Genome of the Komodo dragon reveals adaptations in the cardiovascular and chemosensory systems of monitor lizards

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Monitor lizards are unique among ectothermic reptiles in that they have high aerobic capacity and distinctive cardiovascular physiology resembling that of endothermic mammals. Here, we sequence the genome of the Komodo dragon Varanus komodoensis, the largest extant monitor lizard, and generate a high-resolution de novo chromosome-assigned genome assembly for V. komodoensis using a hybrid approach of long-range sequencing and single-molecule optical mapping. Comparing the genome of V. komodoensis with those of related species, we find evidence of positive selection in pathways related to energy metabolism, cardiovascular homoeostasis, and haemostasis. We also show species-specific expansions of a chemoreceptor gene family related to pheromone and kairomone sensing in V. komodoensis and other lizard lineages. Together, these evolutionary signatures of adaptation reveal the genetic underpinnings of the unique Komodo dragon sensory and cardiovascular systems, and suggest that selective pressure altered haemostasis genes to help Komodo dragons evade the anticoagulant effects of their own saliva. The Komodo dragon genome is an important resource for understanding the biology of monitor lizards and reptiles worldwide.

The evolution of form and function in non-avian reptiles contains numerous examples of innovation and diversity. There are an estimated 10,000 reptile species worldwide, found on every continent except Antarctica, with a diversity of lifestyles and morphologies corresponding to a broad range of anatomic and physiological adaptations. Understanding how these adaptations evolved through changes to biochemical and cellular processes will reveal fundamental insights into areas ranging from anatomy and metabolism to behaviour and ecology.

The varanid lizards (genus Varanus, or monitor lizards) are an unusual group within squamate reptiles (lizards and snakes). Varanids exhibit the largest range in size among reptiles, varying in mass by over five orders of magnitude (8 g–100 kg). Varanids have a unique cardiopulmonary physiology and metabolism with numerous parallels to the mammalian cardiovascular system. For example, their cardiac anatomy is characterized by well-developed ventricular septa (‘muscular ridge’ and ‘bulbus lamellae’) resulting in a functionally divided heart. This enables a dual-pressure cardiovascular system characterized by high systemic and low pulmonary blood pressures. Furthermore, varanid lizards can achieve and sustain higher aerobic metabolic rates and endurance capacity than similar size non-varanid squamates, which enables intense, sustainable movements while hunting prey or in bouts of male–male combat. The largest of the varanid lizards, the Komodo dragon Varanus komodoensis, can grow to 3 m in length and run up to 20 km h\(^{-1}\), allowing them to hunt large prey including deer and boar. Komodo dragons have a higher metabolism than predicted by allometric scaling relationships for varanid lizards, which may explain their capacity for daily movement to locate prey. Their ability to locate injured or dead prey through scent tracking over several kilometres is enabled by a powerful olfactory system, and their hunting is aided by serrate teeth, sharp claws, and saliva with
anticoagulant and shock-inducing properties\textsuperscript{2,4}. Furthermore, Komodo dragons engage in aggressive intraspecific conflicts over mating, territory and food, and wild individuals often bear scars from previous conflicts\textsuperscript{1}. To understand the genetic underpinnings of Komodo dragon physiology, we sequenced its genome and present a de novo assembly, generated with a hybrid approach of Illumina short-read sequencing with long-range sequencing using 10x Genomics, PacBio and Oxford Nanopore sequencing, and single-molecule optical mapping using the Bionano platform. This suite of technologies allowed us to confidently assemble a high-quality reference genome for the Komodo dragon, which can serve as a template for other varanid lizards. We used this genome to understand the relationship of varanids to other reptiles using phylogenomics. We uncovered Komodo dragon-specific positive selection for genes encoding regulators of muscle metabolism, cardiovascular homoeostasis, and haemostasis. Furthermore, we discovered multiple lineage-specific expansions of a family of chemoreceptor genes in several squamates. Finally, we generated a high-resolution chromosomal map by assigning genomic scaffolds to chromosomes, enabling us to address questions about karyotype and sex chromosome evolution in squamates.

**Results**

**De novo genome assembly.** We sequenced the Komodo dragon genome principally from DNA isolated from peripheral blood of two male Komodo dragons housed at Zoo Atlanta: Slasher, offspring of the first Komodo dragons given to US President Reagan from President Suharto of Indonesia, and Rinca, an unrelated juvenile. A third individual from Gran Canaria was used for PacBio DNA sequencing. The Komodo dragon genome is distributed across 20 pairs of chromosomes, comprising eight pairs of large chromosomes and 12 pairs of microchromosomes\textsuperscript{11,16}. De novo assembly was performed with a combination of 10x Genomics linked-read sequencing, Bionano optical mapping data, PacBio sequencing and Oxford Nanopore MinIon sequencing (Methods). The final assembly contained 1,411 scaffolds (\textgtr10 kb) with an N50 scaffold length of 24 Mb (longest scaffold: 138 Mb; Table 1). The assembly is 1.51 Gb in size, \textasciitilde 32\% smaller than the genome of the Chinese crocodile lizard *Shinisaurus crocodilurus*, the closest relative of the Komodo dragon for which a sequenced genome is available, and \textasciitilde 15\% smaller than the green anole *Anolis carolinensis*\textsuperscript{11}, a model squamate lizard (Supplementary Table 1). An assembly-free error corrected k-mer counting estimate of the Komodo dragon genome size\textsuperscript{11} is 1.69 Gb, while a flow cytometry-based estimate of the Komodo dragon genome size is 1.89 Gb (Ref.\textsuperscript{14}; estimated 3.86 pg of DNA per nucleus, with a conversion factor of 978 Mb pg\textsuperscript{-1} (Ref.\textsuperscript{15})). Gaps comprise 0.97% of the assembly. We assessed the completeness of the Komodo dragon genome assembly by searching for 2,586 single-copy vertebrate genes using BUSCO\textsuperscript{16}. The Komodo dragon genome has a similar distribution of single-copy (95.7\%), duplicated (0.4\%), fragmented (2\%) and missing (1.9\%) universal vertebrate genes as other reptile genomes (Supplementary Table 3). The GC content of the Komodo dragon genome is 44.0%, similar to that of the *S. crocodilurus* genome (44.5%) but higher than the GC content of *A. carolinensis* (40.3%; Supplementary Table 1). Repetitive elements accounted for 32\% of the genome, most of which were transposable elements (Supplementary Table 2). As repetitive elements account for 49.6\% of the *S. crocodilurus* genome\textsuperscript{11}, most of the difference in size between the Komodo dragon genome and that of its closest sequenced relative can be attributed to repetitive element content.

**Chromosome scaffold content.** We isolated chromosome-specific DNA pools from a female Komodo dragon embryo from Prague zoo stock through flow sorting\textsuperscript{16} and performed Illumina short-read sequencing on 15 DNA pools containing all Komodo dragon chromosomes (VKO1-20, VKOZ, VKOW; Supplementary Table 4). For each chromosome, we determined scaffold content and homology to *A. carolinensis* and chicken *Gallus gallus* chromosomes (Table 2 and Supplementary Tables 5 and 6). For pools where chromosomes were mixed, we determined partial scaffold content of single chromosomes. A total of 243 scaffolds containing 1.14 Gb (75\% of total 1.51 Gb assembly) were assigned to 20 Komodo dragon chromosomes. As sex chromosomes share homologous pseudoautosomal regions, scaffolds enriched in both mixed 17/18/Z and 11/12/W regions, scaffolds enriched in both mixed 17/18/Z and 11/12/W regions. As sex chromosomes have homologous pseudoautosomal regions, scaffolds enriched in both mixed 17/18/Z and 11/12/W chromosomes pools most likely contained sex chromosome regions. As sex chromosomes share homologous pseudoautosomal regions, scaffolds enriched in both mixed 17/18/Z and 11/12/W chromosomes pools most likely contained sex chromosome regions. As male varanid lizards are homogametic (ZZ) and the embryo used for flow sorting was female (ZW), scaffolds from the male-derived chromosome pools most likely contained sex chromosome regions. As sex chromosomes share homologous pseudoautosomal regions, scaffolds enriched in both mixed 17/18/Z and 11/12/W chromosomes pools most likely contained sex chromosome regions.

### Table 1 | Genome statistics of the Komodo dragon genome

| Assembly size | 1.51Gb (1,507,945,839 bp) |
| Number of scaffolds | 1,411 |
| Minimum scaffold length | 10 kb |
| Maximum scaffold length | 138 Mb |
| N50 scaffold length | 29 Mb (29,129,838) |
| Number of protein-coding genes | 18,457 |
| GC content | 44.04\% |

### Table 2 | Results of scaffold assignments to chromosomes of *V. komodoensis*

| *V. komodoensis* chromosome | *G. gallus* homology | *A. carolinensis* homology | Total number of assigned scaffolds | Total length of assigned scaffolds (bp) |
|-----------------------------|----------------------|---------------------------|-----------------------------------|---------------------------------------|
| Chr1                        | Chr1, 3, 5, 18, Z    | Chr1, 2, 3                | 94                                | 245,019,529                           |
| Chr2                        | Chr1, 3, 5, 7        | Chr1, 2, 6                | 14                                | 156,023,568                           |
| Chr3                        | Chr1, 4             | Chr3, 5                   | 11                                | 115,571,927                           |
| Chr4                        | Chr1, 2, 5, 27       | Chr1, 4, 6                | 39                                | 117,170,416                           |
| Chr5                        | Chr1                | Chr3                      | 6                                 | 75,951,376                            |
| Chr6, 7, 8                  | Chr2, 6, 8, 9, 20    | Chr1, 2, 3, 4             | 25                                | 200,178,831                           |
| Chr9, 10                    | Chr11, 22, 24        | Chr7, 8                   | 8                                 | 69,008,218                            |
| Chr11, 12                   | Chr4, 10            | Chr10, 11                 | 6                                 | 52,491,606                            |
| Chr12                       | Chr1, 5, 23         | Chr9                      | 9                                 | 19,625,567                            |
| Chr13                       | Chr14               | Chr12                     | 3                                 | 21,537,982                            |
| Chr14                       | Chr15               | ChrX                      | 4                                 | 14,821,201                            |
| Chr15                       | Chr17               | Chr16                     | 2                                 | 13,367,238                            |
| Chr16                       | Chr19               | Chr17, 21                 | 10                                | 17,262,365                            |
| Chr17, 18                   | Chr1, 9, 15, 17     | Chr14                     | 6                                 | 11,765,548                            |
| Chr19                       | Chr1, 28            | Chr18                     | 6                                 | 10,642,498                            |
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A. carolinensis chromosome 18, and mostly to G. gallus chromosome 28, in agreement with recent transcriptome analysis. Gene annotation. To annotate genes in the Komodo dragon genome, we performed RNA sequencing (RNA-seq) of heart tissue, and then used the MAKER pipeline with assembled RNA-seq transcripts, protein homology and de novo predictions as evidence (Methods). A total of 18,457 protein-coding genes were annotated in the Komodo genome, 17,189 (93%) of which have at least one annotated Interpro functional domain (Table 1). Of these protein-coding genes, 63% were expressed (reads per kilobase of transcript per million mapped reads > 1) in the heart. Most (89%) Komodo dragon protein-coding genes are orthologous to A. carolinensis genes. The median amino acid identity of single-copy orthologues between Komodo dragon and A. carolinensis is 68.9%, whereas it is 70.6% between one-to-one orthologues in Komodo dragon and S. crocodilurus (Supplementary Fig. 1).

Phylogenetic placement of Komodo dragon. Recent analyses estimate that varanid lizards and their closest extant relative, the earless monitor lizard of the Lanthanotus genus, diverged 62 Myr ago, and that varanid lizards and the Shinisauridae family diverged 115 Myr ago. We used 1,394 orthologous proteins from the Komodo dragon genome, 14 representative non-avian reptile species (seven squamates, three turtles and four crocodilians), three avian species (chicken, wild turkey and zebra finch) and four mammalian species (platypus, mouse, dog and human) to estimate a species tree (Fig. 1). Our analysis supports a sister relationship between anguimorphs (monitor lizards, anguids, Chinese crocodile lizards and relatives) and iguanians (dragon lizards, chameleons and iguanas), with snakes as sister to these two groups. This is in agreement with previously published analyses, including the most comprehensive marker gene-based molecular phylogenetic analyses, and in disagreement with a proposed sister relationship between anguimorphs and snakes or other topologies.

Expansion of vomeronasal genes across squamate reptiles. The vomeronasal organ is a chemosensory tissue shared across most amphibians, reptiles and mammals that detects chemical cues including pheromones and kairomones. There are two classes of vomeronasal chemosensory receptors, both of which have undergone repeated gene family expansions and contractions across vertebrate evolution. The gene family encoding vomeronasal type 2 receptors (V2Rs) has expanded in amphibians, snakes and some mammalian lineages. In contrast, crocodilian and turtle genomes contain very few V1R and V2R genes, and birds have entirely

Fig. 1 | Estimated species phylogeny of 15 non-avian reptile species, 3 avian species and 4 mammals. Maximum likelihood phylogeny constructed from 1,394 one-to-one orthologous proteins. Support values from 10,000 bootstrap replicates are shown. All silhouettes reproduced from PhyloPic. Credits: python silhouette, V. Deepak under a Creative Commons licence CC BY 3.0; lizard silhouette, Ghedo and T. Michael Keesey under a Creative Commons licence CC BY-SA 3.0. Photograph of Slasher, a Komodo dragon sampled for DNA in this study. Credit: photo courtesy of Adam Thompson/Zoo Atlanta.
To clarify the relationship between vomeronasal organ function and the evolution of vomeronasal receptor gene families, we analysed the coding sequences of 15 reptiles, including the Komodo dragon, for presence of V1R and V2R genes (Fig. 2a). We found a large repertoire of V2Rs, comparable to that of snakes, in the Komodo dragon, other anguimorphan lizards and geckos. We confirmed that there are few V1R genes across reptiles generally, and few to zero V2R genes in crocodilians and turtles (Supplementary Table 7). The low number of V2R genes in *A. carolinensis* and the Australian dragon lizard (*Pogona vitticeps*) suggests that V2R genes are infrequently expanded in iguanians, though more iguanian genomes are needed to test this hypothesis.

We next constructed a phylogeny of all V2R gene sequences across squamates (Fig. 2b) to understand the dynamic evolution of this gene family. The topology of this phylogeny supports the hypothesis that V2Rs expanded in the common ancestor of squamates, as there are clades of gene sequences containing members from all species (Fig. 2b). In addition, there are many well-supported single-species clades (that is, Komodo dragon only or Burmese python only) dispersed across the gene tree, consistent with multiple duplications of V2R genes later in squamate evolution, including in the Komodo dragon and gecko lineages (Fig. 2b).

Because V2Rs expanded in rodents through tandem gene duplications that produced clusters of paralogues, we examined clustering of V2R genes in our Komodo dragon assembly to determine whether a similar mechanism was at play. Of 129 V2 genes, 77 are organized into 21 gene clusters ranging from 2 to 13 paralogues (Fig. 3a and Supplementary Table 8). A phylogeny of all Komodo dragon V2R genes (Fig. 3b) showed that the genes in the largest 13-gene cluster group together in a gene tree of Komodo dragon V2R genes (Fig. 3). Of the remaining 52 V2R genes, 35 are on scaffolds less than 100 kb in size, so our estimate of V2R clustering is a lower bound due to fragmentation in the genome assembly (Supplementary Table 8). These results support the hypothesis that expansions of V2R genes in multiple squamate reptile lineages arose through tandem gene duplication.

**Positive selection.** To evaluate adaptive protein evolution in the Komodo dragon genome, we tested for positive selection across one-to-one orthologues in squamate reptiles using a branch-site model (Supplementary Table 9). Our analysis revealed 201 genes with signatures of positive selection in Komodo dragons (Supplementary Table 10). Of these, 188 had a one-to-one orthologue in humans, 93 mapped to pathways in the Reactome database and 34 had an annotated functional interaction with at least one other positively selected gene (Supplementary Fig. 2). These 34 genes are enriched for...
12 pathways (false discovery rate <5%), including three related to mitochondrial function, four related to coagulation and five related to immune function (Supplementary Table 11).

Many of the genes under positive selection point towards important adaptations of the Komodo dragon’s mammalian-like cardiovascular and metabolic functions, which are unique among non-varanid ectothermic reptiles. These include mitochondrial function and cellular respiration, haemostasis and the coagulation cascade, and angiotensinogen (Supplementary Table 11 and Supplementary Fig. 2)51. Innate and adaptive immunity genes, which are frequently under positive selection in vertebrates, are well represented among positively selected genes52. Finally, 106 positively selected genes do not have an annotated function and 25% of positively selected genes were not detectably expressed in the heart and likely represent adaptations in other aspects of Komodo dragon biology.

**Positive selection of genes regulating mitochondrial function.** In the Komodo dragon genome, we found evidence of positive selection of electron transport chain components including multiple subunits and assembly factors of the type I NADH dehydrogenase—NDUFA7, NDUFA7, NDUFAF2, NDUFB5—as well as components of the cytochrome c oxidase protein complexes, COX6C and COA5 (Fig. 4, Supplementary Fig. 3 and Supplementary Table 10). We also found signatures of positive

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**Fig. 3 | Gene clusters of V2Rs evolved through gene duplication.** a. Cluster of 13 V2R genes in the Komodo dragon genome. Pink genes are V2R genes and grey genes are non-V2R genes. Gene labels correspond to labels in b. b. Unrooted phylogeny of 129 V2R genes in the Komodo dragon. As most of the genes in this gene cluster group together in a gene phylogeny of all Komodo dragon V2R genes, it is likely that this cluster evolved through gene duplication events. Branches with bootstrap support less than 80 are collapsed. Clades without genes in this V2R gene cluster are collapsed. Genes in the V2R cluster are coloured pink and labelled as in a.
selection for other elements of mitochondrial function in the Komodo dragon lineage (Fig. 4). For example, we detected positive selection for \textit{ACADL}, which encodes a critical enzyme for mitochondrial fatty acid β-oxidation, the major postnatal metabolic process in cardiac myocytes\textsuperscript{35}. Furthermore, two genes that promote mitochondrial biogenesis, \textit{TFB2M} and \textit{PERM1}, have undergone positive selection in the Komodo dragon. \textit{TFB2M} regulates mitochondrial DNA (mtDNA) transcription and dimethylates mitochondrial 12S ribosomal RNA\textsuperscript{54,55}, while \textit{PERM1} regulates the expression of select \textit{PPARGC1A/B} and \textit{ESRRB/B/G} target genes with roles in glucose and lipid metabolism, energy transfer, contractile function, muscle mitochondrial biogenesis and oxidative capacity\textsuperscript{36}. \textit{PERM1} also enhances mitochondrial biogenesis, oxidative capacity and fatigue resistance when overexpressed in mice\textsuperscript{37}. Finally, we observed positive selection of \textit{MDH1}, encoding malate dehydrogenase, which together with mitochondrial \textit{MDH2} regulates the supply of NADPH and acetyl coenzyme A to the cytoplasm, thus modulating fatty acid synthesis\textsuperscript{38}.

Multiple factors regulating mitochondrial translation have also undergone positive selection in the Komodo dragon (Fig. 4). These include four components of the mitochondrial 28S small ribosomal subunit (\textit{MRPS15}, \textit{MRPS23}, \textit{MRPS31} and \textit{AURKAIP1}) and two components of the mitochondrial 39S large ribosomal subunit (\textit{MRPL28} and \textit{MRPL37}). We also found positive selection of \textit{ELAC2} and \textit{TRMT10C}, which are required for maturation of mitochondrial transfer RNA, and \textit{MRM1}, which encodes a mitochondrial rRNA methyltransferase\textsuperscript{39-41}.

Overall, these instances of positive selection in genes encoding proteins important for mitochondrial function could underlie the remarkably high aerobic capacity in the Komodo dragon. Additional genome sequences are needed to determine whether these changes are specific to the Komodo dragon, shared across varanid lizards generally, or found in unsequenced reptiles.

**Positive selection of angiotensinogen.** We detected positive selection for angiotensinogen (\textit{AGT}), which encodes the precursor of several peptide regulators of cardiovascular function, the most well-studied being angiotensin II (AngII) and angiotensin 1-7 (A1-7). AngII has a vasoactive function in blood vessels and inotropic effects on the heart\textsuperscript{42}. In mammals, the level of AngII increases in response...
to intense physical activity, contributing to arterial blood pressure and regional blood regulation\textsuperscript{61-66}. Reptiles have a functional renin–angiotensin system (RAS) that is important for their cardiovascular response to aerobic activity\textsuperscript{77-80}. The positive selection for AGT points to important adaptations in cardiovascular physiology and the renin–angiotensin system in the Komodo dragon.

**Positive selection of haemostasis-related genes.** We found evidence for positive selection across regulators of haemostasis, which reduces blood loss after injury. Four genes that regulate platelet activities, MRVII, RASGRP1, LCP2 and CD63, have undergone positive selection in the Komodo dragon genome. MRVII is involved in inhibiting platelet aggregation\textsuperscript{75}, RASGRP1 coordinates calcium-dependent platelet responses\textsuperscript{81}, LCP2 is involved in platelet activation\textsuperscript{72} and CD63 has a role in controlling platelet spreading\textsuperscript{73}. In addition, two coagulation factors, F10 (factor X) and F13B (coagulation factor XIII B chain) have undergone positive selection in the Komodo dragon genome. Activation of factor X is the first step in initiating coagulation\textsuperscript{75} and factor 13 is the final factor activated in the coagulation cascade\textsuperscript{73}. Further, FGB, which encodes one of the three subunits of fibrinogen, the molecule converted to the clotting agent fibrin\textsuperscript{76}, has undergone positive selection in the Komodo dragon genome.

**Discussion**

We have sequenced and assembled a high-quality genome of the Komodo dragon that will be a template for analysis of other varanid genomes, and for further investigation of genomic innovations in the varanid lineage. We were able to assign 75% of the genome to chromosomes, providing a significant contribution to comparative genomics of squamates and vertebrates generally. As the number of squamate whole-genome sequences continues to grow, there will be opportunities to examine the evolution of noncoding DNA in these reptiles.

Varanid lizards have genotypic sex determination and share ZZ/ZW sex chromosomes with other anguimorphan lizards\textsuperscript{16,18}. Here, we were able to assign genomic scaffolds to the Z chromosome of the Komodo dragon. All Z chromosome scaffolds were homologous to A. carolinensis chromosome 18 and mostly to chicken chromosome 28, in agreement with a recent transcriptome-based analysis\textsuperscript{18}. Within Iguania, the sister group of anguimorphs\textsuperscript{20-24}, there are environmental sex-determination systems without sex chromosomes as well as conserved XX/XY sex chromosomes homologous to anguimorph autosomes\textsuperscript{77-81}. Sex chromosomes in most snakes (pythons and all families of caenophidian snakes\textsuperscript{82}) are homologous to chromosome 6 of A. carolinensis and thus to autosomes of the Komodo dragon, suggesting an independent origin of sex chromosomes in snakes and anguimorphs. However, the ancestral sex determination of snakes remains unresolved\textsuperscript{82-83}. The regions of sex chromosomes shared by the common ancestor of varanids and several other lineages of anguimorph lizards contain the amh gene\textsuperscript{84}, which has a crucial role in vertebrate testis differentiation. Homologues of amh are strong candidates for sex-determining genes in several lineages of teleost fishes and in monotremes\textsuperscript{84-86}, and should be considered candidate sex-determining genes in varanids and other anguimorphs.

Our comparative genomic analysis identified previously undescribed species-specific expansion of V2Rs across multiple squamates, including lizards and at least one snake. Komodo dragons, like other squamates, are known to have a sophisticated lingual–vomeronasal system for chemical sampling of their environment\textsuperscript{87}. This sensory apparatus allows Komodo dragons to perceive environmental chemicals for social and ecological activities, including kin recognition, mate choice\textsuperscript{88-90}, predator avoidance\textsuperscript{91,92} and hunting prey\textsuperscript{93,94}. Komodo dragons are unusual as they adopt different foraging tactics across ontology, with smaller juveniles preferring active foraging for small prey and large adult dragons targeting larger ungulate prey via ambush predation\textsuperscript{95}. However, utilization of the vomeronasal system across ontogeny seems likely, given the exceptional capacity for Komodo dragons of all sizes to locate prey. Future work will be able to explore the role of V2R expansion in the behaviour and ecology of Komodo dragons, including their ability to locate prey at long distances\textsuperscript{96}.

We found evidence for positive selection in the Komodo dragon genome across genes involved in regulating mitochondrial biogenesis, cellular respiration and cardiovascular homeostasis. Komodo dragons and other monitor lizards have a high aerobic capacity and exercise endurance, and our results reveal selective pressures on biochemical pathways that are likely to be the source of this high aerobic capacity. Reptile muscle mitochondria typically oxidize substrates at a much lower rate than mammalian mitochondria, partly based on substrate-type use\textsuperscript{97}. The findings that Komodo dragons experienced selection in several genes encoding mitochondrial enzymes, including one involved in fatty acid metabolism, points towards a more mammalian-like mitochondrial function. Future work on additional varanid species, and other squamate outgroups, will test these hypotheses. Selective pressures acting on these mitochondrial genes in Komodo dragons is consistent with the increased expression of genes associated with oxidative capacity found in pythons after feeding\textsuperscript{98,99}.

In addition, we found positive selection for angiotensinogen, which encodes two potent vasoactive and inotropic peptides with central roles in cardiovascular physiology. In mammals, AngII contributes to the mean arterial blood pressure and to the redistribution of cardiac output\textsuperscript{100-102}. A compelling hypothesis is that these changes to angiotensinogen may be an important component in the ability of the Komodo dragon to rapidly increase blood pressure and cardiac output as required for hunting, extended periods of locomotion including inter-island swimming, and male–male combat during the breeding season. Direct measures of cardiac function have not been made in Komodo dragons, but in other varanid lizards, a large aerobic scope during exercise is associated with a large factorial increase in cardiac output\textsuperscript{103}. Future physiological studies measuring the hemodynamic responses to exercises with respect to AngII expression can test this hypothesis. Giraffes, which have evolved high blood pressure to maintain cardiovascular homeostasis in their elongated bodies, have experienced positive selection on several blood pressure regulators, including the angiotensin-converting enzyme\textsuperscript{104}. It is possible that positive selection in animals with high blood pressures converges on angiotensin regulators. Overall, the evolution of these genes suggests a profoundly different cardiovascular and metabolic profile relative to other squamates, endowing the Komodo dragon with unique physiological properties.

We also found evidence for positive selection across genes that regulate blood clotting. Like other monitor lizards, the saliva of Komodo dragons contains anticoagulants, which is thought to aid in hunting\textsuperscript{105}. During conflict with conspecifics over food, territories or mates, Komodo dragons use their serrate teeth to inflict bite wounds, raising the possibility that these anticoagulants may enter their bloodstream. The extensive positive selection of genes encoding their coagulation system may reflect a selective pressure for Komodo dragons to evade the anticoagulant and hypotensive effects of the saliva of conspecifics. While all monitor lizards tested contain anticoagulants in their saliva, the precise mechanism by which they act varies\textsuperscript{106}. It is possible that different species of monitor lizards evolved adaptations that reflect the diversity of their anticoagulants, or that coevolution has occurred between monitor lizard coagulation systems and anticoagulant saliva. Further, as Komodo dragons have high blood pressure, changes to their coagulation system may reflect increased protection from vascular damage.
Methods
DNA isolation and processing for Bonnie optical mapping. Komodo dragon whole blood was obtained from one of two individuals housed at Zoo Atlanta (Rinca). Samples from the animals at Zoo Atlanta were collected with the approval of the Zoo Atlanta Institutional Animal Care and Use Committee. High-molecular-weight genomic DNA was extracted for genome mapping. Blood was centrifuged at 2,000 × g for 2 min, plasma was removed and the sample was stored at −20 °C. Then, 2.5 μl of blood was embedded in 100 μl agarose gel plugs to give ~7 μg DNA per plug, using the Bio-Rad CHEF Mammalian Genomic DNA Plug Kit (Bio-Rad Laboratories). Plugs were treated with proteinase K overnight at 50 °C. The plugs were then washed, melted and solubilized with Gelase (Epicycle). The purified DNA was subjected to 4h of drop dialysis. DNA concentration was determined using Qubit 2.0 Fluorometer (Life Technologies), and the quality was assessed with pulsed-field gel electrophoresis.

The high-molecular-weight DNA was labelled according to commercial protocols using the IrysPrep Reagent Kit (Bionano Genomics). Specifically, 300 ng of purified genomic DNA was nicked with 7 U nicking endonuclease NSB001 (NEB) at 37 °C for 2h in NEB Buffer 2. The nicked DNA was labelled with a fluorescent-dUTP nucleotide analogue using Taq polymerase (NEB) for 1 h at 72 °C. After labelling, the nicks were repaired with Taq ligase (NEB) in the presence of DPNTs. The backbone of fluorescently labelled DNA was stained with DNA stain (Bionano).

Bionano mapping and assembly. Using the Bionano Irys instrument, automated electrophoresis of the labelled DNA occurred in the nanochannel array of an IrysChip (Bionano Genomics), followed by automated imaging of the linearized DNA. The DNA backbone (outlined by YOYO-1 staining) and locations of fluorescent labels along each molecule were detected using IrysChip's software. The length and set of labels for each molecule defines an individual single-molecule map. Raw Bionano single-molecule maps were de novo assembled into consensus maps using the Bionano IrysSolve assembly pipeline (version 5134) with default settings, with noise values calculated from the 10x Genomics Supernova assembly. The Bionano optical mapping data comprised 80x genome coverage, and the scaffold N50 of the assembly was 1.2 Mb.

DNA processing for 10x Genomics linked-read sequencing. Blood samples from two individuals housed at Zoo Atlanta (Slasher and Rinca) were used. High-molecular-weight genomic DNA extraction, sample indexing and generation of partition barcoded libraries were performed by 10x Genomics according to the Chromium protocol. Genomic DNA was used as input to the Chromium system.

10x Genomics sequencing and assembly. The 10x Genomics barcoded library was sequenced on the Illumina HiSeq2500. A total of 660 million of the raw reads comprising 57x genome coverage were assembled using the company’s Supernova software (version 1.0) with default parameters. Output fasta files of the Supernova genome coverage were assembled using the company’s Supernova software (version 1.0) with default parameters. Output fasta files of the Supernova assembly were generated in pseudohaploid format, which links phased and unphased regions of the assembly into ‘pseudo-haplotype’ scaffolds. This generated an initial assembly with a scaffold N50 length of 10.2 Mb and a contig N50 length of 95 kb.

Oxford Nanopore sequencing. DNA isolated from Slasher was sequenced to 0.75x coverage on an Oxford Nanopore MinIon sequencer following the manufacturer’s instructions. MinKNOW was used for basecalling and output to FASTQ files.

DNA processing for PacBio sequencing. Komodo dragon whole blood collected in EDTA from an individual housed at Reptilandia zoo, Gran Canaria under institutional approval, stored at −20 °C, was used to extract high-molecular-weight DNA for single-molecule real-time sequencing. Extraction was performed using gravity-flow, anion-exchange tips (Qiagen genomic-tip 100 G kit) to a final DNA concentration of 130 ng/μl assessed using a Qubit 2.0 Fluorometer. Size of extracted DNA was determined by a 16h pulse-field gel electrophoresis, which resolved high-molecular-weight fragments from 15 kb to 85 kb. We constructed a PacBio library using the GS-FLX Titanium platform. High-molecular-weight genomic DNA extracted DNA was determined by a 16h pulse-field gel electrophoresis, which was extracted using a Qubit 2.0 Fluorometer. Size of extracted DNA was determined by a 16h pulse-field gel electrophoresis, which resolved high-molecular-weight fragments from 15 kb to 85 kb. We constructed a PacBio library using the GS-FLX Titanium platform.

We also compared the genome organization at the chromosome level among V. komodoensis, A. carolinensis and G. gallus. We determined homology of each V. komodoensis scaffold to A. carolinensis and G. gallus (Acaro2.0) genome generating alignment between genomes with LAST107 and PRR was calculated as ratio of positions on scaffold to positions on chromosome based on distance between positions. Finally, several statistics were calculated for each scaffold. Calculated parameters included: mean pairwise distance between positions on scaffold and number of positions on scaffold, number of positions on scaffold, position representation ratio (PRR) and P value of PRR. PRR of each scaffold was used to evaluate enrichment of given scaffold on chromosomes. PRR was calculated as ratio of positions on scaffold to positions in genome divided by ratio of scaffold length to genome length. PRRs > 1 correspond to enrichment, while PRRs < 1 correspond to depletion. As the PRR value is distributed lognormally, we use its logarithmic form for our calculations. To filter out only statistically significant PRR values, we used thresholds of logPRR > 0 and its P value ≤ 0.01. Scaffolds with logPRR > 0 were considered enriched in the given sample. If one scaffold was enriched in several samples we chose the highest PRR to assign scaffold as top sample.

RNA sequencing. RNA was extracted from heart tissue obtained from an adult male specimen that died of natural causes at the San Diego Zoo. This was approved by the Institutional Animal Care and Use Committee and Biomaterials Review Group of San Diego Zoo. Trizol reagent was used to extract RNA following the manufacturer’s instructions. Two RNA-seq libraries were produced using a NuCea RNA Library Prep kit v2 and 8 μl of RNA were used to construct each library using the TruSeq RNA sample preparation kit. The RNA-seq libraries were then sequenced on an Illumina Nextseq 500 with 150 bp paired-end strand-specific reads.

Genome annotation. RepeatMasker v4.0.7 was used to mask repetitive elements in the Komodo dragon genome using the Squarmania repeat database from the NCBI.
in the Komodo dragon lineage were then tested for positive selection at all nodes in the phylogeny. This resulted in 201 genes being under positive selection in the Komodo dragon lineage (Supplementary Table 10).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The assembled Komodo dragon genome is available in the National Center for Biotechnology Information (NCBI) under the accession SJP/D00000000. All DNA sequencing used to generate the assembly is available in the NCBI Sequence Read Archive (SRA) database under accession PRJNA523222. Illumina sequencing data from chromosome pools is available in the NCBI SRA under accession PRJNA594893. RNA-seq data of heart tissue is available in the NCBI SRA under accession PRJNA527513. Original protein annotations, noncoding RNA annotations, all alignments for phylogenetic analyses and selection analyses, and newick files of phylogenetic trees are available in the following Figshare repositories: https://doi.org/10.6084/m9.figshare.7961135.v1, https://doi.org/10.6084/m9.figshare.7955891.v1, https://doi.org/10.6084/m9.figshare.7955879.v1, https://doi.org/10.6084/m9.figshare.7949483.v1, https://doi.org/10.6084/m9.figshare.7799496.v1, https://doi.org/10.6084/m9.figshare.7967300. The project folder for all Figshare data is available at https://figshare.com/projects/Data_for_Komodo_dragon_genome_paper/61271.

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Author contributions
A.L.L. did genome annotation and all comparative genomics analyses. Y.Y.Y.L. led the sequencing and assembly efforts with Y.M. and A.C.Y.M. A.K.H. led the initial development of the project. A.L. sequenced isolated chromosomes with M.R., M.J.P. and M.A. under supervision of L.K., and assigned sequences with A.I.M., I.G.K. and V.A.T. M.F. and V.O. contributed to genome assembly in the lab of R.F. with C.C. W.L.E. initially assembled the transcriptomes and annotated the genome. M.M. and M.F. isolated samples and obtained PacBio sequence in the lab of T.P. and C.C. E.S. performed PacBio sequencing. P.V. and I.R. provided embryos for cell line establishment. O.A.R. provided frozen tissue samples. J.R.M. collected specimen blood.

Competing interests
The authors declare no competing interests.

Additional information
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

No software was used for data collection.

Data analysis

Bionano IrysSolve assembler version 5134, 10x Genomics Supernova assembler v1.0, SSPACE-LongRead, dopseq, cupadapt v1.13, pybedtools v0.7.10, LAST, RepeatMasker, STAR version 2.6.0, Trinity version 2.4.0, exonerate version 2.2.0, MAKER version 3.01.02, SNAP, Augustus version 3.1.1, InterProScan version 5.31.70, OrthoFinder version 2.0.0, trRNAscan-SE version 1.3.1, Infernal software suite, PRANK v.170427, IQ-TREE version 1.6.7.1, CAFE v4.2, Protein-Protein BLAST 2.6.0+, TMHMM v2.0, MAFFT v7.310, trimAL, PAL2NAL, HYPHY, aBSREL, EMBOS needle version 6.6.0.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The assembled Komodo dragon genome is available in NCBI under the accession SJPD00000000. All DNA sequencing used to generate the assembly is available in the NCBI SRA database under accession PRJNA523222. Illumina sequencing data from chromosome pools is available in the NCBI SRA under accession PRJNA529483. RNA-seq data of heart tissue is available in the NCBI SRA under accession PRJNA527313. Original protein annotations, noncoding RNA annotations, all alignments for phylogenetic analyses and selection analyses, and newick files of phylogenetic trees are available in the following Figshare repositories: doi:10.6084/m9.figshare.7961135.v1, doi:10.6084/m9.figshare.7955891.v1, doi:10.6084/m9.figshare.7955879.v1, doi:10.6084/m9.figshare.7949483.v1, doi:10.6084/m9.figshare.7759496.v1, doi:10.6084/m9.figshare.7967300. The project folder for all Figshare data is available at https://figshare.com/projects/
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | The comparative genomics work in this study was performed using single genomes from individual species, so no sample size calculations were performed. |
| Data exclusions | No data were excluded. |
| Replication | No biological experiments were performed in this work that could be replicated. |
| Randomization | Randomization is not relevant to this paper, as the main hypotheses in this work refer to individual species. |
| Blinding | No blinding was possible for analyses carried out in this study as hypotheses were tested on single genomic sequences. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
| n/a | Involved in the study |
| - | [ ] Antibodies |
| - | [ ] Eukaryotic cell lines |
| - | [ ] Palaeontology |
| - | [ ] Animals and other organisms |
| - | [ ] Human research participants |
| - | [ ] Clinical data |
| n/a | Involved in the study |
| - | [ ] ChiP-seq |
| - | [ ] Flow cytometry |
| - | [ ] MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Varanus Komodoensis, ages 20 and 6, house at Zoo Atlanta, one individual at Reptilandia zoo in Gran Canaria in the Canary Islands, and one deceased individual (tissue previously collected) and San Diego Zoo. All maintained under ambient conditions. |
| Wild animals | No wild animals |
| Field-collected samples | No field collected samples |
| Ethics oversight | Zoo Atlantata IACUC |

Note that full information on the approval of the study protocol must also be provided in the manuscript.