

Shedding light on the Imps of Darkness: an integrative taxonomic revision of the Galápagos marine iguanas (genus *Amblyrhynchus*)

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Received 27 July 2016; revised 13 January 2017; accepted for publication 26 January 2017

Marine iguanas are among the most highly emblematic taxa of the Galápagos archipelago but have paradoxically received little attention from taxonomists. *Amblyrhynchus* is currently considered as a monotypic genus with a total of seven subspecies, *A. cristatus cristatus*, *A. c. nanus*, *A. c. venustissimus*, *A. c. albemarlensis*, *A. c. hassi*, *A. c. mertensi* and *A. c. sielmanni*. Although consensually followed for more than half a century, this classification does not properly reflect the main natural subdivisions inferred by more recent molecular evolutionary studies. We integrate population genetics, phylogenomics and comparative morphology to propose an updated taxonomy reflecting the evolutionary history of this group. We recognize a single species with 11 divergent population clusters at the level of subspecies: *A. c. albertmarlensis* is recognized as a junior synonym of *A. c. cristatus*, and five new subspecies are described: ***A. c. godzilla* subsp. nov.** (San Cristóbal-Punta Pitt), ***A. c. jeffreysi* subsp. nov.** (Wolf and Darwin), ***A. c. hayampi* subsp. nov.** (Marchena), ***A. c. trillmichi* subsp. nov.** (Santa Fé) and ***A. c. wikelskii* subsp. nov.** (Santiago). Recognizing the genetically divergent population clusters as subspecies also highlights several of them as management units in need of conservation efforts, such as the two subspecies endemic to San Cristóbal.

ADDITIONAL KEYWORDS: *Amblyrhynchus* – Galápagos – integrative taxonomy – management units (MUs) – Marine Iguana – new subspecies.

INTRODUCTION

The world's only contemporary truly marine lizard, the Galápagos marine iguana (*A. cristatus*) represents, together with giant tortoises and Darwin's finches, one of the most emblematic taxa of the Galápagos archipelago. The remarkable aquatic behaviour and adaptations of marine iguanas have led to the publication of many

studies focusing on their ecological, ethological and physiological peculiarities, as well as on their conservation (e.g. Bartholomew & Lasiewski, 1965; Trillmich & Trillmich, 1986; Laurie, 1990; Drent, Van Marken Lichtenbelt & Wikelski, 1999; Steinfartz *et al.*, 2007). Given that these lizards are a regular feature in documentaries dealing with Charles Darwin's biography or with the wildlife endemic to the archipelago, their popularity might even have led to them being the inspiration for a terrifying Hollywood protagonist (*Godzilla*, Emmerich, 1998).

Paradoxically – but somehow in line with Darwin's aversion against these 'hideous looking', 'disgusting'

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and ‘stupid’ ‘imps of darkness’ – taxonomists seem to have largely neglected the genus *Amblyrhynchus*, despite the fact it has remarkably dispersed and diversified across the whole archipelago. Only three taxa were described in the course of the 19th century (*Amblyrhynchus cristatus* Bell, 1825, *Iguana* (A.) *ater* Gray, 1831 and *A. nanus* Garman, 1892). Their taxonomy and systematics were addressed by two papers published in the mid-1950s by Irenäus Eibl-Eibesfeldt. Mostly known as a pioneer of human ethology, Eibl-Eibesfeldt took the opportunity of the Xarifa expedition to the Galápagos to collect several dozen specimens of iguanas, and subsequently proposed major taxonomic changes in two different contributions (Eibl-Eibesfeldt, 1956, 1962). Considering *Amblyrhynchus* to be a monotypic genus, he recognized *A. nanus* as a subspecies of *A. cristatus*, and described five additional new subspecies (Fig. 1): *A. c. venustissimus* (1956) from the island of Española, *A. c. albemarlensis* from Isabela, *A. c. hassi* from Santa Cruz, *A. c. mertensi* from San Cristóbal and Santiago and *A. c. sielmanni* from Pinta (1962). Despite the rapid development of molecular approaches over the last two decades, and the publication of several phylogenetic and population genetic studies addressing various evolutionary issues related

to this taxon (Rassmann, 1997; Rassmann *et al.*, 1997; Steinfartz *et al.*, 2009, MacLeod *et al.*, 2015a), no taxonomic revision has taken insights on the phylogeny and evolution of marine iguanas into account. As a consequence, Eibl-Eibesfeldt’s subspecific classification has remained unchanged for decades and has been used as a basis for the designation and prioritization of conservation units of this threatened and protected species (ITWG, 2011; IUCN, 2016; Uetz & Hošek, 2016).

Eibl-Eibesfeldt’s work provided important insights into the diversification of this taxon across the archipelago, but could not provide a comprehensive picture of the diversity of marine iguanas: (1) his studies were based on morphological examination of specimens from only seven islands of the archipelago, notably excluding several major islands (e.g. Floreana and Marchena) and the remote northern islands of Darwin and Wolf; (2) several subspecific entities were justified and briefly diagnosed largely on intuitive comparisons, probably on the basis of observation of colour pattern in the field, but not on quantitative statistical assessments of inter- and intrapopulational phenotypic variation and (3) several molecular studies, not yet available at the time of Eibl-Eibesfeldt’s taxonomic work, led to entirely new perspectives on the geographic distribution of genetic

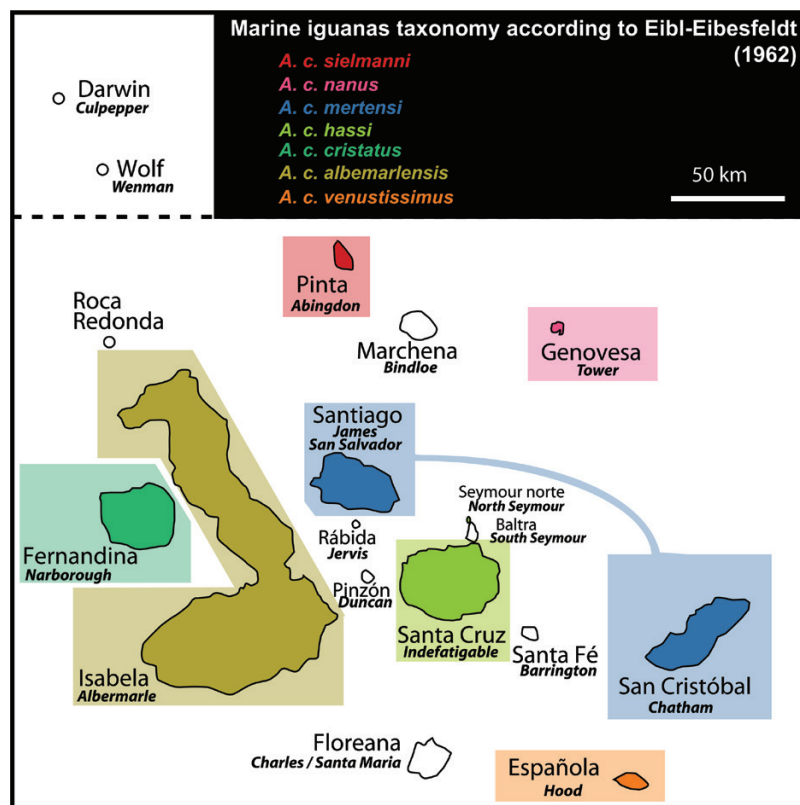


Figure 1. Distribution of the different subspecies of Galápagos marine iguanas according to the classification of Eibl-Eibesfeldt (1962). In addition to currently used names, historical name(s) for each island are also given.

population clusters (Rassmann *et al.*, 1997; Steinfartz *et al.*, 2009; MacLeod *et al.*, 2015a).

The discrepancy between the current classification of subspecies and observed genetic differentiation between island populations of marine iguanas is particularly problematic with respect to the use of subspecies as conservation units by leading conservation organizations worldwide, such as the International Union for Conservation of Nature (IUCN). As an endangered and protected species, marine iguanas are listed under CITES Appendix II and are ranked as 'vulnerable' according to the IUCN red list, meaning that formally the status of marine iguana populations has to be monitored and managed. Currently, conservation assessments are based on the taxonomy of subspecies as suggested by the works of Eibl-Eibesfeldt (1956, 1962) and do not take any information derived from molecular datasets into account. As a consequence, certain clearly separate and evolutionarily distinct populations are not being afforded the protection they may need. For instance, the iguanas of San Cristóbal and Santiago islands are designated as one subspecies in the current classification, and are thus managed as such (Fig. 1); yet the genetic data indicate three clearly separate population clusters from these islands, one from Santiago and two from San Cristóbal (see fig. 2 of MacLeod *et al.*, 2015a). Failure to recognize the distinction between those units could lead to local extinctions of genetically distinct populations, which could result in the loss of potentially vital genetic diversity that is critical for the survival of the whole species. Conversely, the well-intermixed populations found on Isabela and Fernandina islands are viewed as two separate subspecies, and therefore may be awarded a higher conservation priority than is indeed needed. As a consequence, finite resources and management efforts may be misdirected. Although efforts to redirect focus towards the most important populations that need to be separately managed are underway (MacLeod & Steinfartz, 2016), units for conservation are almost always taxonomic entities, and therefore without taxonomic reform, this sub-optimal and potentially dangerous situation persists.

Recent studies on marine iguanas based on comprehensive molecular datasets including nuclear and mitochondrial DNA, microsatellite loci and genome-wide single nucleotide polymorphisms (SNPs) have greatly furthered our knowledge on *Amblyrhynchus* phylogeny and evolution. We are now in the position to integrate this knowledge with an extensive study of comparative morphology of marine iguanas. On this basis, we propose an updated taxonomy, ranking *A. cristatus* as a single species with 11 genetically divergent population clusters that are here awarded the status of subspecies. These subspecies should be seen and treated as management units in the context of major conservation planning and actions in the future.

MATERIAL AND METHODS

SAMPLES FOR MOLECULAR ANALYSES

Samples and data used in this work were collected for various studies over recent decades (Rassmann, 1996; Rassmann *et al.*, 1997; Steinfartz *et al.*, 2007, 2009; MacLeod *et al.*, 2015a; MacLeod & Steinfartz, 2016). Most samples were collected in the years 1991, 1993, 2004, 2012 and 2013, by the authors and C. Rassmann.

MICROSATELLITE LOCI DATA AND POPULATION GENETIC STRUCTURE

From an original dataset of 1600+ samples genotyped at 12 microsatellite loci, we created a subset of 643 samples by random subsampling certain populations which were overrepresented in the dataset. This was to avoid artefacts within population structure analysis (Chikhi *et al.*, 2010; Shringarpure & Xing, 2014). Most islands were represented by 50 samples, although some sets were slightly smaller; sample sizes are given in Table S1. Population structure was assessed using a model-based Bayesian clustering method (STRUCTURE v2.3, Pritchard, Stephens & Donnelly, 2000). Parameters used were identical to those of MacLeod & Steinfartz (2016). We interpreted the results following Evanno, Regnaut & Goudet (2005), as implemented in STRUCTURE HARVESTER (Earl & Holdt, 2012) and permuted data from replicated simulations using CLUMPP (Jakobsson & Rosenberg, 2007). Islands were assigned to the cluster identity for which the majority of individuals had the highest probability of belonging. To evaluate the strength of these clusters further, assignment tests were conducted in GENEALLEX version 6.5, using the 'leave one out' method (Peakall & Smouse, 2006), and the same software was used to calculate private alleles per cluster.

MITOCHONDRIAL DATASET

Using previously analysed data ($N = 1203$, Steinfartz *et al.*, 2009) and newly collected samples ($N = 264$) from MacLeod *et al.* (2015a), we constructed a dataset based on a 1181 bp DNA segment of the mitochondrial control region in 1467 individuals. New sequences were generated with primers and methods developed by Hanley & Caccone (2005). Sequences were edited using CODONCODE ALIGNER (CodonCode Corporation) and aligned using CLUSTAL W (Larkin *et al.*, 2007), as implemented in MEGA (Tamura *et al.*, 2013). The haplotype network was constructed using information from phylogenetic trees as implemented in HAPVIEW (Salzburger, Ewing & Von Haeseler, 2011). Maximum likelihood trees were estimated with PHYML (Guindon & Gascuel, 2003) using the best-fitting substitution model as identified by the Akaike

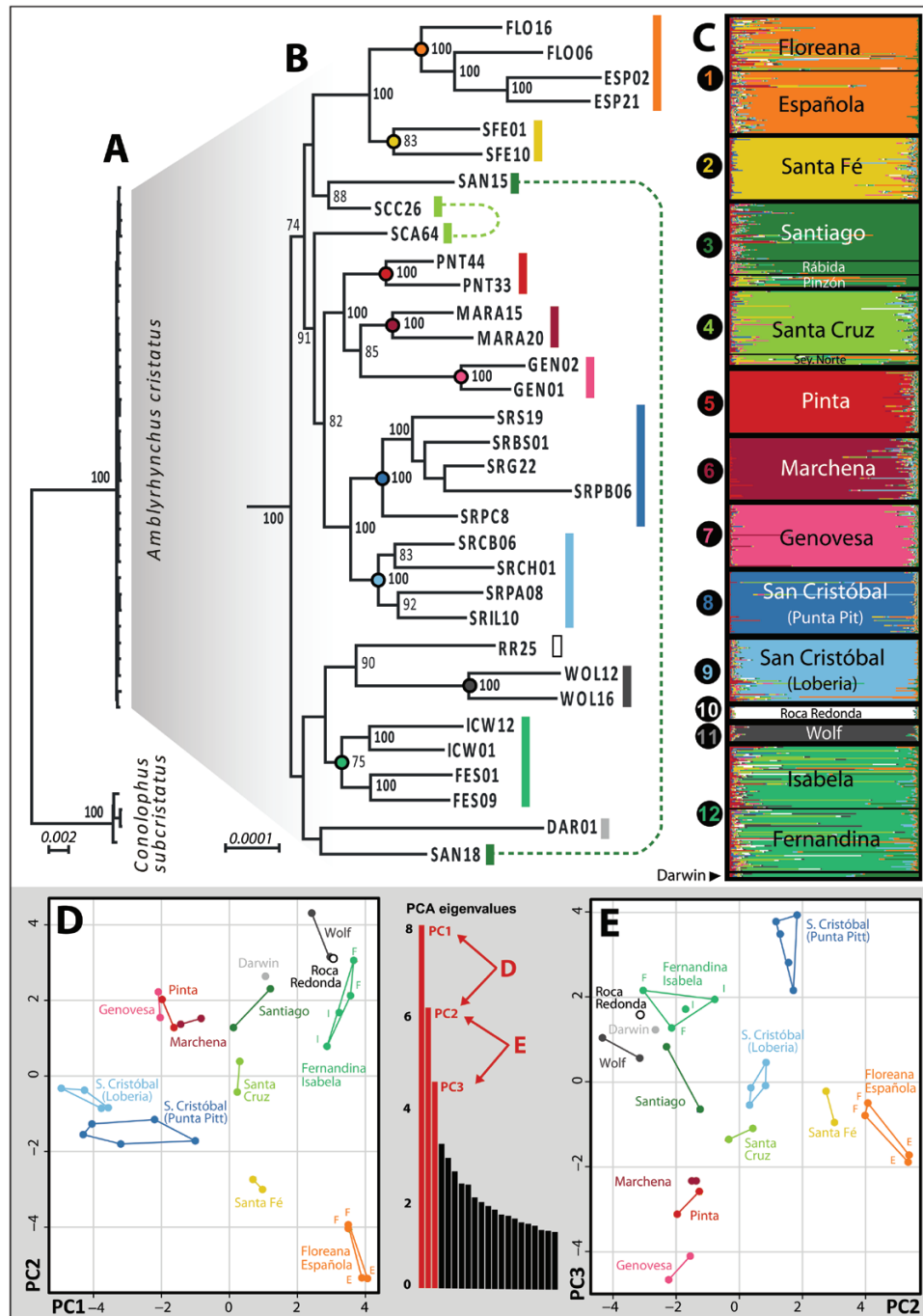


Figure 2. Genetic differentiation among Galápagos marine iguanas. (A, B) Phylogenetic tree realized from concatenated nuclear DNA sequences derived from RADseq. Basal nodes characterizing relevant clusters of specimens are highlighted by coloured circles, whereas the different samples belonging to a microsatellite-derived genetic cluster but retrieved paraphyletically in the tree are linked by a dashed line. Sample labels are abbreviated (cf. Material and Methods for more details). (C) STRUCTURE-based assignment of samples from the microsatellite dataset to genetic clusters, with an optimal value of $K = 12$. (D) PC1-2 and (E) PC2-3 plots from the SNP-PCA analysis using allele frequency of 1086 bi-allelic SNPs after mapping against the *Anolis* genome. Colours match in all sub-figures and refer to genetic clusters identified in the microsatellite analysis in (C).

information criterion (Akaike, 1974) in J-MODELTEST (Posada, 2008). Haplotype numbers were in keeping with those of Steinfartz *et al.* (2009), with the exception of newly detected haplotypes. The network figure was prepared combining HAPVIEW, POPART (Leigh & Bryant, 2015) and ADOBE ILLUSTRATOR CS2. To find haplotypes that were private to islands, we used the haplotype collapse tool available at FABOX (Villesen, 2007). Here, the two San Cristóbal populations were treated separately in recognition of their high level of distinctiveness, as noted by several previous studies (e.g. Steinfartz *et al.*, 2009; MacLeod *et al.*, 2015a).

SNP GENOTYPING ANALYSIS OF RADSEQ DATA

We used the original sequence data received from restriction site-associated DNA sequencing (RADseq) from MacLeod *et al.* (2015a). The sampling included the following specimens: five individuals from San Cristóbal (Punta Pitt population) [SRBS01, SRPB06, SRPC08, SRS19, SRG22], four from San Cristóbal (Lobería population) [SRCH01, SRCB06, SRPA08, SRIL10], two from Española [ESP02, 21], Isabela [ICW01, 12], Fernandina [FES01, 09], Floreana [FLO06, 16], Genovesa [GEN01, 02], Santa Cruz [SCC26, SCA64], Santa Fé [SFE01, 10], Santiago [SAN15, 18], Marchena [MARA15, 20], Pinta [PNT33, 44], Wolf [WOL12, 36] and a single one from Roca Redonda [RR25] and from Darwin [DAR01]. In addition to these ingroup samples, we included as outgroups four samples from Galápagos Land Iguanas (*Conolophus subcristatus*) [LSF04, LSF06, LSC05, LIV05] also included in previous phylogenetic analyses and showing a sister clade relationship with the genus *Amblyrhynchus* (see MacLeod *et al.*, 2015a, b).

We used two different approaches to describe the genetic variation observed in the RADseq dataset. First, we used a phylogenetic approach based on the short DNA sequence stretches obtained from RADseq, with individuals as terminal taxa. Such an approach might have restrictions in reconstructing phylogenetic relationships, but allows inference of clustering of individuals using a phylogenetic approach, that is not simply driven by sequence similarity. A *de novo* clustering and assembly approach was performed with PyRAD software (Eaton, 2014), using identical settings as reported in MacLeod *et al.* (2015a), except here we utilized a more relaxed clustering similarity threshold (85%) and a higher threshold for sample coverage (sample coverage = 50%, i.e. including only those loci where more than 19 samples had data). It has been shown that values of clustering similarity threshold between 60 and 90% can maximize the number of output loci of PyRAD algorithm (Takahashi, Nagata & Sota, 2014; Pante *et al.*, 2015) and therefore these

settings were selected to increase the number of output loci, while enforcing a relatively high sample coverage. This filtering scheme resulted in 52 396 RAD-Seq loci and their nucleotide sequences were concatenated in a 4 419 638 bp matrix alignment. The alignment was used for maximum likelihood phylogenetic inference under a GTR + Γ model of nucleotide substitution in RAxML v. 8 (Stamatakis, 2014), testing the statistical support of nodes with 100 bootstrap replicates.

In addition to the phylogenetic approach, we used a genotypic approach to characterize the genetic variation among marine iguana individuals and populations by analysing SNPs from the RADseq dataset. Starting with the de-multiplexed and quality-trimmed 90 bp single-end Illumina reads of the 33 marine iguana samples, we used bioinformatic tools to align the reads against the *Anolis carolinensis* genome (Alföldi *et al.*, 2011), call deep coverage variants and score SNPs well-represented across samples. Aligning was performed with BOWTIE2 (Langmead & Salzberg, 2012) using '--very-sensitive-local' settings. SNPs were sorted and indexed using SAMTOOLS (Li *et al.*, 2009) and later filtered using VCFTOOLS (Danecek *et al.*, 2011). Filtering settings included all SNPs with > 10 reads coverage (--minDP 10), >25% sample coverage (--max-missing-count 25) and excluded indel variants (--remove-indels). The genotype information of the resulting 21 792 SNPs was recoded in PLINK format (minor allele states coded as 0/1/2). To exclude monomorphic loci and rare alleles from the calculations, we enforced a 0.03 minor allele frequency threshold (therefore excluding those alleles present in only one individual), leaving only 1086 bi-allelic SNPs which were then used to represent the genetic diversity using a Principal Component Analysis (PCA), based on allele frequencies as implemented in ADEGENET package for R (Jombart & Ahmed, 2011).

To obtain a first hypothesis on the pattern of splits and mixtures among subspecies, and to identify those that might have regularly been receiving migrants and thus genetic material from others, we used the bi-allelic SNP data and reconstructed drift trees using the software TREEMIX (Pickrell & Pritchard, 2012), running 100 iterations and assuming between zero and five migration events among subspecies. Obviously, the number of migration events among subspecies of marine iguanas is largely unknown and migration is likely to be a rather continuous processes among these populations and not discrete and restricted to relatively short periods of time. Despite these violations of the underlying models in TREEMIX we think that our analyses would highlight the most important migration vectors and could be informative for our taxonomic decisions. Samples from Darwin and Wolf were used to root the tree based on the results of MacLeod *et al.* (2015a).

MORPHOLOGY

We examined a total of 166 specimens, most of them preserved in 70% ethanol. Most were entire specimens or sometimes only the skin, with few others being either stuffed or plastined, from the collections of the California Academy of Sciences, San Francisco (CAS, CAS-SUR), the Hessisches Landesmuseum, Darmstadt (HLMD), the Natural History Museum, London (NHM), the Oxford University Museum of Natural History (OUMNH) and the Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt am Main (SMF). A small number of skeletons collected in the field were deposited in the collection of the Charles Darwin Research Station on Santa Cruz, Galápagos Islands, Ecuador (VCCDRS). Additionally, 225 living specimens from 12 different localities of San Cristóbal (see [Macleod *et al.*, 2015a](#)) were photographed in the field for subsequent morphological examination.

Seven distinct metric measurements were recorded to the nearest 0.1 mm using a digital caliper, and 18 meristic (scales counts) or qualitative (pholidotic patterns) measurements were taken: Number of scales across the rostral field (RF), across the frontonasal field (FN) and the prefrontal field (PF), number of supralabials (SL) and infralabials (IL), configuration of the rostral scale regarding the adjacent supralabials (Ro) and of the pair of mental (Me) scales, number of scales around midbody (MSC), along the occipital gap of the crest (Cog), the nuchal crest (Cnc), the nuchal gap (Cng), the dorsal crest (Cdc) and the caudal crest (Ccc) [sums of Cnc and Cdc (C46) and sums of Cnc, Cng and Cdc (C456) have also been considered as distinct characters], number of lamellae under the third (Lam3) and the fourth toes (Lam4), number of femoral pores (FePo), snout-vent length (SVL), tail length (TL), fourth toe length (ToL), two different measures of the head length (HL1 and HL2), head width (HW) and head height (HH). Detailed explanations about these measurements, including pholidotic definitions presently used, are available in Appendix S1.

Statistical analyses of morphometric data were carried out with STATISTICA, v.7.1 (StatSoft Inc). Given the rather unbalanced dataset, with large numbers of specimens measured from particular islands and very few from other islands, and numerous missing data, we refrained from performing sophisticated multivariate tests. Furthermore, we emphasize that our goal was to probe for possible morphological divergence among genetic clusters for purposes of taxonomic diagnosis, and not to accurately describe shape differences. We chose to perform pairwise comparisons among all clusters with non-parametric Mann–Whitney U-tests, separately for males and females, and using size-ratio values (raw data divided by SVL) for morphometric

measurements. We performed a Bonferroni correction for multiple tests. However, we also kept only those results that were significant before applying this correction, given that the correction counteracted our main purpose, that is understanding which clusters show morphometric differences in a higher or lower number of characters when compared to other clusters. Considering criticism on Bonferroni correction (e.g. [Perneger, 1998](#); [Nakagawa, 2004](#)), we are convinced this procedure is justified in our case. Besides Mann–Whitney U-tests, we also visualized differences among clusters by performing a PCA on the raw data, including specimens without missing data only and leaving PCA axes non-rotated.

DELIMITATION OF SUBSPECIFIC UNITS WITHIN
AMBLYRHYNCHUS CRISTATUS

Previous studies have characterized *A. cristatus* as a single species with distinct genetic clusters that have not reached species-level differentiation ([Macleod *et al.*, 2015a](#)). We here follow this view and will classify these units as subspecies. Although an objective definition of subspecies does not exist, we herein follow [Vences *et al.* \(2013\)](#) and [Hawltischek, Nagy & Glaw \(2012\)](#), considering the subspecies rank as useful to refer to distinguishable population-level units that have not attained a status of independent evolutionary lineages, and thus do not satisfy species criteria.

To reach a meaningful delimitation of marine iguana subspecies, we integrated the various sets of molecular and morphological data. Our informal delimitation procedure started with the population genetic analysis of microsatellite loci data using STRUCTURE, as this method is able to identify genetic clusters among individuals based on their genotypic information (e.g. shown for marine iguanas in previous work; [MacLeod & Steinfartz, 2016](#)). We then compared representative samples of these clusters for congruent differentiation in SNP loci, and subsequently also took their differentiation of mitochondrial DNA and external morphology into account. Specifically, we considered support for a subspecies-level unit (initially defined as separate cluster on the basis of microsatellite loci analysis) to increase if it (1) represents a cluster (reconstructed as monophyletic group) in the RADseq tree, (2) is not closely related to other clusters in the RADseq tree, (3) is separated from other clusters by non-overlapping space in the SNP-PCA analysis and (4) shows significant differences to other clusters in many morphometric characters. We also inspected the SNP-based PCA to understand if other alternative groupings of samples were evident, or whether samples belonging to the same microsatellite loci-based cluster might include distinctly divergent subunits.

To assign subspecies-level nomina to clusters, we followed a general principle of minimal change with respect to existing names (cf. [Vences *et al.*, 2013](#)), in order to modify the existing classification only where strictly suggested by the data, and to make, as much as possible, use of previously available nomina rather than erecting new ones.

RESULTS

POPULATION GENETIC STRUCTURE INFERRED FROM MICROSATELLITE LOCI

STRUCTURE analysis showed that the most likely number of genetic clusters inferred from the microsatellite loci data was 12. This is in keeping with the ten clusters indicated by previous work ([MacLeod & Steinfartz, 2016](#)), including the addition of one new cluster each for the islands of Wolf and Roca Redonda (Fig. 2C). For most islands, the assignment of a genetic cluster was clear, with the general pattern being one genetic cluster per island. The exceptions were Fernandina and Isabela, Floreana and Española, and Santiago and Rábida – in these cases each pair of islands share a genetic cluster. San Cristóbal is a further exception, with two rather distinct clusters present. For the small islands of Darwin, Pinzón and Seymour Norte, there was no clear pattern of individual assignment to other clusters. The results for these islands should be regarded as inconclusive, especially since sampling sizes here are small. Seymour Norte and Pinzón are herein assigned to the Santa Cruz and Santiago clusters, respectively, based on the highest assignment probability of the majority of individuals; see discussion (page 30) below regarding the identity of the population from Darwin. Across the assignment tests, average self-assignment (number of individuals that were assigned to their own cluster, i.e. island of origin) was 95% (range: 85–100%). The number of private alleles per cluster ranged from 0 to 10, with Fernandina and Isabela having the most (cf. more details in Tables S2, S3, S7, S8).

RADSEQ DATA

In the phylogenetic tree (Fig. 2A, B), marine iguanas are retrieved as monophyletic, and show a relatively low level of intraspecific divergence (<0.05%). Most of the island-dependent population clusters are retrieved as monophyletic units. We will, in the following, refer to these mostly as clusters to avoid implying common ancestry, given that the concatenated analysis is not able to distinguish between common ancestry of these individuals and similarity due to possible secondary admixture. In the phylogenetic

tree, the following clusters formed monophyletic units: Española (bootstrap score of 100%), Santa Fé (83%), Pinta (100%), Marchena (100%), Genovesa (100%), San Cristóbal (100%), Wolf (100%), Isabela (100%) and Fernandina (100%). In contrast, specimens from Santiago, Santa Cruz and Floreana do not form clusters. However, all the specimens from Floreana and Española together form a strongly supported clade (100%), sister to Santa Fé (100%). Populations from the three north-eastern islands of Pinta, Marchena and Genovesa together also form a well-supported clade (100%). Populations from Fernandina and Isabela are inferred as a moderately supported clade (75%), which is sister to a clade containing the specimens from Wolf and Roca Redonda (90%). The tree retrieves as reciprocally monophyletic units (100% each) the two divergent units ('Punta Pitt' and 'Lobería' populations) previously reported by [MacLeod *et al.* \(2015a\)](#) on the island of San Cristóbal.

The first three axes of the PCA based on SNP data (Fig. 2D, E) explain 18.9% of the variability (PC1: 8.1%, PC2: 6.2%, PC3: 4.6%). These three axes allow the unambiguous discrimination of most of the insular populations, the pairs of islands Pinta/Marchena and Fernandina/Isabela presenting the weakest degree of differentiation, respectively. PCA also retrieves a relevant divergence between the two allopatric San Cristóbal populations ('Punta Pitt' and 'Lobería').

Analysis of population mixture using TREEMIX led to the subspecies tree represented in Appendix S2. With one and two assumed mixture events, the analysis reconstructed successively the following instances of mixture (1) from San Cristóbal 'Punta Pitt' to Santa Fé; (2) from a branch connecting the cluster of Santa Fé/Floreana/Española to the remaining populations to San Cristóbal 'Punta Pitt'. With higher assumed numbers of mixture events, alternative cases were reconstructed, but these never involved any direct mixture between the two separate populations from San Cristóbal.

ANALYSIS OF THE MITOCHONDRIAL DATASET

The 1467 individual sequences could be collapsed into 113 distinct haplotypes (Fig. 3), including the 106 found in earlier work ([Steinfartz *et al.*, 2009](#)) plus seven newly detected haplotypes. Fernandina had by far the largest number of private haplotypes, followed by Isabela. Both Santa Cruz and the Punta Pitt population of San Cristóbal harboured each a single haplotype that was private to that island or population (cf. also Table S4). The three haplogroups identified by [Steinfartz *et al.* \(2009\)](#) were maintained, each group having notable geographic structure. Individuals with haplotypes from group C were generally from the northern islands of Pinta, Genovesa, Marchena and the eastern islands of

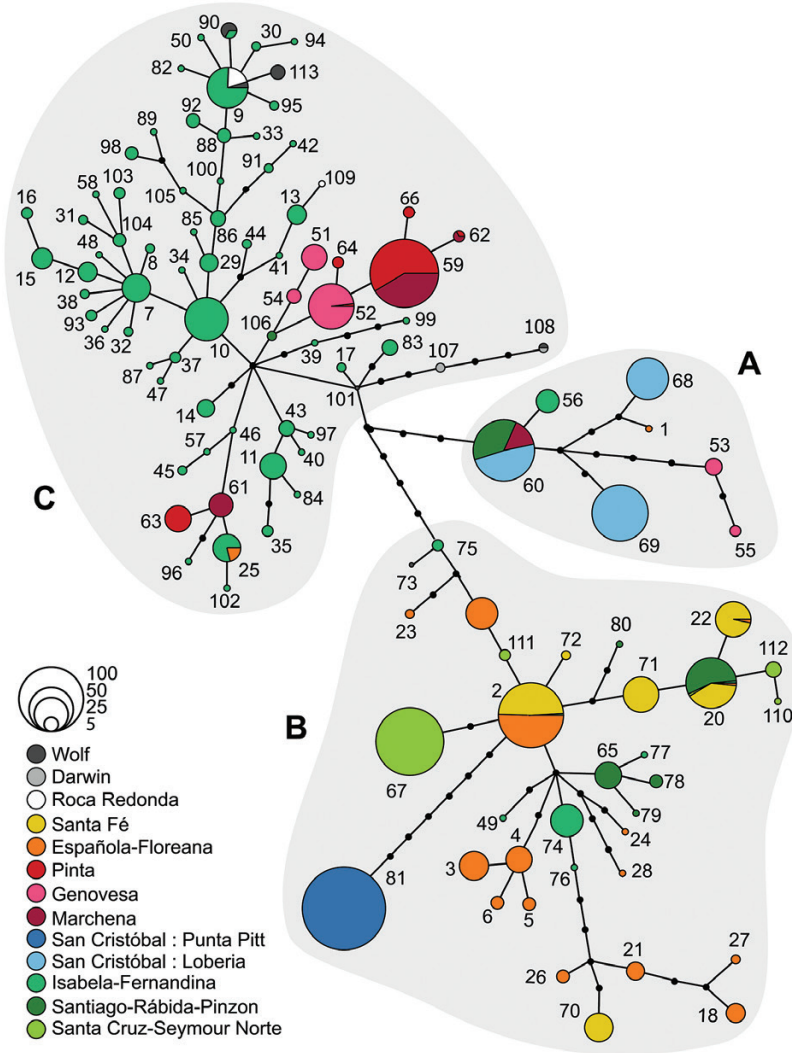


Figure 3. Haplotype network inferred from 1181 bp of the mitochondrial control region in 1467 individuals. Circles represent haplotypes (size proportional to the number of individuals), black lines represent mutational steps and black dots missing haplotypes. A, B and C refer to the three main haplogroups previously identified by [Steinfartz *et al.* \(2009\)](#).

Fernandina and Isabela. Group A had the fewest haplotypes and is dominated by individuals from Lobería on San Cristóbal Island. Group B is dominated by individuals from the central, southern and eastern islands and includes the distinctive haplotype 81, unique to animals from the Punta Pitt population of San Cristóbal Island.

MORPHOLOGICAL STUDY

Comparisons of the phenotypic traits examined show a significant level of divergence between the different insular populations of marine iguanas ([Fig. 4](#), [Tables 1](#), S5, S6). Among the most obviously differentiated traits, body size (SVL) is remarkably variable: populations of large body size such as Santa Cruz, Fernandina/Isabela or San Cristóbal–Lobería have maximum male

SVL values almost double those measured for the dwarf populations from Darwin, Wolf or Genovesa. Individuals from Santa Cruz (largest SVL) and Darwin (smallest SVL) are the extremes ([Fig. 4A](#)). Although the different meristic and morphometric traits examined do not present unambiguous diagnostic properties and values overlap for most pairwise comparisons among the genetically defined clusters, significant differences in at least a few of these variables were detected in the vast majority of pairwise comparisons of male specimens, despite occasionally low sample sizes. Due to the large amount of pairwise comparisons, most of the significance values did not hold after Bonferroni correction over the total of 2047 tests. Nevertheless, for many of the comparisons having substantial sample sizes, some variables remained statistically significant at the required

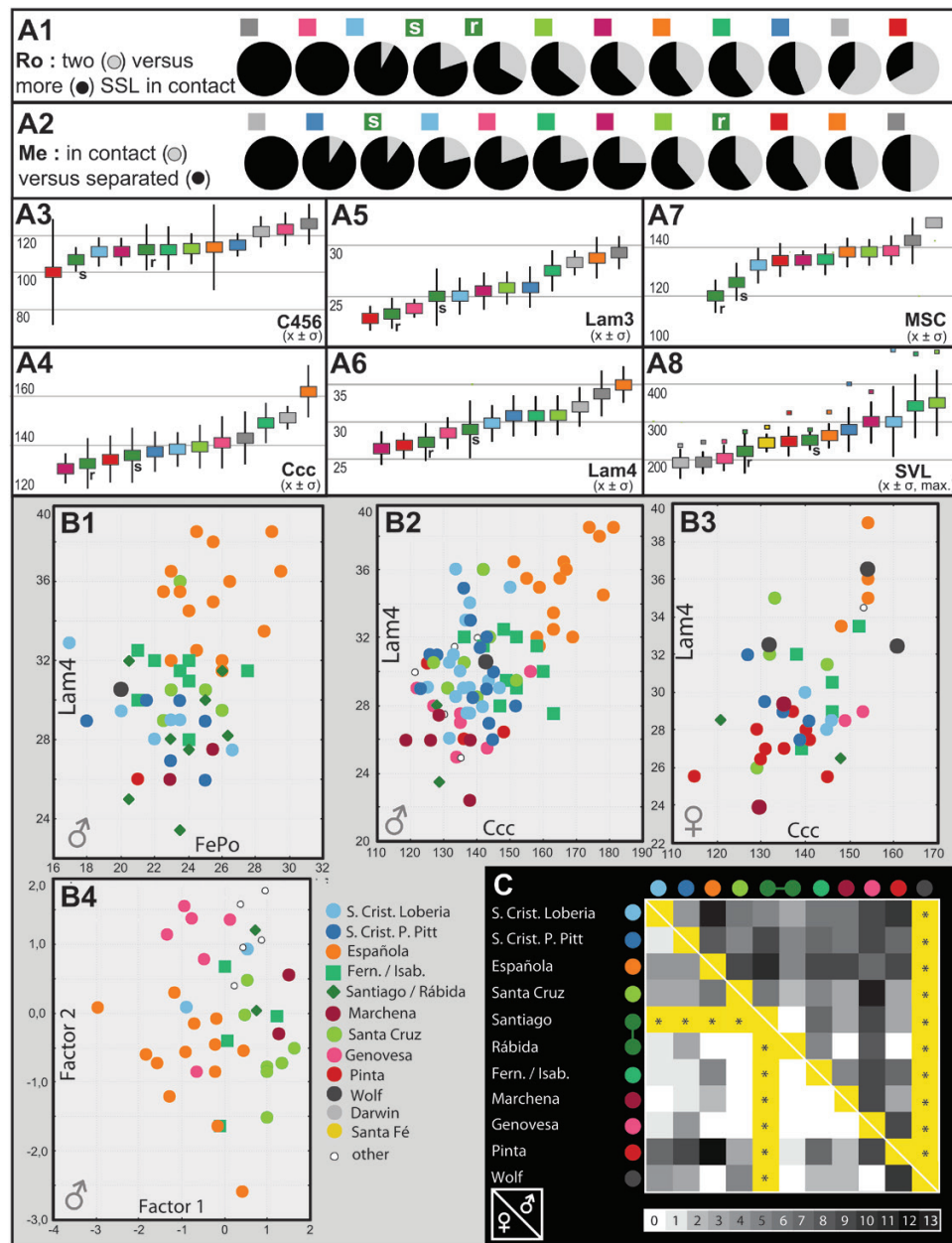


Figure 4. Morphological differentiation among the different clusters of Galápagos marine iguanas. (A) Phenotypic variation for a selection of the most relevant morphological traits: (A1, 2) rostral (Ro) and mental (Me) scales configuration, (A3, 4) number of crest spines along body (C456) and tail (Ccc), (A5, 6) number of lamellae under the third (Lam3) and fourth toes (Lam4), (A7) number of scale around midbody (MSC, mean from both sides) and (A8) snout-vent length (SVL, in mm). (B) Bivariate scatterplots showing variation among individuals in selected scale counts showing distinct differences among populations: femoral pores (FePo) vs. Lam4, (B2–B3) tail crest spines vs. Lam4, separately for males and females; B4 is a bivariate plot of first and second factors from a PCA of morphological variables (males without missing data only). (C) Graphical abstract showing the levels of divergence between each pair of clusters. The number of morphological characters with significant differences in each pairwise comparison (Mann–Whitney U-tests) is represented with different shades of grey (from 0 to 13 variables with significant differences). Males above, females below diagonal. Statistical comparisons not testable due to an insufficient sampling size are represented by yellow cells with an asterisk (Darwin and Santa Fé populations not included for the same reason). See Table 1 for detailed descriptive dataset and Table S5 for the statistical tests. Genetic clusters derived from microsatellite analysis are represented with the same colours in all figures.

Table 1. Comparison between the main genetic clusters of Galápagos marine iguanas for the most relevant morphological characters examined for the present study.

		<i>A. c. cristatus</i>	<i>A. c. godzilla</i>	<i>A. c. hassi</i>	<i>A. c. jeffreysi</i>		<i>A. c. mertensi</i>
(1/2)		Isab–Ferna	San Cristóbal PP	Santa Cruz	Wolf	Darwin	SanCris LOB
Ro	<i>contact 2 SSL :</i>	40.0%	44.1%	36.0%	0.0%	60.0%	23.1%
	<i>more than 2 :</i>	60.0%	55.9%	64.0%	100.0%	40.0%	76.9%
	(<i>n</i>)	(25)	(68)	(25)	(8)	(5)	(13)
Me	<i>in contact :</i>	21.7%	9.1%	38.9%	50.0%	0.0%	30.8%
	<i>not in contact :</i>	78.3%	90.9%	61.1%	50.0%	100.0%	69.2%
	(<i>n</i>)	(23)	(33)	(18)	(8)	(5)	(13)
Cog	$\bar{x} \pm \sigma$	3.9 $\pm$ 2.0	N/A	5.4 $\pm$ 2.8	4.0 $\pm$ 0.8	7.8 $\pm$ 1.7	5.3 $\pm$ 2.1
	m–M (<i>n</i>)	0–9 (25)		1–12 (19)	3–5 (8)	6–10 (4)	3–10 (12)
Cnc	$\bar{x} \pm \sigma$	15.4 $\pm$ 3.0	16.8 $\pm$ 2.8	14.8 $\pm$ 3.1	16.5 $\pm$ 4.8	16.8 $\pm$ 1.7	16.6 $\pm$ 3.9
	m–M (<i>n</i>)	5–20 (24)	11–24 (28)	10–22 (19)	7–24 (8)	15–19 (4)	7–24 (12)
Cng	$\bar{x} \pm \sigma$	7.4 $\pm$ 6.1	10.8 $\pm$ 3.2	9.3 $\pm$ 7.1	14.4 $\pm$ 12.9	14.0 $\pm$ 2.2	14.3 $\pm$ 10.3
	m–M (<i>n</i>)	0–16 (23)	5–17 (28)	0–26 (18)	0–43 (8)	12–17 (4)	7–43 (12)
Cdc	$\bar{x} \pm \sigma$	91.0 $\pm$ 7.4	87.4 $\pm$ 5.2	88.9 $\pm$ 8.1	95.6 $\pm$ 9.7	91.5 $\pm$ 5.8	93.3 $\pm$ 8.6
	m–M (<i>n</i>)	78–107 (24)	78–97 (30)	76–104 (18)	84–113 (8)	86–99 (4)	84–113 (12)
Ccc	$\bar{x} \pm \sigma$	149.1 $\pm$ 8.0	137.4 $\pm$ 8.2	139.4 $\pm$ 8.7	142.9 $\pm$ 10.8	151.2 $\pm$ 4.9	146.1 $\pm$ 9.7
	m–M (<i>n</i>)	136–163 (21)	122–152 (28)	127–161 (18)	128–161 (8)	144–157 (5)	128–161 (13)
c46	$\bar{x} \pm \sigma$	106.3 $\pm$ 8.2	104.6 $\pm$ 6.0	103.9 $\pm$ 9.1	112.1 $\pm$ 11.4	108.3 $\pm$ 6.2	110.8 $\pm$ 9.8
	m–M (<i>n</i>)	94–126 (24)	95–116 (28)	88–122 (18)	100–133 (8)	103–115 (4)	100–133 (12)
c456	$\bar{x} \pm \sigma$	113.5 $\pm$ 11.6	115.3 $\pm$ 6.1	113.2 $\pm$ 8.1	126.5 $\pm$ 10.8	122.3 $\pm$ 8.2	125.1 $\pm$ 9.9
	m–M (<i>n</i>)	95–134 (24)	103–125 (27)	101–129 (18)	112–145 (8)	115–132 (4)	112–145 (12)
MSC*	$\bar{x} \pm \sigma$	135.1 $\pm$ 6.3	N/A	138.0 $\pm$ 5.3	142.7 $\pm$ 9.4	150.0 $\pm$ 1.4	144.3 $\pm$ 8.7
	m–M (<i>n</i>)	124.5–144.0 (21)		130.5–148.5 (12)	124.0–149.5 (7)	149.0–151.0 (2)	124.0–151.0 (9)
SL*	$\bar{x} \pm \sigma$	9.6 $\pm$ 0.4	9.4 $\pm$ 0.7	9.1 $\pm$ 0.8	9.2 $\pm$ 0.5	9.8 $\pm$ 0.8	9.4 $\pm$ 0.7
	m–M (<i>n</i>)	9.0–10.5 (24)	8.0–11.5 (61)	7.5–11.0 (17)	8.5–10.0 (8)	8.5–10.5 (5)	8.5–10.5 (13)
IL*	$\bar{x} \pm \sigma$	11.0 $\pm$ 1.0	11.4 $\pm$ 1.0	11.1 $\pm$ 0.9	11.6 $\pm$ 1.0	11.9 $\pm$ 0.2	11.7 $\pm$ 0.8
	m–M (<i>n</i>)	9.0–13.0 (23)	9.0–15.0 (58)	10.0–13.0 (17)	10.5–13.0 (8)	11.5–12.0 (5)	10.5–13.0 (13)
RF	$\bar{x} \pm \sigma$	4.3 $\pm$ 0.8	4.5 $\pm$ 0.6	4.3 $\pm$ 0.6	3.8 $\pm$ 0.5	5.2 $\pm$ 0.4	4.3 $\pm$ 0.9
	m–M (<i>n</i>)	3–6 (24)	4–6 (34)	3–5 (19)	3–4 (8)	5–6 (5)	3–6 (13)
FN	$\bar{x} \pm \sigma$	3.3 $\pm$ 0.8	3.1 $\pm$ 0.5	3.6 $\pm$ 0.5	3.4 $\pm$ 0.5	3.4 $\pm$ 0.5	3.4 $\pm$ 0.5
	m–M (<i>n</i>)	2–5 (24)	2–4 (25)	3–4 (18)	3–4 (8)	3–4 (5)	3–4 (13)
PF	$\bar{x} \pm \sigma$	4.0 $\pm$ 0.8	3.6 $\pm$ 0.9	3.9 $\pm$ 0.8	3.5 $\pm$ 0.5	4.2 $\pm$ 0.4	3.8 $\pm$ 0.6
	m–M (<i>n</i>)	3–5 (24)	3–6 (23)	3–5 (17)	3–4 (8)	4–5 (5)	3–5 (13)
FePo*	$\bar{x} \pm \sigma$	24.0 $\pm$ 2.5	21.4 $\pm$ 2.6	24.1 $\pm$ 1.3	22.3 $\pm$ 3.2	22.5	22.3 $\pm$ 2.3
	m–M (<i>n</i>)	21.0–29.0 (11)	17.0–25.5 (18)	22.0–26.0 (10)	20.0–24.5 (2)	(1)	20.0–24.5 (3)
Lam4*	$\bar{x} \pm \sigma$	30.8 $\pm$ 2.6	30.8 $\pm$ 2.7	30.9 $\pm$ 2.6	33.8 $\pm$ 3.0	32.0 $\pm$ 2.6	33.2 $\pm$ 2.9
	m–M (<i>n</i>)	25.5–36.0 (24)	26.0–38.0 (58)	26.0–36.0 (16)	30.5–40.0 (8)	29.5–34.5 (4)	29.5–40.0 (12)
Lam3*	$\bar{x} \pm \sigma$	27.5 $\pm$ 2.0	25.9 $\pm$ 2.0	25.8 $\pm$ 1.6	29.3 $\pm$ 1.6	28.3 $\pm$ 1.2	29.0 $\pm$ 1.5
	m–M (<i>n</i>)	23.0–31.5 (24)	22.5–30.0 (58)	23.0–28.5 (16)	26.0–31.0 (8)	27.5–30.0 (4)	26.0–31.0 (12)
ToL*	$\bar{x} \pm \sigma$	58.4 $\pm$ 11.6	50.2 $\pm$ 8.4	53.0 $\pm$ 11.2	36.7 $\pm$ 3.9	37.5 $\pm$ 6.4	37.0 $\pm$ 4.6
	m–M (<i>n</i>)	44.0–81.7 (18)	32.3–67.0 (70)	37.9–69.2 (13)	32.8–42.5 (8)	31.7–43.5 (4)	31.7–43.5 (12)
HL1	$\bar{x} \pm \sigma$	59.2 $\pm$ 12.5	N/A	54.8 $\pm$ 15.6	36.3 $\pm$ 4.9	35.9 $\pm$ 7.6	36.1 $\pm$ 5.6
	m–M (<i>n</i>)	40.9–79.6 (18)		13.1–74.9 (15)	30.4–44.8 (8)	29.2–44.3 (4)	29.2–44.8 (12)
HL2	$\bar{x} \pm \sigma$	53.7 $\pm$ 11.8	49.3 $\pm$ 10.4	49.2 $\pm$ 13.8	32.2 $\pm$ 4.3	31.9 $\pm$ 7.9	32.1 $\pm$ 5.4
	m–M (<i>n</i>)	36.1–74.9 (18)	29.2–72.7 (68)	13.3–67.5 (15)	25.9–37.4 (8)	24.8–41.2 (4)	24.8–41.2 (12)
HH	$\bar{x} \pm \sigma$	50.5 $\pm$ 13.2	41.5 $\pm$ 8.8	44.0 $\pm$ 14.7	27.0 $\pm$ 3.8	27.6 $\pm$ 6.6	27.2 $\pm$ 4.6
	m–M (<i>n</i>)	31.8–69.2 (18)	24.5–57.9 (70)	10.0–64.5 (15)	21.6–31.8 (8)	21.1–33.5 (4)	21.1–33.5 (12)
HW	$\bar{x} \pm \sigma$	54.5 $\pm$ 14.2	40.6 $\pm$ 8.5	52.6 $\pm$ 11.8	30.9 $\pm$ 4.1	29.3 $\pm$ 5.7	30.4 $\pm$ 4.5
	m–M (<i>n</i>)	34.2–77.3 (18)	22.6–56.4 (70)	34.2–73.1 (14)	25.3–37.5 (8)	24.7–37.1 (4)	24.7–37.5 (12)
TL	$\bar{x} \pm \sigma$	543.1 $\pm$ 156.3	482.6 $\pm$ 98.3	476.4 $\pm$ 116.5	309.1 $\pm$ 52.7	303.5 $\pm$ 59.8	307.3 $\pm$ 52.4
	m–M (<i>n</i>)	330–843 (22)	260–680 (71)	289–640 (14)	239–379 (8)	246–360 (4)	239–379 (12)
SVL	$\bar{x} \pm \sigma$	341.9 $\pm$ 83.6	280.1 $\pm$ 58.3	350.6 $\pm$ 86.6	192.3 $\pm$ 29.1	189.5 $\pm$ 38.9	191.3 $\pm$ 30.9
	m–M (<i>n</i>)	160–480 (23)	160–400 (71)	210–485 (14)	155–245 (8)	148–236 (4)	148–245 (12)

Table 1. continued

<i>A. c. nanus</i>	<i>A. c. hayampi</i>	<i>A. c. sielmanni</i>	<i>A. c. trillmichi</i>	<i>A. c. venustissimus</i>	<i>A. c. wikelskii</i>		
Genovesa	Marchena	Pinta	Santa Fé	Española	Santiago	Rábida	TOTAL
0.0%	37.5%	66.7%	N/A	40.0%	20.0%	33.3%	25%
100.0%	62.5%	33.3%		60.0%	80.0%	66.7%	75%
(16)	(8)	(18)		(25)	(10)	(6)	(16)
20.0%	25.0%	41.2%	N/A	45.8%	10.0%	40.0%	20%
80.0%	75.0%	58.8%		54.2%	90.0%	60.0%	80%
(15)	(8)	(17)		(24)	(10)	(5)	(15)
6.6 ± 2.9	4.5 ± 2.2	4.6 ± 2.7	N/A	4.1 ± 1.9	4.1 ± 1.5	5.2 ± 1.9	4.5 ± 1.7
3–12 (16)	3–10 (8)	2–12 (17)		2–9 (24)	2–7 (10)	3–8 (5)	2–8 (15)
16.1 ± 3.8	15.0 ± 4.1	16.0 ± 2.5	N/A	17.2 ± 2.7	14.5 ± 2.6	16.4 ± 5.0	15.1 ± 3.5
11–25 (16)	5–20 (8)	10–19 (17)		12–24 (26)	10–20 (10)	9–23 (5)	3–23 (15)
15.1 ± 4.2	10.1 ± 4.4	12.6 ± 3.2	N/A	12.1 ± 8.0	10.7 ± 6.2	14.0 ± 7.7	11.8 ± 6.7
8–23 (16)	5–18 (8)	6–18 (17)		0–30 (24)	0–21 (10)	6–25 (5)	0–25 (15)
92.6 ± 6.5	86.4 ± 3.4	77.8 ± 13.6	N/A	89.2 ± 5.4	82.2 ± 6.4	83.6 ± 10.7	82.7 ± 7.7
84–104 (16)	81–91 (8)	28–93 (17)		81–102 (24)	75–98 (10)	70–95 (5)	70–98 (15)
141.0 ± 10.8	130.5 ± 6.0	134.2 ± 9.6	N/A	161.7 ± 10.6	135.9 ± 11.2	132.6 ± 10.3	134.8 ± 10.6
122–156 (15)	119–138 (8)	115–148 (15)		138–181 (21)	121–161 (10)	121–148 (5)	121–161 (15)
108.7 ± 8.4	101.4 ± 4.7	93.8 ± 14.1	N/A	106.5 ± 7.0	96.7 ± 6.9	100.0 ± 14.8	97.8 ± 9.8
99–123 (16)	92–106 (8)	44–112 (17)		93–125 (24)	88–112 (10)	79–117 (5)	79–117 (15)
123.8 ± 8.9	111.5 ± 7.3	100.6 ± 28.4	N/A	114.1 ± 22.9	107.4 ± 6.4	114.0 ± 12.3	109.6 ± 9.1
111–137 (16)	99–122 (8)	0–127 (18)		18–134 (24)	99–117 (10)	102–129 (5)	99–129 (15)
138.8 ± 5.9	134.7 ± 3.9	134.8 ± 6.8	N/A	137.9 ± 5.9	125.8 ± 7.7	119.9 ± 6.7	123.2 ± 7.5
128.5–145.0 (12)	129.0–140.0 (9)	123.5–148.0 (15)		130.0–148.5 (19)	114.0–135.5 (5)	110.0–125.0 (4)	110–135.5 (9)
9.3 ± 0.6	8.9 ± 1.0	9.3 ± 0.6	N/A	9.5 ± 0.6	8.7 ± 0.5	8.9 ± 0.5	8.8 ± 0.5
8.5–10.5 (16)	7.0–10.0 (8)	8.5–10.0 (16)		8.0–10.5 (24)	7.5–9.5 (10)	8.0–9.5 (5)	7.5–9.5 (15)
10.8 ± 0.9	10.3 ± 0.5	11.3 ± 0.9	N/A	11.5 ± 0.9	10.6 ± 0.7	10.8 ± 1.0	10.6 ± 0.8
10.0–13.0 (15)	9.5–11.0 (8)	9.5–12.5 (16)		9.5–13.0 (23)	9.5–12.0 (10)	9.5–12.0 (5)	9.5–12 (15)
4.7 ± 0.5	4.3 ± 0.4	4.8 ± 0.6	N/A	4.3 ± 0.6	4.1 ± 0.7	4.4 ± 0.5	4.2 ± 0.7
4–5 (16)	4–5 (8)	4–6 (17)		3–5 (26)	3–5 (10)	4–5 (5)	3–5 (15)
3.1 ± 0.8	3.1 ± 0.3	3.1 ± 0.4	N/A	3.4 ± 0.6	3.3 ± 0.7	3.0 ± 0.7	3.2 ± 0.7
2–5 (16)	3–4 (7)	2–4 (17)		3–5 (26)	2–4 (10)	2–4 (5)	2–4 (15)
3.7 ± 0.7	3.1 ± 0.3	3.6 ± 0.9	N/A	4.0 ± 0.9	3.3 ± 0.5	3.8 ± 1.1	3.5 ± 0.7
3–5 (16)	3–4 (7)	3–5 (17)		3–5 (26)	3–4 (10)	3–5 (5)	3–5 (15)
28.6 ± 1.8	24.3 ± 1.3	23.5 ± 3.5	N/A	25.3 ± 2.2	23.2 ± 2.6	23.3 ± 0.4	23.2 ± 2.1
25.0–31.0 (10)	23.0–25.5 (2)	21.0–26.0 (2)		22.5–29.5 (16)	20.5–26.0 (5)	23.0–23.5 (2)	20.5–26.0 (7)
28.5 ± 2.1	26.5 ± 2.2	26.8 ± 1.7	N/A	35.0 ± 2.4	29.0 ± 3.8	27.3 ± 2.5	28.4 ± 3.4
25.0–33.0 (16)	22.5–30.0 (10)	23.0–30.5 (15)		31.5–39.0 (22)	22.5–34.0 (10)	23.5–30.0 (5)	22.5–34.0 (15)
23.8 ± 0.9	25.5 ± 1.8	22.8 ± 1.2	N/A	28.8 ± 2.0	24.9 ± 2.8	23.3 ± 1.5	24.4 ± 2.5
22.0–25.5 (16)	22.0–29.0 (10)	20.0–24.5 (16)		24.5–32.5 (23)	21.0–29.5 (10)	21.5–25.0 (5)	21.0–29.5 (15)
36.3 ± 4.8	44.6 ± 5.2	39.9 ± 4.4	46.8 ± 5.2	47.3 ± 4.3	42.3 ± 4.0	41.8 ± 2.8	42.1 ± 3.4
27.3–43.1 (16)	39.5–53.6 (7)	33.2–46.6 (15)	36.5–55.4 (12)	38.9–54.8 (19)	36.9–45.8 (5)	39.0–44.8 (4)	36.9–45.8 (9)
38.5 ± 5.8	47.7 ± 5.7	42.6 ± 6.0	43.4 ± 5.4	48.7 ± 5.0	43.5 ± 5.3	42.4 ± 3.1	43.0 ± 4.2
27.2–44.8 (16)	39.7–56.3 (7)	34.5–52.7 (15)	35.8–52.4 (12)	39.5–58.5 (19)	37.5–50.1 (5)	39.7–46.8 (4)	37.5–50.1 (9)
34.0 ± 5.9	42.7 ± 6.3	37.2 ± 5.7	N/A	44.5 ± 4.7	38.2 ± 5.1	37.8 ± 1.9	38.0 ± 3.8
22.3–41.0 (16)	34.7–53.0 (7)	30.6–49.6 (15)		36.3–55.3 (19)	33.2–44.2 (5)	35.8–40.3 (4)	33.2–44.2 (9)
28.8 ± 4.6	39.2 ± 6.2	33.8 ± 4.6	30.9 ± 4.5	36.8 ± 5.5	34.6 ± 4.8	32.2 ± 2.8	33.5 ± 4.0
19.9–33.8 (16)	29.9–48.4 (7)	28.2–42.0 (14)	22.6–37.8 (12)	27.1–47.6 (19)	28.3–40.1 (5)	29.6–35.3 (4)	28.3–40.1 (9)
34.1 ± 5.4	42.9 ± 5.6	37.3 ± 5.4	30.8 ± 3.5	41.2 ± 5.0	38.4 ± 5.5	36.9 ± 3.8	37.7 ± 4.6
24.4–40.3 (16)	34.7–51.0 (7)	30.9–46.0 (15)	25.6–37.3 (12)	32.1–49.9 (18)	32.5–43.6 (5)	32.8–42.0 (4)	32.5–43.6 (9)
324.8 ± 56.8	415.8 ± 76.0	340.6 ± 41.5	395.8 ± 47.9	448.4 ± 60.8	394.0 ± 35.3	305.6 ± 54.9	349.8 ± 63.7
212–395 (15)	336–545 (10)	287–422 (13)	330–485 (12)	330–555 (19)	351–431 (5)	217–367 (5)	217–431 (10)
204.8 ± 33.9	298.5 ± 54.6	248.5 ± 38.6	244.7 ± 23.6	263.5 ± 32.4	251.0 ± 25.0	221.0 ± 56.4	236.0 ± 44.0
146–247 (16)	206–379 (10)	200–323 (15)	215–285 (12)	200–325 (19)	221–278 (5)	125–271 (5)	125–278 (10)

For each given meristic and mensural character we give mean ± SD, followed by range and sample size in parentheses. For bilateral meristic characters (*), the mean has been calculated from both sides. For abbreviations of variables, see Material and Methods.

level of $P < 0.00003$ (Table S5). This was true for the comparison of specimens from San Cristóbal–Lobos to those from San Cristóbal–Punta Pitt (variable TL/SVL), Española (Ccc, Lam3, Lam4, SVL), Santa Cruz (TL/SVL) and Genovesa (SVL); of specimens from San Cristóbal–Punta Pitt to those from Española (SVL, Ccc, Lam3), Santa Cruz (TL/SVL), Marchena (HL2/SVL) and Genovesa (SVL, HW/SVL); of specimens from Española to those from Santa Cruz (Ccc), Isabela–Fernandina (SVL, Lam4) and Genovesa (Lam3, Lam4) and specimens from Isabela–Fernandina to those from Genovesa (SVL, Lam3). These data suggest that in particular, variation in the number of lamellae under the toes is a strongly diagnostic character in some population comparisons.

Statistically supported differences between females were less common in pairwise inter-cluster comparisons. This certainly can be in part explained by even lower sample sizes for females, but it also suggests that females might be characterized by a less pronounced inter-population phenotypic divergence than males, and that many morphological differences between clusters might refer to sexually dimorphic traits differentially expressed in males (Fig. 4C).

It was unfortunately not possible to formalize, quantify and objectively compare colour pattern because of the high complexity and variability of this trait, and because of the variable states of preservation and degrees of colour patterns alteration in old collection specimens we examined. Furthermore, our dataset might include specimens with brighter breeding coloration and other more dull specimens collected in other times of the year, with unequal distribution across the islands. It nevertheless appears from our observations that males from different islands tend to show obvious chromatic differentiation (e.g. males from Española are characterized by a remarkably bright coloration with a turquoise vertebral stripe running over a red dorsal background, whereas those from Wolf and Darwin are almost completely black). In contrast, females tend to be more uniform across the archipelago and are remarkably darker, less contrasted and more homogeneous than males belonging to the same population. Examples for differences in male colour pattern in inter-cluster comparisons are the small-sized populations from Genovesa, Darwin and Wolf which have a dark overall coloration, those from Marchena or Isabela which tend to have a contrasted tabby pattern, those from Santa Cruz and San Cristóbal–Lobos with a dark background with a lighter vertebral wide stripe and those from Española and Floreana which are highly contrasted and relatively colourful, combining large black dots on a light orange background and a olive/grey vertebral wide stripe (Figs 5–7). Interestingly, Española–Floreana is also one of the clusters showing the highest divergence in other morphological characters examined (e.g. number of digital lamellae and spiny scales along the caudal crest).

RECOGNIZED UNITS AT THE SUBSPECIES LEVEL

Given the overall congruence of the microsatellite loci-based genetic clusters with the additional molecular and morphological evidence, we feel it is justified to modify the current intraspecific classification of *A. cristatus* as proposed by Eibl-Eibesfeldt (1962) in a way that considers each valid cluster as separate subspecies. The following is a summary of the integrative rationale applied for this classification and anticipates our taxonomic conclusion (Fig. 8) which is then formally applied in the subsequent subspecies accounts. In the following, we review each of the clusters as obtained in microsatellite loci analysis, and the additional evidence for its taxonomic distinctness.

Fernandina and Isabela cluster

Populations from these two islands are considered to form a single cluster based on microsatellite loci data. They also form a cluster in the RADseq tree and are closely related in the SNP-PCA. We therefore consider these populations to belong to a single subspecies which shows distinct morphological differences to all other subspecies. As Fernandina is the type locality of *A. cristatus*, this cluster is the nominal subspecies *A. c. cristatus*, with junior synonyms *A. c. ater* and *A. c. albemariensis* (see justification and nomenclatural consequences below).

Genovesa cluster

The two included specimens are a cluster in the RADseq tree. SNP data suggest the Genovesa cluster is related to those from Marchena and Pinta, but it is separated by SNP-PC3, has different dominant mitochondrial haplotypes and appears to be morphologically quite distinct from all other populations. We therefore consider the Genovesa cluster as a separate subspecies, *A. c. nanus*.

Pinta cluster

RADseq data place the included specimens as cluster closely related to the clusters from Genovesa and, especially, Marchena. We here consider this unit as subspecies *A. c. sielmanni*.

Marchena cluster

Closely related in SNP-PCA to *A. c. sielmanni* from Pinta. Also shares mitochondrial haplotypes, and only rather weakly differentiated morphologically from Pinta specimens. To ensure consistency among subspecies delimitation and microsatellite loci clustering, and considering that the RADseq tree does not support a cluster of (Pinta + Marchena), we here suggest considering

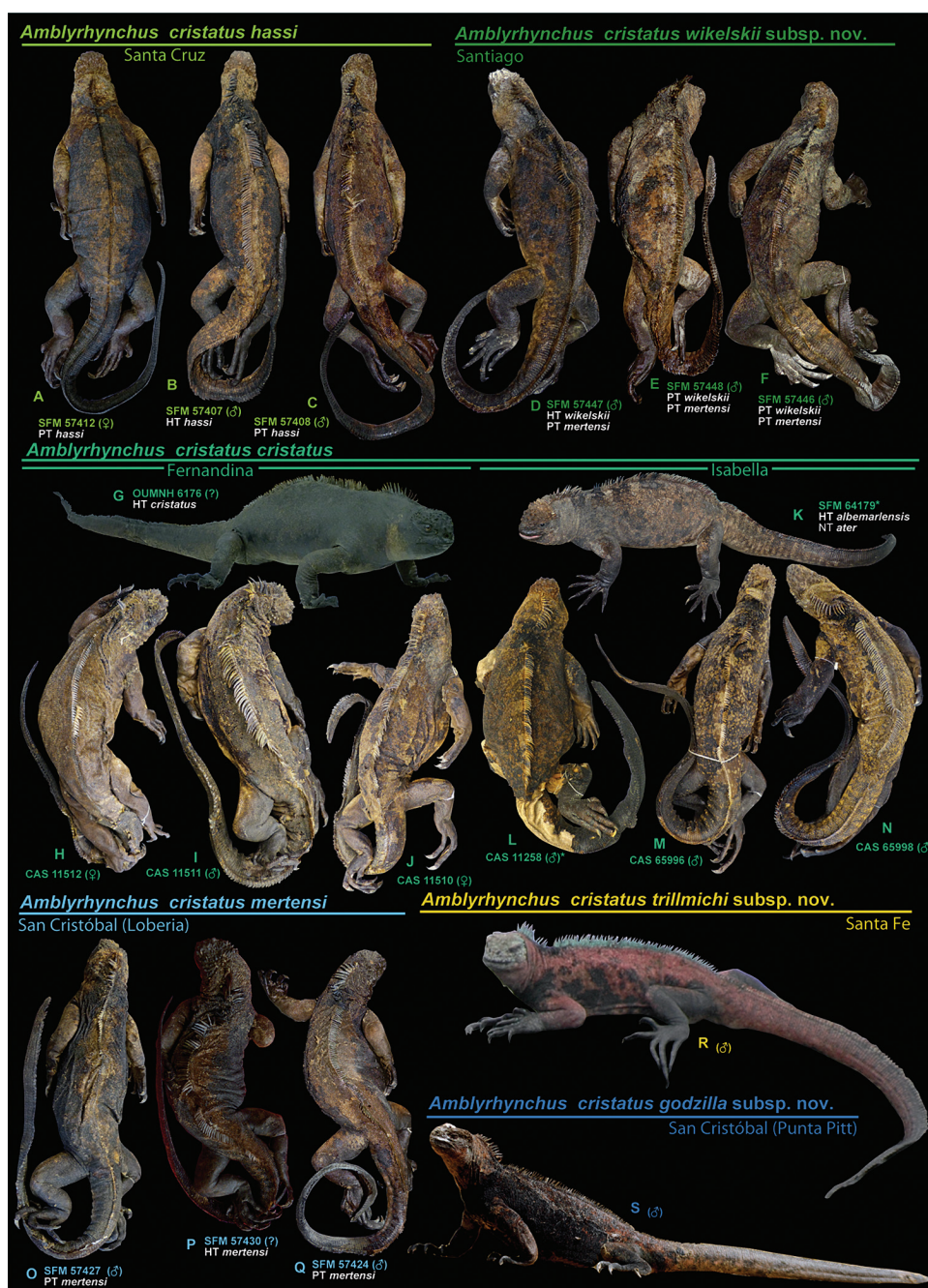


Figure 5. Overview of the phenotypic diversity among *Amblyrhynchus cristatus* subspecies: (A–C) *Amblyrhynchus cristatus hassi* from Santa Cruz (SFM 57412, 57407, 57408) (D–F) *A. c. wikelskii* subsp. nov. from Santiago (SFM 57447, 57448, 57446), (G–N) *A. c. cristatus* from Fernandina (OUMNH 6176, CAS 11512, 11511, 11510) and Isabela (SFM 64179, 11258, 65996, 65998), (O–Q) *A. c. mertensi* from San Cristóbal (SFM 57427, 57430, 57424), (R) *A. c. trillmichi* subsp. nov. (uncollected living specimen), (S) *A. c. godzilla* subsp. nov. (uncollected living specimen). HT: holotype, NT: neotype, PT: paratype.

the Marchena population as a new subspecies *A. c. hayampi* subsp. nov., but we are aware that future studies might reveal this population to be better classified as *A. c. sielmanni* along with the Pinta population, and this nomen to become a junior synonym of *sielmanni*.

Santa Cruz cluster

Samples are close to each other in SNP-PCA, although they do not form a cluster in the RADseq tree. These iguanas are also characterized by mitochondrial haplotypes exclusive to the island and are morphologically



Figure 6. Overview of the phenotypic diversity among *Amblyrhynchus cristatus* subspecies. (A–D) *Amblyrhynchus cristatus venustissimus* from Champion (CAS 11062) and Española (SFM 57415, 49853, 57413), (E–G) *A. c. sielmanni* (CAS 12565, SFM 57417, CAS 12570), (H–J) *A. c. nanus* from Genovesa (NHM 1946.8.30.20, SFM 57432, SFM 57445), (K–N) *A. c. hayampi* subsp. nov. from Marchena (CAS 12467, 12458, 12449, 12471), (O–U) *A. c. jeffreysi* subsp. nov. from Wolf (CAS 12690, CAS-SUR 4777, CAS 12693, CAS 12961) and Darwin (CAS 12694, 12696, 12695). HT: holotype, PT: paratype.

well differentiated from most other island clusters, except for Rábida, Marchena and Pinta. We consider this cluster, which also includes specimens from Seymour Norte, as *A. c. hassi*.

Santiago cluster

The two included samples do not form a cluster in the RADseq tree but are close to each other in the SNP-PCA. Morphologically well differentiated from most



Figure 7. Photographic plate showing the different subspecies of Marine Iguana (living mature males exclusively). (A) *Amblyrhynchus cristatus nanus*, Genovesa, (B) *A. c. hayampi* subsp. nov., Marchena, (C) *A. c. sielmanni*, Pinta, (D, E) *A. c. venustissimus*, Española and Floreana, respectively, (F) *A. c. trillmichi* subsp. nov., Santa Fé, (G) *A. c. hassi*, Santa Cruz, (H) *A. c. cristatus*, Fernandina, (I) *A. c. cristatus*, Isabela, (J) *A. c. mertensi*, San Cristóbal, (K) *A. c. godzilla* subsp. nov., San Cristóbal, (L) *A. c. wikelskii* subsp. nov., Santiago. Pictures from A. Ibañez (E, K), M. Krueger (A), H. Snell (B, D, H), S. Steinfartz (C, I, L), M. Vences (G, J), M. Vitousek (F).

other islands except Rábida, Marchena and Pinta. Here described as *A. c. wikelskii* subsp. nov. This subspecies probably also occurs on Rábida and Pinzón, which were included in the same microsatellite loci cluster (inconclusively in the case of Pinzón).

Santa Fé cluster

Sister to the Floreana/Española cluster in the RADseq tree, but distinguished from it in the SNP-PCA. Despite sharing the most common mitochondrial haplotype, overall the Santa Fé specimens appear to have in their majority different haplotypes than the

Floreana/Española ones. No morphological data of Santa Fé specimens are available. We describe the Santa Fé populations as *A. c. trillmichi* subsp. nov.

Floreana and Española cluster

Populations from these two islands form a single cluster in the microsatellite loci analysis. They also are closely related in SNP-PCA and form a cluster in the RADseq tree, but specimens from the two islands are not reciprocally monophyletic in the RADseq tree. We therefore consider specimens from both islands as belonging to a single subspecies for which the name

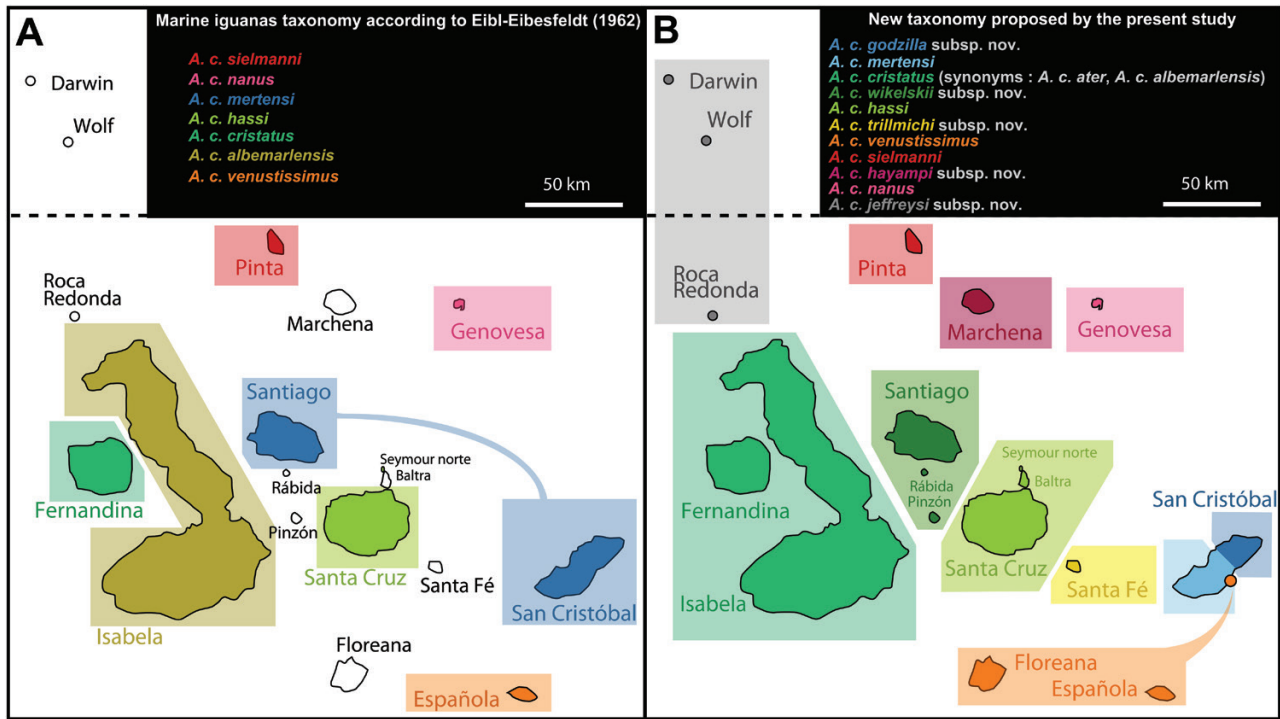


Figure 8. Distribution of the different subspecies of Galápagos marine iguanas according to the classification of Eibl-Eibesfeldt (1962) and the new taxonomy proposed herein. The orange dot on the southern coast of San Cristóbal represents the population of putative migrant specimens of *A. c. venustissimus*.

A. c. venustissimus is available, and which is morphologically highly divergent from all other subspecies.

San Cristóbal (Lobería) cluster

Samples from the two San Cristóbal clusters form reciprocally monophyletic sister groups in the RADseq tree. They are consistently distinguished from each other in the SNP-PCA and by not sharing mitochondrial haplotypes. Morphological divergence is low, but consistently detectable if analysed sample size is high. We apply the subspecies name *A. c. mertensi* to the Lobería cluster.

San Cristóbal (Punta Pitt) cluster

Sister to the San Cristóbal–Lobería cluster (*A. c. mertensi*) in the RADseq tree, but distinguished from that cluster by multiple lines of evidence as specified above. No nomen is available for this population which we therefore describe as *A. c. godzilla* subsp. nov.

Wolf cluster

Specimens from Wolf form a cluster in the RADseq tree and cluster separately from most other islands except Roca Redonda in the SNP-PCA. This population has affinities to *A. c. cristatus* from Fernandina and Isabela

as suggested by SNP-PCA and mitochondrial haplotypes, but female specimens from Wolf show a weak morphological differentiation despite low sample size. We describe the Wolf population as *A. c. jeffreysi* subsp. nov., and furthermore include in this subspecies the population from Roca Redonda in a preliminary way, based on its placement by the SNP-PCA. Once morphological data from Roca Redonda become available, the identity of this population should be re-evaluated. We also include in this same subspecies the population from Darwin in a preliminary way because of (1) affinities in the SNP-PCA, (2) sharing of the only Darwin non-exclusive mtDNA haplotype with Wolf specimens, (3) morphological similarities and (4) biogeographic affinities given that both Darwin and Wolf located in the north of the Galápagos archipelago, geographically isolated from other islands. More morphological data for appropriately sexed specimens are needed to test this taxonomic hypothesis.

TAXONOMIC REVISION

We here follow the rationale of MacLeod *et al.* (2015a) to consider the evolutionary divergence among populations of Galápagos marine iguanas insufficient to warrant a status of separate species for any of them.

Therefore, all marine iguanas are classified in a single species, *A. cristatus*, subdivided into 11 subspecies:

AMBLYRHYNCHUS CRISTATUS BELL, 1825

AMBLYRHYNCHUS CRISTATUS

CRISTATUS BELL, 1825

(FIGS 5G–N, 7)

Iguana (*Amblyrhynchus*) *Ater* Gray, 1831. Holotype.

Type locality: ‘From the Galápagos’. Specimen not located (according Frost & Etheridge, 2012) and here considered to be lost. Synonymy by Boulenger, Cat. Liz. Brit. Mus. Nat. Hist., 2: 185.

Amblyrhynchus cristatus albemarlensis Eibl-Eibesfeldt, 1962. Holotype: SMF 64179. Type locality: ‘Insel Albemarle’ [= Isla Isabela]. No paratypes.

Holotype: OUMNH 6176. Type locality: allegedly Mexico: ‘Habitat in Mexico (...) Sent from Mexico by Mr. Bullock’ (Bell, 1825: 204). Eibl-Eibesfeldt (1956: 88) designated as new ‘Terra typica’ the island of ‘Narborough’ [= Isla Fernandina]. No paratypes.

Specimens examined (*N* = 30): **Isabela:** SFM 64179, coll. by I. Eibl-Eibesfeldt in 1957, Elisabeth Bay (holotype of *A. c. albemarlensis* and neotype of *Iguana ater*, stuffed specimen; see nomenclatural acts below). CAS 11253–11260, coll. by R.H. Beck in 1906, Iguana Cove. CAS 11311, 11313, 11314, coll. by J.R. Slevin and E.S. King in 1906, vicinity of Tagus Cove. CAS 65996–65998, coll. by H. Moffitt in 1905, Tagus Cove. **Tortuga (off Isabela):** CAS 10284–10289, coll. by J.R. Slevin in 1905, no exact locality. **Fernandina:** HLMD-RA-3067, coll. by G. Scheer in 1954. CAS 11510–11514, coll. by J.R. Slevin and E.S. King in 1906, no exact locality. SFM 57401, 57402, coll. by I. Eibl-Eibesfeldt in 1957, NE coast of the island, Punta Espinosa. **Unknown island:** OUMNH 6176 (holotype of *A. c. cristatus*).

Comment on type locality of *A. cristatus* Bell, 1825: Ascertaining the plausibility of the designation (emendation) of the type locality of this nomen by Eibl-Eibesfeldt (1956) is complex. Before 1825, few expeditions took place to the Galápagos, such as the 1789–1794 Malaspina expedition and the 1793 expedition by James Colnett. However, whaling ships regularly visited the archipelago, and one of these, the *Thames*, visited Fernandina in 1806 (Langdon, 1984). For the sake of economy in classificatory change, we therefore here follow the type locality emendation of Eibl-Eibesfeldt (1956), given that the provenance of the type specimen of *A. cristatus* from Fernandina is a definite possibility.

Designation of a neotype for *I. ater* Gray, 1831: In *A synopsis of the species of Class Reptilia*, Gray (1831)

gave only very rudimentary details about the material he examined to describe this new species. He nevertheless mentioned in the introduction, the specimens he personally examined: ‘In forming this list, the collection of reptiles of the British Museum, the College of Surgeons, and of Mr. Bell, in London, of the Gardens of Plants and Ecole de Medecine at Paris, of the Royal Museum of Leyden and Berlin, and the Free Town of Francfort, have been studied with attention (...)’. Later, Duméril & Bibron (1837: 197) will likely be the only other scientists having had the opportunity to study the type material examined by Gray, stating that ‘*Amblyrhincus ater*. Gray’ [sic]: ‘C’est encore une espèce qui n’est connue des naturalistes que par un seul sujet qui fait partie des richesses erpétologiques que renferme le musée britannique’ [this is also a species which is known to naturalists only by a single subject, part of the herpetological treasures held at the British museum]. Such a statement clearly confirms that (1) only a single specimen has been examined by Gray and that (2) it was at that time deposited in the collection of the British Museum (now NHM). To our knowledge, this specimen has never been examined by subsequent authors. Examination of the type catalogue at the NHM did not yield any explicit mentions of this type specimen, and we did not find any specimen in the collection that could be regarded as type of *ater*. Apparently, all the catalogued marine iguanas in the NHM were collected at least several decades after the original description, in accordance with the statement made by Frost & Etheridge in 2012 who cautiously considered the original type material as ‘not located’.

Two specimens at the Museum of Comparative Zoology at Cambridge (Harvard), MCZ R-6369 and R-6370, are catalogued as ‘syntypes’ of *A. ater* (whereas additional annotations mention the contrary ‘not a type?’). Both specimens were collected on Duncan (= Pinzón) during the *Albatross expedition* in 1891, and therefore cannot represent the original type material.

The nomen *I. ater* would have priority over the majority of subspecific names available for island populations of marine iguanas. We here suggest defining a neotype for this nomen, with the express purpose of stabilizing the subspecies-level taxa defined in the following accounts. As further qualifying conditions for a neotype designation according to §75.3 of the *Code*, we have above elaborated why we consider the holotype of *I. ater* to be lost, and it is also clear that the neotype selected in the following is morphologically consistent with the very rudimentary information available on the former name-bearing type (from the original description; see above).

The *Code* also requires that the neotype comes as near as practicable from the original type locality. We selected as neotype the specimen SMF 64179 from Isabela (deposited in a recognized scientific or

educational institution and here documented morphologically in Fig. 5 as required by the *Code*). Isabela was visited by at least three whaling ships before 1831 (Langdon, 1984) and therefore this island has a high likelihood to have been the source of the lost holotype.

Finally, the *Code* requires a statement of the characters differentiating the nominal taxon for which a neotype is designated from other taxa. In this study, we summarize the characters distinguishing *A. c. cristatus* (and thus its synonym *ater*) from all other subspecies of marine iguanas (e.g. Table 1). As we consider *ater* to be a synonym of the nominal subspecies *A. c. cristatus*, it cannot be distinguished from this taxon at present. Yet, we feel that the designation of a neotype is justified in this case as it contributes to resolving taxonomic uncertainty, stabilizing all other subspecies names in a species where subspecific units are at the core of intensive conservation efforts (see Discussion).

The selected neotype specimen (SMF 64179) is at the same time also the holotype of *A. cristatus albe-marlensis* Eibl-Eibesfeldt, 1962 and this latter nomen therefore becomes an objective junior synonym of *I. ater* Gray, 1831.

Geographic distribution: Isabela, Tortuga, Fernandina and very likely their satellite islets (Fig. 8).

Diagnostic description: *A. cristatus cristatus* is a large-sized marine iguana subspecies (mean SVL 342 mm, up to a maximum of 480 mm). Mature males are characterized by a remarkably well-developed crest of spines and pronounced prominent conical supracephalic scales. In preservative, males have a yellowish dorsal background, marbled or speckled with small black dots, frequently aligning and merging to each other to form five to ten spindle-shaped transversal stripes along the body. This subspecies does not seem to present particularly remarkable pholidotic features (see also Figs 4, 5G–N, Table 1).

Redescription of the holotype: OUMNH 6176 (Fig. 5G), adult unsexed stuffed specimen very poorly preserved, presenting a long sutured slit running from the throat to the cloaca, missing eyes (replaced by glass marbles) and toes II to IV of the left foot. Skin apparently varnished and/or painted, and coated in some parts, preventing detailed examination of several characteristics of scalation. SVL (345 mm) shorter than tail length (483 mm). Details of the cephalic scalation barely visible. Subdigital lamellae 25 under the right third toe, 32 under the right fourth toe. Approximately 280 visible transversal rows of minute scales at mid-body. Dorsal crest moderately developed, 15 spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of approximately 10 very

reduced scales (Cng), 82 spiny scales running along the dorsal side of the trunk (Cdc), then at least 136 visible scales on the dorsal side of the tail.

Skin very likely painted or varnished, uniformly dark grey, ventral side and lateral sides of the throat slightly lighter.

AMBLYRHYNCHUS CRISTATUS
NANUS GARMAN, 1892

(Figs 6H–J, 7)

Holotype: NHM 1946.8.30.20 (formerly numbered BMNH 99.5.4). Type locality: 'Tower Island' [= Isla Genovesa].

Specimens examined (N = 17): **Genovesa:** NHM 1946.8.30.20, don. by Dr. Baur (holotype of *A. c. nanus*). CAS 12272–12274, coll. by J.R. Slevin in 1906, N coast of the island. CAS 104674, 104675, coll. by I.L. Wiggins in 1967, no exact locality. SFM 57432–57435, SFM 57437–57441, SFM 57444, SFM 57445, coll. by I. Eibl-Eibesfeldt in 1957, Darwin Bay.

Geographic distribution: Genovesa and very likely its satellite islets (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus nanus* is a small-sized subspecies of marine iguana (mean SVL 205 mm, up to a maximum of 247 mm). Mature males have a moderately developed crest of spines and moderately conical supracephalic scales. In preservative, males are almost uniformly dark brown or blackish dorsally, with small light ochre dots, most often aligning and sometimes merging to form five to ten transversal stripes along the body. More specifically, this subspecies is also characterized by a rostral scale always in contact with three or more adjacent supralabials (100%), a low number of lamellae under the third (23.8 ± 0.9) and fourth (28.5 ± 2.1) toe and the highest number of femoral pores (28.6 ± 1.8) (see also Figs 4, 6H–J, Table 1).

Redescription of the holotype: NHM 1946.8.30.20 (Fig. 6H), juvenile or subadult unsexed specimen preserved in 70% ethanol, in relatively good condition, with exception of a 3 cm long incision running along the ventral side of the body. SVL (155 mm) shorter than tail length (199 mm). Head 25.1 mm long (HL1), 23.4 mm high and 18.9 mm wide. Fourth finger length of 24.7/23.9 mm. Rostral scale in contact with two adjacent supra-supralabials (barely in point contact with the third (median) one). Mental separating the anteriormost infralabials. Eight supralabials on the right side, nine on the left side. Ten infralabials on both sides. Internasal and rostral scales separated by a row of five small granular scales. Four large and flat

frontonasal scales, posterior to and in contact with the internasal. Subdigital lamellae 23/22 under the third toe, 26/24 under the fourth toe; 280 transversal row of minute scales at midbody. Dorsal crest weakly developed, 16 spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of 11 very reduced scales (Cng), 85 spiny scales running along the dorsal side of the trunk (Cdc), then 141 scales on the dorsal side of the tail.

After more than 124 years of preservation in ethanol, original colour pattern still visible, although likely darker and less contrasted than originally. Background coloration of the dorsal and lateral sides of the whole body dark grey to blackish, with four to five transversal stripes made of aligned small brownish/ochre dots. Dorsal and lateral sides of the tail and of limbs uniformly dark grey. Dorsal and lateral sides of the head as dark as the rest of the body, with the largest scale of the parietal region being lighter. Crest spines as dark as the dorsum, with exception of a series of five consecutive olive/greyish spines of the nuchal crest, and few series of two to four consecutive brownish spines corresponding with the lighter transversal stripes of the dorsum. Ventral side light cream, darker on the anteriormost part and on the extremities; mental region, chest, the ventral sides of limbs and tail greyish; collar, palms and soles almost as dark as the dorsal side.

AMBLYRHYNCHUS CRISTATUS
VENUSTISSIMUS EIBL-EIBESFELDT, 1956
(FIGS 6A–D, 7)

Holotype: SMF 49851. Type locality: 'Nordküste der Insel Hood' [= Isla Española, N coast].

Paratypes: SMF 49852, from the type locality, 49853, from 'Gardener-Bucht der Insel Hood' [= Gardner Bay, Isla Española], SMF 49853, from 'nördlich von Hood liegenden Insel Gardener' [= Isla Gardner, off N coast of Isla Española].

Specimens examined (N = 30): **Española:** SFM 49851, 49852, coll. by I. Eibl-Eibesfeldt in 1954, N coast, Gardner Bay (respectively holotype and paratype of *A. c. venustissimus*). SFM 49854, coll. by I. Eibl-Eibesfeldt in 1954, no exact locality (paratype of *A. c. venustissimus*). SFM 57413–57416, coll. by I. Eibl-Eibesfeldt in 1957, north-western point of the island. CAS 9308, 9382, 9390, 10960–10969, CAS 11016–11018, coll. by J.R. Slevin in 1906, no exact locality. **Gardner (off Española):** SFM 49853, HLMD-RA-3046, 3049, 3050, coll. by I. Eibl-Eibesfeldt in 1954, no exact locality (all four paratypes of *A. c. venustissimus*). **Champion (off Floreana):** CAS 11062, coll. by F.T. Nelson in 1906, no exact locality.

Floreana: NHM 1876.6.21.1, 1876.6.21.26, coll. by Com. W.E. Cookson, no exact locality.

Geographic distribution: Española, Gardner, Floreana, Champion and very likely their satellite islets. A population of putative migrant specimens that might be assignable to this subspecies has also been put into evidence by MacLeod *et al.* (2015a) on the southern coast of San Cristóbal (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus venustissimus* is a medium-sized subspecies of marine iguana (mean SVL 264 mm, up to a maximum of 325 mm). Mature males are characterized by a moderately developed crest of spines and moderately conical supracephalic scales. In preservative, the male pattern of coloration is characterized by an olive/grey vertebral stripe (turquoise in life) running over an orange dorsal background coloration (red in life) speckled with large black dots on the flanks. More specifically, this subspecies is also characterized by a high number of lamellae under third (28.8 ± 2.0) and fourth (35.0 ± 2.4) toes and a very high number of scales along the dorsal (114.1 ± 22.9) and caudal crest (161.7 ± 10.6) (see also Figs 4, 6A–D, Table 1).

Redescription of the holotype: SMF 49851. Specimen could not be accessed without destruction of the original, historical jar and therefore was only examined through the glass of the jar; some morphological details could therefore not be reliably scored. Adult male preserved in 70% ethanol, in a very good condition. Hemipenes everted. SVL (≈ 430 mm) shorter than tail length (≈ 430 mm), according to Eibl-Eibesfeldt (1956). Rostral scale in contact with two adjacent supra-supralabials. Anteriormost supralabials in contact below the mental. Nine supralabials on the right side. Eleven infralabials on the right side. Internasal and rostral scales separated by a row of five small granular scales. Three large and conical frontonasal scales, posterior to and in contact with the internasal. Subdigital lamellae 30 under the right third toe. Twenty-four femoral pores on the right side. At midbody 132 transversal rows of minute scales on the right side (left side not visible). Dorsal crest well developed, 17 spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of 13 very reduced scales (Cng), 82 spiny scales running along the dorsal side of the trunk (Cdc), then 138 scales on the dorsal side of the tail.

After 62 years of preservation in ethanol, original colour pattern well preserved and sharply contrasted. Background coloration of the dorsal side of the body light dull orange/ochre, with a wide vertebral light olive/grey stripe running from shoulders to tail insertion. Nuchal region black and strongly contrasting with the rest of the dorsal side. Several large and strongly contrasting black dots sporadically maculating the flanks

dorsolaterally, almost completely covering them ventrolaterally. Dorsal and lateral side of the head grey, with deep black nasal, labial and ocular regions. Crest spines mostly greyish, with sporadically few deep black isolated ones. Tail uniformly black (with exception of the pale cream anteriormost part of the ventral side). Dorsal and lateral sides of forelimbs mostly olive/grey, with contrasting black extremities (distal third of forearms and hand). Dorsal and lateral sides of hindlimbs mostly grey, darker distally than proximally. Ventrums, ventral sides of the limbs and of the tail basis uniformly light cream, with a contrasting deep black thin gular collar. Throat, ventral side of manus and pes darker than ventrum.

AMBLYRHYNCHUS CRISTATUS HASSI
EIBL-EIBESFELDT, 1962

(Figs 5A–C, 7)

Holotype: SMF 57407. Type locality: ‘*Indefatigable Südküste, westliche Akademiebucht (...) Indefatigable, Galápagos Inseln*’. [= South coast of Isla Santa Cruz, western part of Academy Bay].

Paratypes: SMF 57403–57405, 57408–57412, all from the type locality; SMF 57456 and 57457 (skins only), from Santa Cruz, without further details.

Specimens examined (*N* = 22): **Santa Cruz**: SFM 57407, coll. by I. Eibl-Eibesfeldt in 1960, S coast, western Academy Bay (holotype of *A. c. hassi*). SFM 57405, coll. by I. Eibl-Eibesfeldt in 1957, 3 km W of Academy Bay (paratype of *A. c. hassi*). SFM 57408–57412, western Academy Bay (paratypes of *A. c. hassi*). SFM 57456, SFM 57457, 57406, 58100, coll. by I. Eibl-Eibesfeldt in 1957, no exact locality. SFM 60334, coll. by I. Eibl-Eibesfeldt in 1962, no exact locality. HLMD-RA-3066, coll. by G. Scheer in 1954. CAS 10283, 10787–10791, coll. by J.R. Slevin in 1905, no exact locality. CAS 87101–87103, coll. by F. Nied and T. Belland in 1958, Academy Bay. CAS-SUR 18153, collector unknown, no exact locality.

Geographic distribution: Santa Cruz and very likely its satellite islets, including Seymour Norte (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus hassi* is the largest subspecies of marine iguana (mean SVL 351 mm, up to a maximum of 485 mm). Mature males have a remarkably well-developed crest of spines and moderately conical supracephalic scales. In preservative, the male pattern of coloration is characterized by a dull orange/ochre dorsal background, with many black dots mostly concentrated (and frequently merging together) on the flanks. This subspecies does not seem to present particularly remarkable pholidotic features (see also Figs 4, 5A–C, Table 1).

Redescription of the holotype: SMF 57407 (Fig. 5B), large adult male preserved in 70% ethanol, in very good condition. SVL (439 mm) shorter than tail length (624 mm). Head 69.3 mm long (HL1), 55.5 mm high and 65.4 mm wide. Fourth finger length 65.9/65.5 mm. Rostral scale in contact with three adjacent supra-supralabials (barely with the median one). Anteriormost supralabials not in contact below the mental. Ten supralabials on the right side, nine on the left side. Ten infralabials on the right side, 12 on the left side. Internasal and rostral scales separated by a row of five small granular scales. Four large and conical frontonasal scale, posterior to and in contact with the internasal. Subdigital lamellae 36 under the third toe (on both sides), 32 under the fourth toe (on both sides); 24 femoral pores on the left side (hardly distinguishable on the left side); 279 transversal rows of minute scales at midbody. Dorsal crest well developed, 15 spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of 14 reduced scales (Cng), 89 spiny scales running along the dorsal side of the trunk (Cdc), then 143 scales on the dorsal side of the tail.

After 56 years of preservation in ethanol, original colour pattern well preserved and contrasted. Background coloration of the dorsal side of the body black, with an irregularly edged, wide light brown/ochre stripe running from the anteriormost fifth of the tail to the shoulder, and extending on the lateral sides of the nuchal region. Dorsal and lateral side of the head light brown, with nasal, labial and ocular regions being lighter, almost cream. Crest spines mostly brownish, darker on the anterior half of the body. Dorsal and lateral sides of forelimbs light brown, with the distal third of forearms and hands contrasting blackish. Dorsal and lateral sides of hindlimbs mostly blackish, with exception of the light brown anterior side of thigh. Ventrums, chin, ventral sides of the limbs and of the tail light cream to ochre, with two large and irregular black dots on the chest, and a remarkable contrasting large and triangular deep black gular collar partly extending anteriorly on the throat. Ventral side of manus and pes slightly darker than ventrum.

AMBLYRHYNCHUS CRISTATUS
MERTENSI EIBL-EIBESFELDT, 1962

(Figs 5O–Q, 7)

Holotype: SMF 57430. Type locality: ‘*etwa 3 km südwestlich der Wrack-Bucht der Insel Chatham, Galápagos Inseln*’ [= Isla San Cristóbal, about 3 km SW to Wreck Bay (vicinity of Puerto Baquerizo Moreno)].

Paratypes: SMF 57424–57431, all from the type locality; SMF 57446–57449, from ‘*Insel James, Crab-point, südlich der Jamesbay*’ (= Isla Santiago, Crab-point, S of James Bay), but these four specimens are herein

recognized as member of a distinct and new subspecies, *A. c. wikelskii* subsp. nov. (described below).

Specimens examined ($N = 13$): **San Cristóbal:** SFM 57430, coll. by I. Eibl-Eibesfeldt in 1957, about 3 km SW to Wreck Bay (holotype of *A. c. mertensi*). SFM 57424–57429, SFM 57431, coll. by I. Eibl-Eibesfeldt in 1957, about 3 km SW to Wreck Bay (all of them paratypes of *A. c. mertensi*). CAS 104665–104667, coll. by I.L. Wiggins in 1967, N. end of James Bay. CAS 11020, coll. by E.S. King in 1906, no exact locality. CAS-SUR 4953, coll. by E. Heller and R.E. Snodgrass in 1900, no exact locality.

Geographic distribution: Southern (south-western) part of Isla San Cristóbal and very likely the satellite islets off the southern coast of the island (Fig. 8). For detailed list of localities and geographical coordinates, see MacLeod et al. (2015a).

Diagnostic description: *Amblyrhynchus cristatus mertensi* is a medium to large-sized subspecies of marine iguana (mean SVL 301 mm, up to a maximum of 490 mm). Mature males have a relatively well-developed crest of spines and relatively pronounced conical supracephalic scales. In preservative, males have a dark grey or blackish dorsal background, with a large and irregular lighter brownish/ochre vertebral stripe. More specifically, this subspecies is also characterized by a rostral scale most often (92%) in contact with three or more adjacent supralabials, and by the lowest number of femoral pores (20.7 ± 3.5) (see also Figs 4, 5O–Q, Table 1).

Redescription of the holotype: SMF 57430 (Fig. 6P), adult male preserved in 70% ethanol, in good condition (with exception of a short ventral incision on the left side of the belly). Left hemipenis partly everted. SVL (270 mm) shorter than tail length (430 mm). Head 48.1 mm long (HL1), 34.1 mm high and 44.1 mm wide. Fourth finger length 43.0/42.5 mm. Rostral scale in contact with two adjacent supra-supralabials. Anteriormost supralabials not in contact below the mental. Eleven supralabials on the right side, eight on the left side; 11 infralabials on both sides. Internasal and rostral scales separated by a row of five small granular scales. Four large, textured and conical frontonasal scales, posterior to and in contact with the internasal. Subdigital lamellae 24/24 under the third toe, 26/27 under the fourth toe; 22 femoral pores on the right side, 20 on the left side; 285 transversal rows of minute scales at midbody. Dorsal crest well-developed, 16 spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of 11 very reduced scales (Cng), 77 spiny scales running along the dorsal side of the trunk (Cdc), then 146 scales on the dorsal side of the tail.

After 59 years of preservation in ethanol, original colour pattern relatively well preserved. Background coloration of the dorsal and lateral side of the body almost black, with an irregularly edged, wide dark brown stripe running from the shoulders to the anteriormost fifth of the tail. Head entirely dark brown. Crest spines mostly brownish, darker on the tail. Dorsal and lateral sides of limbs dark brown, hands and feet almost black. Ventrums, ventral sides of the limbs and the anterior-most part of the tail light cream (although the very dark flank colour tends to extend ventrally) and with exception of few irregular large black patches on the belly and of a remarkably contrasting large and deep black gular collar extending over the whole chest. Throat dark grey. Ventral side of manus and pes significantly darker than the rest of the ventral side.

AMBLYRHYNCHUS CRISTATUS SIELMANNI
EIBL-EIBESFELDT, 1962
(Figs 6E–G, 7)

Holotype: SMF 57417. Type locality: ‘Westküste der Insel Abington’ [= Isla Pinta, W coast].

Paratypes: SMF 57418–57423, all from the type locality.

Specimens examined ($N = 18$): **Pinta:** SFM 57417, coll. by I. Eibl-Eibesfeldt in 1957, W coast of the island (holotype of *A. c. sielmanni*). SFM 57421, 57423, coll. by I. Eibl-Eibesfeldt in 1957, W coast of the island (paratypes of *A. c. sielmanni*). CAS 12547, 12548, 12550, 12552, 12553, 12555, 12556, 12560, 12562, 12565, 12570, 12572, 12573, 12579, 12583, coll. by J.R. Slevin and E.S. King in 1906, no exact locality.

Geographic distribution: Pinta and very likely its satellite islets (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus sielmanni* is a medium sized-subspecies of marine iguana (mean SVL 249 mm, up to a maximum of 323 mm). Mature males have a moderately developed crest of spines and relatively pronounced conical supracephalic scales. In preservative, males are dark grey or blackish dorsally (with exception of the head which is lighter), marbled or speckled with small ochre dots, frequently aligning and merging to each other to form around five spindle-shaped transversal stripes along the body. More specifically, this subspecies is also characterized by a very low number of lamellae under third (22.8 ± 1.2) and fourth (26.8 ± 1.7) toes and a low number of scales along the dorsal (100.6 ± 28.4) and caudal crest (134.2 ± 9.6) (see also Figs 4, 6E–G, Table 1).

Redescription of the holotype: SMF 57417 (Fig. 6F), adult unsexed specimen preserved in 70% ethanol, in moderately good condition, with short ventral incisions on the belly and the chest, a large patch of missing tissue on the left shoulder, and the tip of the left fourth toe being amputated. SVL (285 mm) shorter than tail length (415 mm). Head 52.3 mm long (HL1), 39.7 mm high and 43.1 mm wide. Right fourth finger length 44.4 mm. Rostral scale in contact with two adjacent supra-supralabials. Anteriormost supralabials not in contact below the mental. Ten supralabials on the right side, nine on the left side. Eleven infralabials on the right side, 12 on the left side. Internasal and rostral scales are separated by a row of five small granular scales. Three large and slightly convex frontonasal scales, posterior to and in contact with the internasal. Subdigital lamellae 23/22 under the third toe, 26 under the fourth right toe; 26 femoral pores on both sides; 261 transversal rows of minute scales at midbody. Dorsal crest well developed, 16 spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of 15 reduced spiny scales (Cng), 78 spiny scales running along the dorsal side of the trunk (Cdc), then 118 scales on the dorsal side of the tail.

After 59 years of preservation in ethanol, original colour pattern still visible although likely darkened. Background coloration of the dorsal and lateral sides of the body blackish with dark brown/mahogany complex nebulous patterns. Nuchal region almost uniformly blackish. Dorsal and lateral side of the head brownish/olive, the labial and periocular regions blackish. Crest spines dark olive/grey to black. Dorsal and lateral sides of the tail almost uniformly black, with exception of nebulous lighter brownish/ochre scales on the proximal third of the tail. Dorsal and lateral sides of the limbs mostly blackish. Ventrums, ventral sides of the limbs and of the tail uniformly cream/grey, with exception of a wide gular black collar and a few large black spots on the chest. Chin dark grey. Palms and soles slightly darker than the rest of the ventrum.

**AMBLYRHYNCHUS CRISTATUS
HAYAMPI SUBSP. NOV.**

(Figs 6K–N, 7, 9B)

LSID urn:lsid:zoobank.org:act:A5D505A8-63E7-48F8-BCB9-1EFC68ED1FE2

Holotype: CAS 12471, coll. by J.R. Slevin and E.S. King in 1906, NW coast of Isla Marchena.

Paratypes ($N = 11$): CAS 12449, 12451, 12452, 12456, 12458, 12460, 12464, 12467, 12469, 12473, 12474, same data as holotype.

Specimens examined ($N = 12$): **Marchena:** CAS 12449, 12451, 12452, 12456, 12458, 12460, 12464, 12467, 12469, 12471, 12473, 12474, coll. by J.R. Slevin and E.S. King in 1906, NW coast of the island.

Etymology: The subspecific epithet *hayampi* refers to the word used to designate the common iguana in Kichwa (a Quechuan language representing one of the three official languages of Ecuador and currently spoken by a million people in South America).

Geographic distribution: Marchena and very likely its satellite islets (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus hayampi* subsp. nov. is a medium- to large-sized subspecies of marine iguana (mean SVL 299 mm, up to a maximum of 379 mm). Mature males have a well-developed crest of spines and relatively pronounced conical supracephalic scales. In preservative, males are dark grey or blackish dorsally, marbled or speckled with small ochre dots that frequently align and merge to each other to form transversal stripes along the body. This subspecies also has a very low number of lamellae under fourth (26.5 ± 2.2) toes, contrasting with a rather medium number of lamellae under the third toes (25.5 ± 1.8) and a very low number of scales along the caudal crest (130.5 ± 6.0) (see also Figs 4, 6K–N, 9B, Table 1).

Description of the holotype: CAS 12471 (Figs 6N, 9B), adult male preserved in 70% ethanol, in relatively good condition, with exception of long incisions (likely realized during the fixation process) running along the ventral side of the body, limbs, digits and tail, and the removal of the superficial layer of the epidermis in several distinct places. Hemipenes inverted and therefore not visible. SVL (295 mm) shorter than tail length (369 mm). Head 48.5 mm long (HL1), 35.2 mm high and 42.5 mm wide. Fourth toe length 46.3/44.9 mm. Rostral scale in contact with two adjacent supra-supralabials. Anteriormost supralabials in contact below the mental. Nine supralabials on both sides, ten infralabials on both sides. Internasal and rostral scales separated by a row of at least four small granular scales. Three frontonasal scales, posterior to and in contact with the internasal, the two exteriormost being larger and more conical than the smaller and relatively flat medium one. Subdigital lamellae 29/29 under the third toe, 23/22 under the fourth toe. Femoral pores indistinct (not counted); 271 transversal rows of minute scales at midbody. Dorsal crest moderately developed, 15 spiny scales running along the dorsal side of the neck (Cnc), followed by a well-differentiated postnuchal gap of 11 very reduced scales (Cng), 91 spiny scales running

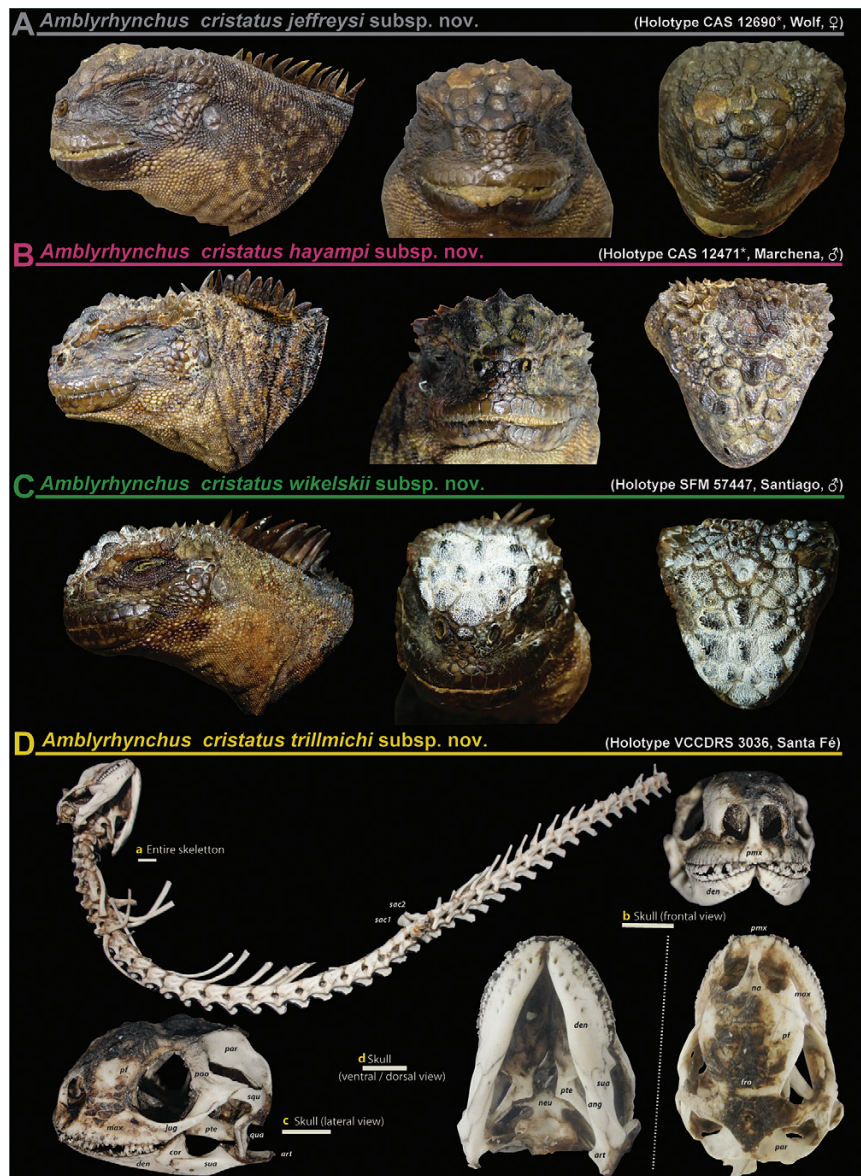


Figure 9. Lateral, frontal and dorsal views of the holotypes of (A) *A. c. jeffreysi* subsp. nov. (CAS 12690, Wolf), (B) *A. c. hayampi* subsp. nov. (CAS 12471, Marchena), (C) *A. c. wikelskii* subsp. nov. (SFM 57447, Santiago) and (D) *A. c. trillmichi* subsp. nov. Asterisks (*) indicate lateral views of the head symmetrically reversed and therefore representing the right side. *ang*: angular; *art*: articular; *cor*: coronoid; *den*: dentary; *fem*: femur; *fib*: fibula; *fro*: frontal; *ha*: hemal arch; *ili*: ilium; *isc*: ischium; *itc*: interclavicle; *jug*: jugal; *max*: maxilla; *mta*: metatarsals; *na*: nasal; *neu*: neurocranium; *par*: parietal; *pf*: prefrontal; *pli*: processus lateralis of interclavicle; *pmx*: premaxilla; *poo*: postorbital; *pte*: pterygoid; *pub*: pubis; *qua*: quadrate; *sac1/2*: first/second sacrals; *sca*: scapulacoracoid; *squ*: squamosal; *ste*: sternum; *sua*: surangular; *tar*: tarsals; *tib*: tibia.

along the dorsal side of the trunk (Cdc), then 138 scales on the dorsal side of the tail.

After 110 years of preservation in ethanol, original colour pattern still visible. Background coloration of the dorsal and lateral sides of the body very dark brown, with lighter, chocolate brown rounded spots (ochre when superficial layer of skin removed) variable in size

and forming complex reticulated patterns. Irregularly distributed across the back, their absence makes visible three or four (hardly distinguishable) transversal spindle-shaped areas of immaculate dark brown background. Anterior part (postnuchal region) and posterior part (pelvic region) of the dorsum region darker than the middle of the dorsum, as less densely maculated.

Dorsal and lateral side of the head ochre to chocolate brown, with large conical scales covering the dorsal side of the head; these scales lighter due to the presence of concretions of undetermined origin at their base (likely deposited during the lifetime of the animal). Crest spines chocolate brown on the nuchal segment, mostly ochre on the trunk segment (with exception of few series of one to three consecutive dark brown spines corresponding with the darker spindle-shape spotless area of the dorsum), and dark brown on the caudal segment. Dorsal and lateral sides of the tail uniformly dark brown (with exception of large ochre patches where the superficial layer of the skin has been removed). Dorsal and lateral sides of the limbs brownish, their distal parts (forearm and manus for forelimbs, toes for hindlimbs) darker than the proximal ones. Mental region, ventrum, ventral sides of the limbs and of the tail uniformly light ochre, with exception of a thin gular collar and the ventral side of manus and pes which are distinctly darker.

***AMBLYRHYNCHUS CRISTATUS*
JEFFREYSI SUBSP. NOV.**

(Figs 60–U, 7, 9A)

LSID urn:lsid:zoobank.org:act:CFCD0F52-D88B-4DC6-A0D6-3C1D9B766476

Holotype: CAS 12690, coll. by J.R. Slevin in 1906, Isla Wolf, no exact locality.

Paratypes ($N = 7$): CAS 12686–12689, 12691–12693, same data as holotype; CAS-SUR 4777, coll. by E. Heller and R.E. Snodgrass in 1905, Isla Wolf, no exact locality.

Specimens examined ($N = 14$): **Wolf**: CAS 12686–12693 CAS-SUR 4777, coll. by E. Heller and R.E. Snodgrass in 1905, no exact locality. **Darwin**: CAS 12693–12696, 12698, coll. In 1906 by J.R. Slevin, Isla Darwin, no exact locality.

Etymology: The subspecies name is a patronym for Jeffreys Málaga, a ranger of the Galápagos National Park with an outstanding interest and enthusiasm for the fauna and flora of the Galápagos, and especially for marine iguanas. Jeffreys Málaga has been awarded several times ‘Guardaparque del Año,’ or Park Warden of the Year and has been an essential part of our team during several excursions to the Galápagos.

Geographic distribution: Wolf, Darwin (tentative assignment), Roca Redonda and very likely their satellite islets (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus jeffreysi* subsp. nov. is the smallest subspecies of marine iguana

(mean SVL 191 mm, up to a maximum of 245 mm). Mature males have a poorly developed crest of spines and weakly conical supracephalic scales. In preservative, males are almost uniformly dark brown or blackish dorsally, with few small ochre dots, and a contrasting lighter crest, mostly ochre. More specifically, this subspecies also has a high number of scale rows at midbody (144.3 ± 8.7), a high number of lamellae under the third (29.0 ± 1.5) and fourth (33.2 ± 2.9) toes and a very high number of scales along the dorsal (125.1 ± 9.9) and caudal crest (146.1 ± 9.7) (see also Figs 4, 60–U, 9A, Table 1).

Description of the holotype: CAS 12690 (Figs 60, 9A), adult female preserved in 70% ethanol, in relatively good condition despite long incisions (likely realized during the fixation process) running along the ventral side of the body, limbs and tail, and the posterior half of the body being slightly discoloured. SVL (208 mm) shorter than tail length (332 mm). Head 36.9 mm long (HL1), 30.2 mm high and 31.5 mm wide. Fourth toe length 38.2/39.4 mm. Rostral scale in contact with three adjacent supra-supralabials. Anteriormost supralabials in contact below the mental. Nine supralabials on both sides; 12 infralabials on the right side, 11 on the left side. Internasal and rostral scales separated by a row of at least four flat and rounded relatively small scales. Four relatively large and weakly convex frontonasal scales, asymmetrically arranged, posterior to and in contact with the internasal. Subdigital lamellae 29/29 under the third toe, 33/32 under the fourth toe; 275 transversal row of minute scales at midbody. Dorsal crest weakly developed, 16 spiny scales running along the dorsal side of the neck (Cnc), followed by a well-differentiated post-nuchal gap of 11 very reduced scales (Cng), 89 spiny scales running along the dorsal side of the trunk (Cdc), remarkably reduced in the posterior half of the body, then 161 scales on the dorsal side of the tail.

After 110 years of preservation in ethanol, original colour pattern still clearly visible, although the posterior half part of the body tends to be lighter, apparently because of the removal of the superficial layer of epidermis. Background coloration of the dorsal and lateral sides of the whole body dark brown. Trunk, lateral sides of the neck and of the base of the tail moderately maculated by tiny ochre spots, whose density increase at the interface of lateral and ventral sides. Dorsal and lateral sides of the head dark to chocolate brown, the larger conical scales covering the top of the head slightly lighter. Crest spines on the nuchal and dorsal segments light ochre, remarkably contrasting with the dark background coloration, whereas spines on the caudal segment almost as dark as the rest of the tail. Dorsal and lateral sides of the tail uniformly dark brown, with exception of few very light ochre spot on the lateral side of the base of the tail. Dorsal and lateral sides of the limbs brownish, immaculate. Mental region, ventrum, ventral sides of the limbs

and of the tail uniformly light ochre, with exception of a gular collar that is distinctly darker.

***AMBLYRHYNCHUS CRISTATUS*
GODZILLA SUBSP. NOV.**

(Figs 5S, 7, 10)

LSID urn:lsid:zoobank.org:act:7039C66A-09BE-4EC7-BF4D-0089533706C5

Holotype: VCCDRS 2967, coll. by A. Macleod in March 2013, Galápagos archipelago, San Cristóbal island, Salinas colony (at Punta Pitt, SRS), at geographical coordinates (decimal format) 0.697247S, 89.27084W. No paratypes.

Specimens examined ($N = 1 + 113$): **San Cristóbal**: VCCDRS 2967 (holotype), Punta Pitt. Morphological characters of 113 specimens have also been examined in life from photographs.

Etymology: The subspecific epithet refers to the fictional saurian monster from the eponym movie franchise created by Tomoyuki Tanaka (IMDb, 2016). Explicitly mentioned in the title sequence of the 1990s American remake (Emmerich, 1998), the physical appearance and swimming behaviour of marine iguanas were a significant source of inspiration to the creature's designer (Patrick Tatopoulos, personal communication). The name is an invariable noun in apposition.

Geographic distribution: Northern (north-eastern) part of San Cristóbal and the satellite islets off the northern coast of San Cristóbal (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus godzilla* subsp. nov. is a medium-sized subspecies of marine iguana (mean SVL 280 mm, up to a maximum of 400 mm). Mature males have a well-developed crest of spines and relatively pronounced conical supracephalic scales. In life, the males are dark grey or blackish dorsally. Dark ochre or brownish dots may align and merge to each other to form a couple of barely discernable and irregular transversal stripes along the body. More specifically, this subspecies is also characterized by mental scales most often (91%) separated (see also Figs 4, 5S, Table 1).

Description of the holotype: VCCDRS 2967 (Fig. 10). Incomplete and dismantled skeleton collected in a fissure of lava rock, consisting in (1) ten cranial elements: premaxilla with a single remaining tooth attached (32.1 mm long), right (toothless) dentary, left ectopterygoid, left angular, right jugal, neurocranium and two incomplete remains of the left (articular and suprarticular, 47.5 mm long in total) and right

– articular – mandibulae, (2) three scapular elements: right scapulocoracoid, interclavicle and right clavicle, (3) a single pelvic element: left hemipelvis, (4) five unidentified relatively short long bones (ranging from 8.5 to 42.1 mm), (5) 12 unidentified ribs (length in linear distance measuring from 38.4 to 57.0 mm) and (6) 24 vertebrae: 15 presacral, both the first and second sacral, and seven caudals (five from the tail basis and two from tail midlength, plus three hemal arches).

***AMBLYRHYNCHUS CRISTATUS*
TRILLMICH SUBSP. NOV.**

(Figs 5R, 7)

LSID urn:lsid:zoobank.org:act:33639AF1-749D-40F6-B408-74754EFF3B38

Holotype: VCCDRS 3036, coll. by A. Ibáñez on 10 January 2016, Galápagos archipelago, Santa Fé island, West coast, El Miedo, geographical coordinates (in decimal format) 0.82581S, 90.02861W.

Specimen examined: **Santa Fé**: VCCDRS 3036.

Etymology: The subspecific epithet refers to Prof. Dr. Fritz Trillmich, a foremost biological researcher in Germany who has spent a lifetime working on the biology of the Galápagos islands. For many years Fritz Trillmich worked together with Martin Wikelski on the island of Santa Fé, where their research on the local population produced important insights into the behaviour and ecology of marine iguanas. In addition to undertaking his own research, Fritz Trillmich is Professor Emeritus of Behavioural Ecology at the University of Bielefeld where he continues to inspire and mentor generations of young researchers who will continue his legacy for years to come.

Geographic distribution: Santa Fé and very likely its satellite islets (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus trillmichi* subsp. nov. is a medium-sized subspecies of marine iguana (mean SVL 245 mm, up to a maximum of 285 mm). Coloration of males (based on two pictures) are apparently similar to (and typical of) *A. cristatus venustissimus* from Floreana and Española, with large black patches and a bluish mid-dorsal stripe over a reddish background (Figs 4, 5R, Table 1).

Description of the holotype: VCCDRS 3036 (Fig. 9D), incomplete skeleton, consisting in complete skull (47.2 mm in maximal length, 37.6 mm in maximal width and 31.4 mm in max height) still in connection with the vertebral spine (24 presacral vertebrae, two sacral and 16 of most proximal caudal vertebrae, total length of



Figure 10. Holotype of *A. c. godzilla* subsp. nov. act: acetabulum; boc: basioccipital; fom: foramen magnum; ili: ilium; isc: ischium; itc: interclavicle; jug: jugal; nrc: neurocranium; occ: occipital condyle; ooc: otooccipital; pas: processus ascendens of soc; pli: processus lateralis of interclavicle; pmx: premaxilla; pop: paraoccipital process; pub: pubis; sac1/2: first/second sacrals; soc: supraoccipital; sua: surangular; too: tricuspid tooth.

410 mm). Appendicular structures from limbs and girdles, most of the ribs, and the distal section of tail are missing.

**AMBLYRHYNCHUS CRISTATUS
WIKELSKII SUBSP. NOV.
(Figs 5D–F, 7, 9C)**

LSID urn:lsid:zoobank.org:act:EBFA5A08-D5F9-41F9-BC2A-2FA65A707637

Holotype: SFM 57447, coll. by I. Eibl-Eibesfeldt in 1957, Crab-point, S of James Bay (Isla Santiago) (specimen originally designated as paratype of *A. c. mertensi*).

Paratypes: SFM 57446, 57448, 57449, same data as holotype (all of them originally designated as paratypes of *A. c. mertensi*).

Specimens examined (N = 13): **Santiago:** SFM 57446–57449, coll. by I. Eibl-Eibesfeldt in 1957, Crab-point,

S of James Bay (all of them being also paratypes of *A. c. mertensi*), SFM 57450, coll. by I. Eibl-Eibesfeldt in 1957, Crab-point, S of James Bay. CAS 12168, 12169, coll. by J.S. Hunter in 1906, no exact locality. **Rábida:** CAS 10613–10616, CAS 11021, coll. by E.S. King in 1905, no exact locality. SFM 25492, coll. during the Hassler expedition in 1872.

Etymology: The subspecies name is a patronym for Prof. Dr. Martin Wikelski in recognition of his outstanding research on marine iguanas for several decades. Martin Wikelski has been fascinated by marine iguanas, which served as a study system for his behavioural and ecological research from his PhD onwards. He has published important papers on marine iguana ecology and conservation, for instance demonstrating the devastating effects of an oil spill on this species, and showing that marine iguanas can shrink in response to starvation induced by El Niño–based climate fluctuations.

Geographic distribution: Santiago, Rábida and very likely their satellite islets (Fig. 8).

Diagnostic description: *Amblyrhynchus cristatus wikelskii* subsp. nov. is a medium-sized subspecies of marine iguana (mean SVL 236 mm, up to a maximum of 278 mm). Mature males have a moderately developed crest of spines and moderately conical supracapalic scales. In preservative, males have dark brown mottling on a yellowish to brownish slightly lighter dorsal background. This subspecies is also characterized by a very low number of scale rows at midbody (125.8 ± 7.7), a low number of lamellae under the third (24.9 ± 2.8) and fourth (29.0 ± 3.8) toes and a low number of scales along the caudal crest (135.9 ± 11.2) (see also Figs 4, 5D–F, 9C, Table 1).

Description of the holotype: SFM 57447 (Figs 5D, 9C), adult male preserved in 70% ethanol, in a good condition with exception of a short ventral incision (likely realized during the fixation process). Hemipenes inverted and therefore not visible. SVL (257 mm) shorter than tail length (411 mm). Head 44.2 mm long (HL1), 35.1 mm high, and 40.5 mm wide. Fourth toe length 45.7/45.9 mm. Rostral scale in contact with three adjacent supra-supralabials. Anteriormost supralabials not in contact below the mental (separated by three granular median scales). Nine supralabials on the right side, eight on the left side. Ten infralabials on both sides. Internasal and rostral scales separated by a row of four small granular scales. Three large and conical frontonasal scales, posterior to and in contact with the internasal. Subdigital lamellae 22/23 under the third toe, 31/32 under the fourth toe. Twenty-six hardly distinguishable femoral pores on the right side (not countable on the left

side); 255 transversal rows of minute scales at midbody. Dorsal crest well developed, ten spiny scales running along the dorsal side of the neck (Cnc), followed by a postnuchal gap of 16 very reduced scales (Cng), 78 spiny scales running along the dorsal side of the trunk (Cdc), then 133 scales on the dorsal side of the tail.

After around six decades of preservation in ethanol, original colour pattern still visible, although the specimen probably became darker during this period. Dorsal and lateral sides of the body dark brown mottled on an irregular yellowish to brownish slightly lighter background. Neck lighter, with an ochre yellowish shade. Lateral side of the head dark brown and ochre yellowish mottled, large conical scales covering the dorsal side of the head whitish, due to the presence of concretions of undetermined origin (likely deposited during the lifetime of the animal). Crest spines mostly chocolate brown, with exception of few series of one to six consecutive dark brown spines corresponding with the darker dots mottling the dorsum. Dorsal and lateral sides of the tail uniformly dark brown, with exception the tail basis yellowish ochre mottled ventrolaterally. Dorsal sides of frontlimbs lighter proximally (brown chocolate and ochre mottled arms) than distally (dark brown forearms), ventral sides light ochre. Dorsal sides of hindlimbs dark brown and ventral sides light ochre. Mental region, ventrum, ventral sides of the limbs and of the tail uniformly light ochre, with exception of a relatively large darker collar and darker ventral sides of manus and pes.

DISCUSSION

THE REVISION OF MARINE IGUANA TAXONOMY – AN OVERDUE NECESSITY FROM AN EVOLUTIONARY AND CONSERVATIONAL PERSPECTIVE

Since the 1990s marine iguanas have been intensively studied using multiple molecular approaches to better understand various aspects of their phylogeny and evolution. These results have shed new light on the initial subspecific division made by I. Eibl-Eibesfeldt.

The first comprehensive phylogenetic study by Rassmann (1997) based on mitochondrial genes, indicated monophyly of Galápagos iguanas, with land iguanas (genus *Conolophus*) as the sister genus of the marine iguanas. The most recent comprehensive phylogenetic approach on Galápagos iguanas, using both mitochondrial and nuclear genes, supports this view and places the Central American genus *Ctenosaura* as the closest relative to the clade of Galápagos iguanas (MacLeod et al., 2015a, b). In a parallel study, Rassmann et al. (1997) also investigated the population structure of marine iguanas across the archipelago on the basis of mitochondrial D-loop haplotype diversity, as well as three microsatellite and

three minisatellite loci. The major finding of this study was a general lack of genetic diversification of island populations, and the authors therefore understandably doubted the justification of subspecific division of marine iguana populations. As a consequence, marine iguanas were henceforth regarded as a single species without any significant population structure, and thus a revision of marine iguana taxonomy seemed to be of no further scientific interest. While the study of [Rassmann *et al.* \(1997\)](#) was well accepted by evolutionary biologists and ecologists, and influenced the interpretation of other biological features in marine iguanas, conservation agencies (e.g. [ITWG, 2011](#); [IUCN, 2016](#)), other biologists (e.g. [Uetz & Hošek, 2016](#); [Jiménez-Uzcátegui, Márquez & Snell, 2016](#)) or field guides on the fauna of the Galápagos (e.g. [Jackson, 2007](#); [Fitter, Fitter & Hosking, 2016](#)) still relied on the Eibl-Eibesfeldt taxonomy, since it was never formally revised.

Based on a comprehensive resampling of island populations, featuring over 1200 marine iguana samples analysed with 13 highly polymorphic microsatellite loci, [Steinfartz *et al.*, \(2009\)](#) revised the view on the population genetic structure of marine iguanas. As population genetic methods had much advanced since the time of the [Rassmann *et al.* study in 1997](#), far deeper insights could be gained. In contrast with the previous results, the 2009 work revealed that most of the island populations formed clear genetic clusters with high self-assignment probabilities. This study also first demonstrated the presence of two highly genetically distinct clusters on the island of San Cristóbal. A follow-up study extended the suite of applied molecular markers to genome-wide SNPs and a more detailed analysis of the situation on San Cristóbal ([MacLeod *et al.*, 2015a](#)). Although this work showed that the lineage of marine iguanas diverged from the land iguana lineage around 4.5 million years ago, it found the divergence among extant populations of *Amblyrhynchus* to be remarkably recent, with the deepest divergence occurring less than 50 000 years ago ([MacLeod *et al.*, 2015a](#)). In contrast, within the lineage of land iguanas, a relatively ancient species (*Conolophus marthae*) was recently described from Isabela Island ([Gentile *et al.*, 2009](#)). Although marine iguanas have clearly not speciated to such an extent, we do see significant population structuring that warrants formal recognition.

EVOLUTIONARY PROCESSES AND INTRASPECIFIC PHYLOGENY

To understand and taxonomically assess the observed differentiation among marine iguanas, it is of relevance to examine in more detail the ongoing

evolutionary processes in these animals. For instance, populations on the island of San Cristóbal are structured by a striking case of within-island speciation between the Lobería and Punta Pitt population clusters, but this process is blurred by hybridization with marine iguanas from other islands and genetic clusters (e.g. Santa Cruz and Española; for details see [MacLeod *et al.*, 2015a](#)). It seems therefore likely that local adaptation and speciation in *Amblyrhynchus* has been continuously arrested and masked by hybridization. We hypothesize that this evolutionary mechanism enhances evolutionary potential and facilitates marine iguana survival across the archipelago, in the context of extreme climatic fluctuations which can result in dramatic starvation events on the different islands ([Laurie, 1990](#); [Wikelski & Trillmich, 1997](#); [Steinfartz *et al.*, 2007](#)). The marine iguana therefore appears to remain as a single species over an evolutionary time scale, but one which absorbs adaptations from local speciation events across various populations into a common gene pool via hybridization ([MacLeod *et al.*, 2015a](#)). In turn, this suggests that maintaining the genetic diversity of marine iguana populations is vital for their long-term survival, as is the case for other threatened Galapagos endemics ([Grant & Grant, 2014](#)).

A further aspect to take into account is the general phylogeography and the direction of inter-island colonization and gene flow in marine iguanas. Our study is based on an unprecedented amount of sequence information from RADseq data, and provides evidence for relationships among a number of subspecies. For instance, *A. c. mertensi* and *A. c. godzilla* (both San Cristóbal) are resolved as sister groups, as are *A. c. trillmichi* and *A. c. venustissimus* (Floreana/Española and Santa Fé). Furthermore, *A. c. sielmanni*, *A. c. hayampi* and *A. c. nanus* (Pinta/Marchena/Genovesa) clearly form one clade. These three cases refer to subspecies from islands in geographical proximity, and interpreting this pattern in a phylogeographic context is intuitively appealing. The resulting hypotheses are (1) a single colonization and intra-island diversification on San Cristóbal ([MacLeod *et al.*, 2015a](#)), (2) colonization of Santa Fé by an ancestral population that subsequently spread to Floreana and from there to Española and (3) an ancestral population first colonizing Pinta, and from there spreading to Marchena and Genovesa. However, with the current methods it is not possible to distinguish whether these groupings are due to phylogeographic ancestry or secondary admixture. Treemix analysis provided some hint that *A. c. godzilla* has been especially affected by mixture events with other island populations (Appendix S2), but not with *A. c. mertensi* that is found on the same island. Given the strong evidence

for inter-island migration and considering that neighbouring islands are relatively close, it is possible that hybridizing migrants have led to genetic similarities among San Cristóbal subspecies that could also influence their grouping in the phylogenetic tree.

SELECTIVE PRESSURE ON EXTERNAL MORPHOLOGY OF MARINE IGUANAS

For his taxonomic classification, Eibl-Eibesfeldt relied on only a few morphological features. For our taxonomic revision we sought to include external morphology as much as possible. However, due to a limited sample sizes for some islands (e.g. Santa Fé, Darwin) and also due to a lack of unambiguous discriminant characters between populations, we must ultimately refrain from an in-depth analysis of morphological data. In contrast to Darwin's finches, which have undergone one of the best documented and understood adaptive radiations, with beak shape and size being the key characters to adapt to different niches across the archipelago (Grant & Grant, 2008, 2014), the external morphology of marine iguanas – beside total length – seems to be rather conserved.

Chiari *et al.* (2016) found that differences in body size among marine iguana populations to be best explained by sea surface temperature and island perimeter, while body shape was best explained by evolutionary history. The influence of these ecological variables on body size can be easily explained by the fact that food shortage (i.e. the depletion of edible algae for the marine iguanas due to sea water temperature increase) caused by El Niño events can cause high mortality in populations, especially among large-sized individuals, thus providing a possible selective force against large body size in those populations where El Niño effects are strongest. Such effects might influence body size evolution in repeatedly affected populations (Wikelski, 2005). The results of Chiari *et al.* (2016) are reliable due to impressive sample sizes, but refer to only a limited number of morphological measurements and no scale counts. Hence, we cannot exclude the possibility that characters other than body size might also be affected by environmental factors.

Evolutionary forces that might influence morphological differences among subspecies of marine iguanas should be considered when evaluating the taxonomic relevance of the differences reported herein. In this context, it is noteworthy that differences between subspecies were most strongly expressed in males, and in some cases this might refer to characters that could be under sexual selection, such as dorsal crest scales or femoral pores, which are far more expressed in males. Body size of males is known to be under positive sexual selection (Wikelski & Trillmich, 1997). Chiari

et al. (2016) have shown that in the vast majority of populations of marine iguanas, males are larger and have higher and shorter heads than females, suggesting that sexual selection also affects other morphometric and possibly meristic characters of these lizards. Therefore, to fully evaluate the taxonomic significance of all morphological differences among marine iguana populations, a better understanding of correlates with sex and body size is needed. If body proportions, for instance, would change allometrically with increasing body size, and in some populations large males are missing, simple statistics would falsely detect morphometric differences between these populations. Although scale counts in squamates are usually thought to be ontogenetically stable and thus reliable taxonomic characters, exceptions have been observed (e.g. Tomovic *et al.*, 2008), suggesting that also some meristic variables could be affected by such effects. However, the large number of differences encountered, many of which also hold for comparisons of females, suggests that the main conclusion of a substantial morphological differentiation among populations is valid.

The apparently stronger expression in males of morphological differences among marine iguana subspecies not only suggests an influence of sexual selection, but also hints to a more general rule, according to which male comparisons have greater power to taxonomically discriminate units at the species and subspecies level. This is obvious for many taxa where male traits directly related to courtship are often seen as decisive species criteria, such as differences in male genitalia of insects and squamates, bioacoustic signals in insects, frogs or birds, complex sexually dimorphic ornamentations of head and body in many animals, or the bright breeding plumage of male birds (Böhme, 1988; Ziegler & Böhme 1997; Seddon *et al.*, 2013).

A NEW SUBSPECIES-LEVEL CLASSIFICATION OF MARINE IGUANAS

As a highly mobile species, the marine iguana has not undergone the deep divergence that characterizes most Galápagos endemics; for example in giant tortoises on the Galápagos 16 distinct species have been described (Poulakakis *et al.*, 2015). We feel that in the light of recent insights, the taxonomy of marine iguanas is best revised by maintaining a single species subdivided into subspecies. Based on our comprehensive microsatellite loci analysis supplemented by mitochondrial sequence data, genome-wide SNP data and an extensive morphological investigation, our new taxonomy of 11 subspecies successfully integrates the most up-to-date information on population structure with the best available morphological comparisons. Nevertheless, it should be noted that due to limited

molecular and/or morphological sampling size for certain islands, future studies focusing on the exact status of the different populations presently recognized as *A. c. jeffreysi* (from Darwin, Wolf and Roca Redonda) may possibly lead to the partitioning of this taxon into several genetic subunits.

Although management units for *Amblyrhynchus* matching the genetic clusters have previously been described (MacLeod & Steinfartz, 2016), it is taxonomic units that remain the default basis for most conservation management plans (Mace, 2004) and therefore taxonomy has a profound influence on conservation management. Despite efforts within the conservation community to define conservation units that represent the dynamic nature of natural systems (Mace, 2004), conservation management remains typically based on species and subspecies lists, sometimes from outdated taxonomies, and such has been the case in marine iguanas. It is therefore of particular importance that taxonomies are accurate in species of conservation concern. In current practice (i.e. based on the taxonomy of Eibl-Eibesfeldt), certain evolutionarily distinct lineages, such as the Punta Pitt population, would be of lower conservation priority than officially recognized subspecies, even when the differentiation of subspecies is of little or no biological relevance (e.g. in the case of the Fernandina and Isabela subspecies; see Fig. 2). The units described herein can serve as a basis for planning future conservation efforts of the Galápagos National Park and will allow finite resources to be directed in such a way that the overall genetic diversity of the species will be better protected. For instance, taxonomic recognition of the two threatened San Cristóbal populations (MacLeod & Steinfartz, 2016) will characterize them as conservation priorities, meaning that they should now be afforded greater protection from local threats, including predation by feral animals and encroachment of urban developments, such as building of new hotel complexes. With the subspecies-level classification proposed here, and almost 60 years after Eibl-Eibesfeldt pioneering work, marine iguana taxonomy now provides a sound systematic basis upon which conservation management can support this species to withstand present and future anthropogenic threats. We hope that our revised taxonomy will also be adopted by the IUCN as a basis for the effective conservation of the marine iguana.

ACKNOWLEDGEMENTS

We are grateful to Lauren Scheinberg and Jesse Vindum (CAS), Jörn Köhler (HLMD), Patrick Campbell (NHM), Mark Carnall (OUMNH) and Linda Acker, Martin Janssen and Gunther Köhler (SMF) for

providing us access to specimens under their care, to Juan M. Guayasamin and Nina Kinti-Moss for providing information on terms in Kichwa language, and to Marcus Krueger, Heidi Snell and Maren Vitousek for making available their precious pictures. Many thanks to Patrick Tatopoulos, designer of *Godzilla* (Emmerich, 1998), for sharing with us some secrets on the creature he gave birth to. This work was supported by a grant from the Swiss Friends of the Galápagos Islands and by a postdoctoral fellowship of the Humboldt Foundation to AI. We would like to thank the Charles Darwin Foundation and the Galápagos National Park Directorate (GNPD) who kindly granted us the permission to carry out this investigation under the permit numbers PC-08-15, PC-09-16, PC-22-14, PC-23-13 and PC-26-12. This publication is contribution number 2151 of the Charles Darwin Foundation for the Galápagos Islands.

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SUPPORTING INFORMATION

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

Appendix S1. Morphological measurements.

Appendix S2. Drift trees depicting evolutionary relationships between marine iguana subspecies as defined in this study.

Table S1. Sample sizes of *Amblyrhynchus cristatus* of the microsatellite and mtDNA haplotype datasets.

Table S2. Proportion of *Amblyrhynchus cristatus* individuals which self-assign to the genetic cluster designated to their island of origin.

Table S3. Private alleles per cluster based on 12 microsatellite loci in *Amblyrhynchus cristatus*.

Table S4. Private haplotypes by island for 1181bp of the mitochondrial control region in *Amblyrhynchus cristatus*.

Table S5. Statistical results of the morphological analysis.

Table S6. Original morphometric measurements and scale counts of *Amblyrhynchus* specimens.

Table S7. Allele frequencies for the 12 microsatellite loci used, measured for each of the 11 proposed subspecies of *Amblyrhynchus cristatus*.

Table S8. Matrix of F_{st} (based on SNP data) and R_{st} values (based on microsatellite data) between the proposed *Amblyrhynchus cristatus* subspecies.