of hybrids in the population was decreased from 28% to 21%, while the proportion of pure *O. tetraspis* increased from 60.5% to 68% (Schmidt, F., 2014).

At the time of writing, the AZA CAG maintains eight managed programmes for the Critically Endangered and Endangered crocodilian species: two yellow Species Survival Plan (SSP) cooperative breeding programmes and six red SSPs. As is the case in EAZA, AZA institutions have a long history of keeping *Osteolaemus*. Since the mid-1970s, many institutions have maintained and bred dwarf crocodiles in their collections. Because of the abundance of specimens, and because the conservation status of dwarf crocodiles was thought to be less dire than that of other crocodilians, the CAG never sought to manage this species within an SSP coordinated programme or other studbook. Our new understanding of taxonomic diversity in *Osteolaemus* and the results of the present genetic analyses, however, clearly require the CAG to contemplate how best to address this newly recognized situation.

Unfortunately, the eight AZA SSP programmes already in place face significant space shortages, making it difficult to bring these populations up to target population sizes, and space issues limit the future sustainability of some programmes. There is growing pressure within the AZA community to reduce the number of managed programmes to help alleviate space shortages. While no decisions have been made, it appears reasonably unlikely that the CAG will choose to create new official studbook programmes for dwarf crocodiles and other means of managing the species may need to be considered. One possible solution previously discussed is for one or more zoo professionals to serve as 'taxon champions', familiarizing themselves with all dwarf crocodiles within AZA institutions, making unofficial recommendations regarding their management and, possibly, maintaining an unofficial studbook for this genus.

The CAG will publish its next Regional Collection Plan in 2015. Decisions regarding the creation of a formal studbook or other population management plan for *Osteolaemus* will need to be made by this time. Whether the CAG initiates a formal programme for dwarf crocodiles or not, this document will include recommendations for AZA collections holding *Osteolaemus*. These recommendations will be quite similar to those detailed above for EAZA facilities. For example, all untested *Osteolaemus* must not be bred until they are tested. Pure populations of *O. tetraspis* and *O. sp. nov. cf. tetraspis* must be maintained by separating individuals found in mixed groups. Hybrid animals should be removed from collections, although these may prove useful as education-programme animals in some cases. Private, responsible facilities capable of caring for these crocodiles could serve the zoo community by taking these specimens. In this case, all hybrids should be permanently marked before leaving AZA facilities to avoid these animals re-entering the population at a later date.

One possible solution to managing *Osteolaemus* within the EAZA and AZA populations is the wholesale exchange of crocodiles across the Atlantic. For instance, transferring all *O. sp. nov. cf. tetraspis* (both haplotype I and haplotype II) from EAZA institutions to the AZA and all *O. tetraspis* from AZA collections to EAZA collections would allow these two species to be managed completely separately without the possibility of accidental hybridization and, furthermore, would strengthen the size and gene diversity of populations of both species. This would also eliminate the issue of managing two species of crocodiles that, at least to the zoo-going public, look absolutely identical to one another, and remove the need to justify this decision to administrators and the public. However, an exchange such as this would be an enormously difficult undertaking and would require significant regulatory oversight because *Osteolaemus* crocodiles are listed in Appendix I of the Convention on International Trade in Endangered Species of

Wild Fauna and Flora (CITES) and in the USA Endangered Species Act. Permits would be required for numerous animals from a multitude of institutions, and this would only address the regulatory hurdles. Institutions would have to agree to the transfers, health checks would have to be performed, and the costs of the transfers would have to be budgeted and approved. Thus, despite its logic, it seems very unlikely that an exchange such as this could ever be carried out successfully. Additionally, further investigation may reveal that it makes more sense to manage *O. sp. nov. cf. tetraspis* on a global level by maintaining the two different haplogroups separately across the Atlantic – a simpler task owing to the smaller number of individuals.

Another notable finding of our study was a significant discrepancy in institutional response rates between EAZA and AZA institutions, likely a result of differences in administrative mechanisms [i.e. through the ESB in EAZA versus Taxon Advisory Group (TAG)-motivated research in AZA]. Zoos and aquariums were much more responsive to the initiative under the ESB. This is additionally noteworthy considering the level of permitting required to send specimens of CITES-listed taxa across borders, in some cases between institutions within Europe (but not necessarily in the European Union) and European Union member states. In addition to the lack of an *Osteolaemus* studbook programme, low participation rates among AZA institutions might indicate a reluctance on the part of veterinary staff to capture and restrain crocodiles solely for the purpose of drawing blood for a research project. It is possible that administrators in AZA considered this project as independent research conducted by a graduate student, rather than an offer to identify their crocodile taxa, and did not feel compelled to participate; even though their participation had been officially requested by the TAG. The results of this investigation clearly indicate the necessity of genetic sampling for the appropriate taxonomic and *ex situ* conservation management of these species in captivity. As the results of our study suggest the need for proper species

identification and pedigree analysis, we strongly recommend that institutions housing unsampled individuals should refrain from breeding until the taxonomic identities of these specimens are determined. Ultimately, such institutions will be required to sponsor their own genetic analyses in order to participate in future studbooks or other coordinated breeding programmes, which heightens the risk that potentially important breeding animals in the AZA population will be excluded from the breeding programme.

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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:

**Appendix S1.** List of 34 African dwarf crocodiles *Osteolaemus* spp from European zoo and aquarium collections that were newly sequenced for this study. A 565 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) and a 642 bp fragment of the nuclear lactate dehydrogenase A (LDH-A) were sequenced. ESB, European studbook; species, *Osteolaemus sp. nov. cf. tetraspis*, *Osteolaemus tetraspis*, *Osteolaemus tetraspis* × *Osteolaemus sp. nov. cf. tetraspis* (hybrids).