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Anatomy, Histology, Aetiology, Development and Functions of Cartilago Cordis: A Systematic Review

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Abstract
Introduction: The cartilago cordis is a structure present within the cardiac skeleton of some, but not all, vertebrate species. This systematic review compared the presence, structure, and function of the cartilago cordis from published works covering all vertebrate species. ***Methods:*** Literature searches were conducted to obtain information relating to the anatomical location, morphology, prevalence, number of structures, development, and function. ***Results:*** The cartilago cordis was most commonly composed of hyaline cartilage but its location within the cardiac skeleton, anatomical, and histological structure varied between species. The cartilago cordis has not been documented in every vertebrate species, or every individual within each species, but it is present in 68 vertebrates including an amphibian, and some mammals, reptiles, and birds. The function of the cartilago cordis is unknown, but theories have ranged from an adaptive mechanism to support cardiac tissue through to roles in conduction and contraction, especially in areas of high mechanical stress. Possible links between the presence of a cartilago cordis and cardiac pathologies were also identified. ***Conclusion:*** The cartilago cordis varied in prevalence, structure, and location; further research is required to understand the function and development. In addition, it is possible there are more vertebrate species containing cartilago cordis than presently known about given its varying prevalence and sometimes small size.

Introduction

Despite historical awareness of its existence, there has been little dedicated research into the cartilago cordis, cartilage located specifically within the right and left trigones (*trigonum fibrosum dextrum et sinistrum*) of the

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heart. Cardiac cartilage also occurs in many other forms, commonly within valves [1], especially in birds [2, 3] and hamsters [4–6] and in the bulbus arteriosus of some fish [7, 8]. Cartilage has also been shown throughout the rest of the heart including the atrial [9] and ventricular walls, and papillary muscles in differing species occurring naturally or in disease states [10–12].

To be defined as a cartilago cordis, the structure(s) must be cartilage positioned within the cardiac skeleton [13–16] and does not include individual cartilage cells or structures within the rest of the heart including the atria, ventricles, or other chambers in lower taxa, blood vessels, suspensory ligaments, or valves. The cardiac skeleton (fibrous skeleton) is a section of fibrous connective tissue separating the atria and ventricles that supports the cardiac valves and resists narrowing of the aorta during contraction [13]. In mammals and birds, the cardiac skeleton has three main components: the *anuli fibrosi*, the right and left fibrous trigones, and the membranous septum (part of the ventricular septum [*septum ventriculorum*]) [17–19]. The fibrous trigones are triangular sections of dense connective tissue separating the aorta and mitral valve, connected by the aortic mitral curtain [19]. The membranous septum is divided into atrioventricular (AV) and interventricular sections, and the central fibrous body is formed from both the right fibrous trigone and this membranous septum [18]. In reptiles, amphibians and fish the various components of the cardiac skeleton often vary greatly between species and even individuals within species due to differing heart morphology. The reptilian heart generally has three chambers (two atria and a common ventricle) instead of the mammalian and avian four chambers (with a full ventricular septa separating the two distinct ventricles) [20], although some suggest that the sinus venosus constitutes a fourth chamber in reptiles [21]. Crocodiles, alligators, and close relatives differ as they tend to have complete have ventricular septation compared to lizards, snakes, and chelonians, which have varying degrees of incomplete ventricular septa or ridges [20, 22, 23]. There are also several other differences in reptiles such as the relative size and histological composition of each anatomical region, and in vascular components and anatomical locations. Commonly, the cartilago cordis in reptiles is within the aorticopulmonary septum and the *pars fibrosa* of the horizontal septum but can be within the connective tissue septum connecting the aortic valves to the aortae and other areas of the cardiac skeleton, as discussed in this review below. The amphibian cardiac skeleton was first described in the greater siren (*Siren lacertina*) [24]. The posterior end of the cardiac skeleton was located in the posterior end of the longitudinal septum of the sinus venosus and continued into the longitudinal septum, with a section extending from the ventricular wall epicardium connecting to the cardiac ligament. The cardiac skeleton then extended beyond the sinuatrial aperture, along the ventral walls of the atrial chambers, to the AV aperture – these apertures were themselves surrounded by connective tissue which connected into the skeleton [24]. The skeleton then extended through the ventricular muscle, connecting with the conal aperture, then through the conus arteriosus and truncus arteriosus. Notably, these latter two structures also have connective tissue within them which is not classified as cardiac skeleton. The anterior terminus of the cardiac skeleton was located at the pericardial cavity near the aortic arches [24]. Fish generally have six main components to their hearts, the sinus venosus, atrium, AV canal, ventricle, conus arteriosus, and the bulbus arteriosus, and there are also valves between the chambers associated vasculature [25]. There is connective tissue in the fish heart but this tends to be concentrated in and around the valves. Connective tissue is also present as thin epicardial associated layers in some tissues, and the conus arteriosus consists of vascularized myocardium, which in the teleost was noted as being rich in collagen fibres [26].

This systematic review describes the prevalence and presentation, structure, shape, size, and histology of cartilago cordis in differing vertebrate species. This review also details the research and theories surrounding the development and functions of cartilago cordis. The cartilago cordis differs from the ossa cordis which is a rare bone located within the same anatomical region [27] as the cartilago cordis. Our previous systematic review investigated the 19 species with an ossa cordis. Therefore, the present review also gives some insights into how the 68 species with cartilago cordis relate to those with ossa cordis and discusses the endochondrial ossification ossa cordis theory. Both cartilago cordis and ossa cordis have been reported as physiologically normal structures in many species, yet both have been reported in relation to pathological situations too. Therefore, pathological cases are presented and discussed in addition to physiologically normally situations.

Methods

In total, 53 search terms (including cartilage, heart, cartilago cordis, cardiac, myocardium, trigone) were used within three databases: PubMed Central, PudMed, and Web of Science. Additionally, further literature was found via these publications and general searches were undertaken in Google Scholar and via Google searches. References were included if they were (a) original

research papers, dissertations or books containing novel research, (b) peer-reviewed, (c) in English, French, Spanish, or German, (d) published on any date, and (f) cartilage was present specifically in the cardiac skeleton.

The limitations of this systematic review include:

1. Variations between regarding publications age, number, and sex of specimens, and the prevalence, anatomical location and histology of cartilago cordis in vertebrate species.
2. The databases were unlikely to contain every publication on cartilago cordis, especially older references, using several databases plus general searches helped mitigate this.
3. Full text was not available for some papers, especially older ones. Full texts were retrieved via The British Library where required and if the full text was not available this was specified.
4. Cartilage within the cardiac skeleton is not always referred to as cartilago cordis in the published literature. This was mitigated by searching for numerous search terms including cartilage, cartilaginous foci, chondrocytes, cartilago cordis, and cartilaginous metaplasia in individually analysing each publication for the presence of cartilago cordis.

The resulting publications are presenting as a PRISMA flow diagram [28] (Fig. 1) and PRISMA checklist (online suppl. Information; for all online suppl. material, see https://doi.org/10.1159/000544776). This systematic review is written in English but Latin names for each species and pertinent anatomical structures are provided upon first use, except where the generally used name is the Latin term according to anatomical nomenclature followed the *Nomina Anatomica Veterinaria* [29]. Given the range of publication dates, the Latin and common names for species and structures names varied, therefore the presently accepted names and terms are used throughout except where specified. Phylogenetic trees were created using iTOL Interactive Tree of Life (https://itol.embl.de/) and PhyloT v2 (https://phylot.biobyte.de/).

Results

Anatomical Presentation and Prevalence of Cartilago Cordis in Different Vertebrate Species

An overview of each of the 68 vertebrate species presenting with a cartilago cordis is presented below subdivided into mammals, birds, reptiles, amphibians, and fish. Furthermore, the prevalence, numbers of specimens, sex, and ages for each species are tabulated in Table 1 (mammals) and Table 2 (birds, reptiles, and amphibians). Cartilago cordis size (Table 3), shape (Table 4), and anatomical position (Table 5) are also presented for each species. Figure 2 and online supplementary Figures 1–3 exhibit the phylogeny tree of species exhibiting cartilago cardis. The overview of each vertebrate species presents both presumed physiology cartilago cordis and pathology/disease associated cases. Following the general overview in each species, there are sections providing insights into cartilago cordis relating to sex, number, size and shape, histological composition, formation and development, functions and then pathological and disease status.

Mammalia

Monotremata

A case report on the platypus (*Ornithorhynchus anatinus*) heart made reference to a cartilago cordis beside the atrioventricular node (AVN) and bundle [30]; however, no other details were provided on this structure.

Primates

In people (*Homo sapiens*), there have been published cases, for example, myxoid and cartilaginous tissue had displaced and/or replace portions of the AV node and bundle of His in a sudden death case of a newborn child [33]. Similarly, a 40-week gestational age stillborn child had cartilaginous metaplasia of the trigone which had displaced and replaced portions of the bundle of His, and the cartilaginous metaplasia had thickened the fibrous ring (*fibrous annulus*) [33]. Likewise other cases have shown fibrocartilage nodules in the central fibrous body of infants [35], in 4.16% of explained infant death cases (n = 4/69) and 5.79% of crib death cases (n = 1/24) [34], and in 20% (n = 3/15) of sudden unexplained infant death (SUID) and in 18% (n = 1/11) of intrauterine explained deaths [32]. A myxoid-cartilaginous formation, approximately 2 cm in diameter, was found in a 10-year-old and a prechondroid area in a myxoid stroma was present 23-week gestation foetus [31]. The authors highlighted their locations as equivalent to where bovine ossa cordis are present, lower and to the right of the trigone, and further noted that in the adults studied the tissue appeared tendinous. As this was the first time cartilaginous/bone structures had explored in relation to the tendinous type tissue within this location, the authors named the structure the fulcrum [31]. The absence of cartilago cordis was also specifically stated in ten healthy adult males [36]. In 1 chimpanzee (*Pan troglodytes*), a single cartilago cordis (shown in Fig. 3a–d) was present on the right side of the fibrous trigone. Notably, three different hearts had ossa cordis and cartilage was present associated with the

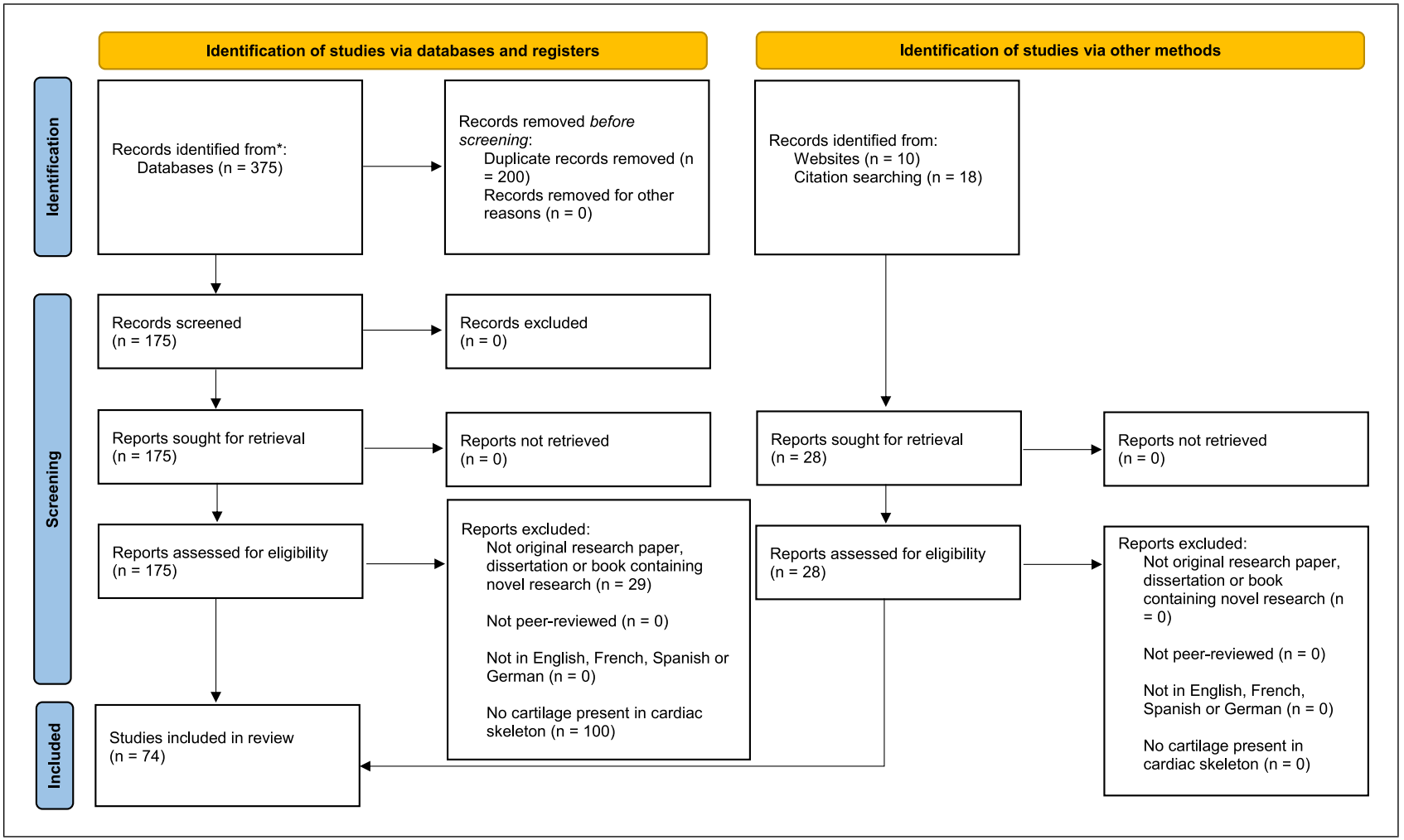


Fig. 1. PRISMA schematic. PRISMA analysis based on published methodology [25].

Table 1. Prevalence, ages, and sex of specimens with cartilago cordis in mammals

Species	Age	Sex	No specimens with cartilago cordis/% prevalence	References
Mammals				
Platypus (*Ornithorhynchus anatinus*)	–	–	1/1 (100%)	[30]
Human (*Homo sapiens*)	23 weeks gest	–	1/1 (100%)	[31]
	25–41 weeks gest	M + F	2/11 (18.18%)	[32]
	25–41 weeks gest	M + F	3/15 (20%)	[32]
	40 weeks gest	M	1/1 (100%)	[33]
	Newborn	M + F	4/69 (4.16%)	[34]
	3–365 days	M + F	1/24 (5.79%)	[34]
	3–365 days	M	1/1 (100%)	[35]
	6 months	F	1/1 (100%)	[35]
	2 years	–	1/1 (100%)	[31]
	10 years	–	1/1 (100%)	[31]
	20–60 years	M	0/10 (0%)	[36]
Chimpanzee (*Pan troglodytes*)	10–59 years (pathology)	M + F	1/16 (6%)	[15, 37]
Rabbit (*Oryctolagus cuniculus*)	–	M + F	–/50	[38]
	–	M + F	9/11 (82%)	[39]
Chinchillas (*Chinchilla lanigera*)	9 months	16M 14F	30/30 (100%)	[40]
Hamster (*Mesocricetus auratus*)	0–708 days	M + F	124/576 (22%)[a]	[17]
	103–843 days	M + F	80/80 (100%)	[41]
White rat (*Rattus norvegicus albus*)	–	–	100/3,000[b] (3%)[b]	[42]
Brown rat (*Rattus norvegicus*)	–	M + F	–/– (100%)	[39]
Mouse (*Mus musculus*)	–	–	15/1,000[b] (2%)[b]	[42]
White rhinoceros (*Ceratotherium simum*)	–	–	1/1 (100%)	[14]
Horse (*Equus caballus*)	2.5–3.5 years	M	7/9 (77.8%)	[36]
	–	M	1/1 (100%)	[39]
Donkey (*Equus asinus*)	Mixed ages	M + F	14/14 (100%)	[43]
Pig (*Sus scrofa domesticus*)	5 months (average)	M	4/8 (50%)	[36]
	Adult	M + F	6/6 (100%)	[44]
Nubian Giraffe (*Giraffa camelopardalis*)	–	–	1/1 (100%)	[45]
Siberian roe deer (*Capreolus pygargus*)	–	M	1/1 (100%)	[39]
Egyptian water buffalo (*Bubalus bubalis*)	1 month-5 years	M + F	1/15 (7%)	[46]
Goat (*Capra hircus*)	–	–	–/38	[47]
Spotted hyena (*Crocuta Crocuta*)	1 month	M	2/2 (100%)	[48]
Leopard cat (*Prionailurus bengalensis*)		M	1/1 (100%)	[48]
Cat (*Felis catus*)	Adults (pathology)	–	58/63 (92.1%)	[49]
Lion (*Panthera leo*)	–	M	1/1 (100%)	[39]
	Juvenile (10 days)	M	1/1 (100%)	[48]

Table 1 (continued)

Species	Age	Sex	No specimens with cartilago cordis/% prevalence	References
Leopard (*Panthera pardus*)	Juvenile (3 months)	F	1/1 (100%)	[48]
Tiger (*Panthera tigris*)	–	0	3/3 (100%)	[48]
Sea lion (*Eumetopias jubatus*)	Pup Sub-adult Adult	M M 1F5M	1/2 (50%) 0/3 (0%) 3/6 (50%)	[50]
Racoon (*Procyon lotor*)	–	–	1/1 (100%)	[48]
Otter (*Lutra lutra*)	– <1 year 1–2 years 2+ years	M + F M + F M + F M + F	2/5 3/3 9/9 8/11	[51]
Japanese weasel (*Mustela itatsi*)	–	M	1/2 (50%)	[39]
Red and silver fox (*Vulpes vulpes*)	–	–	2/2 (100%)	[48]
African wild dog (*Lycaon pictus*)	3 years	F	1/1 (100%)	[48]
Dog (*Canis lupus familiaris*)	2.5 months-10 years (pathology) 5–12 years 5+6 years	– M M	39/40 (97.5%) 3/5 (60%) 2/2 (100%)	[52] [36] [53]
Wolf (*Canis lupus*)	1.5 years	F	1/1 (100%)	[48]

The Matumoto paper specified the samples were not embryonic but did not otherwise state ages [39]. –, Not stated in publication. [a]Numbers were difficult to obtain due to presentation of results. Inferred numbers shown. [b]Approximate figure.

ossa cordis (shown in Fig. 3e–g). All 4 chimpanzees (from 16 sampled) had high levels of idiopathic myocardial fibrosis [15, 37].

Lagomorpha

In the rabbit (*Oryctolagus cuniculus*), the cartilago cordis was described at the border between the ventricular and anterior chambers, at the end of the atrial septum. The study using 50 rabbits showed histologically it was cartilage but did not specify prevalence [38]. Another study in the European rabbit showed 9/11 (males and females) had a cartilago cordis in one (usually the right) or both trigones [39].

Rodentia

Chinchillas (*Chinchilla lanigera*) had cartilago cordis in the left AV valve and left AV opening of all 30 hearts [40]. In Syrian hamsters (*Mesocricetus auratus*), a cartilago cordis was present in 118 of 472 (25%) hearts, plus six of the 118 stained using different methods, all within the right trigone and/or in the membranous septum (within the ventricular septum) [17]. Usually single cartilaginous foci were observed in the individuals with a cartilago cordis, with two being an anomaly found in only 1 hamster from 472, and calcification was observed in 23% of the cartilago cordis [17]. In contrast, cartilago cordis was observed in all 70 males and all 10 females (100%) in an earlier study, and cartilage has also been found in the fibrous tissue surrounding the aortic orifice in some Syrian hamsters [41] and in 11.4% of 351 individuals in the pulmonary valve [5]. Two cartilago cordis were located near the aortic ring in both white rats and mice [42], and a cartilago cordis was present in the right trigones of male and female white rats (*Rattus norvegicus albus*) but not male brown rats (*Rattus norvegicus*) [39]. In mice (*Mus musculus*), fibrocartilage cartilaginous foci were reported within the AV valves [71] and cardiac cartilage was present inbred strain R-Amsterdam rats aged 1 day-132 weeks ($n = 34$), but these were also not classified as cartilago cordis [72].

Perissodactyla

Cartilago cordis has been discovered in a large Perissodactyla, the white rhinoceros (*Ceratotherium simum*), only a single case was investigated one cartilago cordis

Table 2. Prevalence, ages, and sex of specimens with cartilago cordis in birds, reptiles and amphibians

	Age	Sex	No specimens with Cartilago cordis/% prevalence	Reference
Birds				
Quail (*Coturnix coturnix*)[a]	Sub-adult ≤7 days Adult	– – –	0/12 (0%) 10/10 (100%)	[2]
Chicken (*Gallus gallus*)[a]	Sub-adult ≤7 days Adult	– – –	0/9 (0%) 28/28 (100%) –/15	[2] [2] [54]
Wild turkey (*Meleagris gallopavo*)	–	–	1/1 (100%)	[39]
Ryukyu Robin (*Larvivora komadori*)	–	–	1/1 (100%)	[39]
Daurian redstart (*Phoenicurus auroreus*)	–	M + F	1/2 (50%)	[39]
Java sparrow (*Lonchura oryzivora*)	–	M	1/2 (50%)	[39]
Brambling (*Fringilla montifringilla*)	–	M	1/2 (50%)	[39]
Japanese grosbeck (*Eophona personata*)	– –	M F	1/1 (100%) 1/2 (50%)	[39] [39]
Grey-capped greenfinch (*Chloris sinica ussuriensis*)	–	–	1/1 (100%)	[39]
Yellow-breasted bunting (*Emberiza aureola*)	–	–	1/1 (100%)	[39]
Tristram's bunting (*Emberiza tristrami*)	–	M	1/1 (100%)	[39]
Chestnut bunting (*Emberiza rutile*)	–	M + F	1/2 (100%)	[39]
Reptiles				
Tuatara (*Sphenodon punctatus*)	–	–	1/1 (100%)	[55]
Iguana (*Iguana iguana*)	– Adult	– M + F	5/– 7/7 (100%)	[55] [56]
Snakes (42 species): 11 with cartilago cordis = Mexican python (*Loxocemus bicolor*), Calabar python (*Calabaria reinhardtii*), Green tree python (*Morelia viridis*), Ball python (*Python regius*), Western blind snake (*Rena humilis*), Jamaican blind snake (*Typhlops jamaicensis*), Schlegel's beaked blind snake (*Afrotyphlops schlegelii*), King cobra (*Ophiophagus Hannah*), Russell's viper (*Daboia russelii*), Puff adder (*Bitis arietans*), Sri Lankan green vine snake (*Ahaetulla nasuta*)	–	–	11/42 species (26%), $n = 58$ specimens	[16]
Little file snake (*Acrochordus granulatus*)	–	–	–	[57]
Brahminy blind snake (*Indotyphlops braminus*)	–	–	6/–	[58]
Eastern racer (*Coluber constrictor*)	–	–	–	[59]
Green sea turtle (*Chelonia mydas*)	Juvenile	–	11/11 (100%)	[60]
Red-eared turtle (*Trachemys scripta elegans*)	12 years	F	1/1 (100%)	[61]
Chinese pond turtle (*Mauremys reevesii*)	–	–	–	[62]
Japanese pond turtle (*Mauremys japonica*)	–	–	–	[62]
Spanish terrapin (*Mauremys* leprosa)	Embryo 3 months–10 years	– –	~23/23 (100%) 8/8 (100%)	[63] [63]

Table 2 (continued)

	Age	Sex	No specimens with Cartilago cordis/% prevalence	Reference
Mugger crocodile (*Crocodylus palustris*)	–	–	–	[64]
Nile crocodile (*Crocodylus niloticus*)	–	–	21/21 (100%)	[65]
American alligator (*Alligator mississippiensis*)	–	–	–	[64]
Spectacled caiman (*Caiman crocodilus*)	–	–	–	[64]
Amphibians				
Korean salamander (*Hynobius leechii*)	–	M + F	14/14 (100%)	[39]

–, Not stated in publication. ~, Chondrogenesis observed. [a]Not an independent cartilago cordis-extends from valves. The Matumoto paper [39] specified the samples were not embryonic but did not otherwise state ages, and the Singh paper [54] did not specify age but noted about the birds being "old age."

was present and occupied a small part of the right trigone [14]. Cardiac cartilage in the horse (*Equus caballus*) has been found consistently in individuals over 5 years of age in close proximity to the septa of the heart [66, 67]. Buergelt [68] additionally noted it was usually a focal cartilaginous structure, mineralized in older horses, normally located in the left of the heart at the transition from the upper ventricle into the atrium. The cardiac cartilage in horses is usually described as three structures, the cartilago cordis septalis, cartilago cordis sinistra, and cartilago cordis accessoria. A cartilago cordis sinistra was regularly found in horses older than 5 years and an accessory cardiac cartilage was also found in some horses older than 7 years of age, but no specific lengths or positions were noted. In another equine study, seven of the nine (77.8%) individuals presented with cartilago cordis in the central fibrous body [36]. Cartilage was also found in a male horse running from the right trigone to the left AV orifice (ostium atrioventriculare sinistrum), but there was no cartilage in the left trigone [39]. The donkey (*Equus asinus*) presented with two cartilago cordis, one located in the right fibrous trigone and one in the left fibrous trigone, in all 14 donkeys of mixed but unspecified ages, in both sexes, in healthy hearts [43].

Artiodactyla

In a study which examined 8 pig (*Sus scrofa domesticus*) hearts, three contained "cartilaginous metaplasia" in the cardiac fibrous skeleton which resided in/near the AVN [36]. Another study in male and female adult pigs (n = 3/sex) showed all of them had cartilago cordis [44]. An early thesis by Daasch indicated the presence of cartilago cordis in pigs at an early age; however, ossa cordis resided in the same anatomical location in older animals [73]. A case study of 1 giraffe (*Giraffa camelopardalis*) showed the presence of single cartilago cordis. It was positioned in the left fibrous trigone which is more unusual for this structure, but notably an ossa cordis was located within the right trigone [45]. Related to this, sheep (*Ovis aries*), camels (*Camelus dromedarius*), and cattle (*Bos taurus*) did have cartilage in the trigone, but notably this was only in association with ossa cordis (within or directly attached to the bone) and were therefore not classified as cartilago cordis [9, 13, 47, 69, 74–76] (cattle shown in Fig. 4a, b, camel shown in Fig. 4c–f). The Siberian roe deer (*Capreolus pygargus*) had an irregularly square, flattened cartilage-bone plate extending from the right trigone to the ventricular septum [39]. Cartilago cordis was discovered in the central fibrous body of the young Egyptian water buffalo (*Bubalus bubalis*) aged 30 days old (n = 1) whereas ossa cordis were present in the animals aged 3–5 years (n = 14) [46]. Another buffalo study indicated the presence of cartilage surrounding ossa cordis but no cartilago cordis [77]. In a general cardiovascular anatomical study containing 38 goats (*Capra hircus*), cartilago cordis was present in addition to a separate ossa cordis in the annulus fibrosus around the *ostium aortae* [47].

Carnivora

Palpable cardiac cartilage, described as small, was present in the AV septum in two specimens of the Hyaenidae family, in the spotted hyena (*Crocuta crocuta*),

Table 3. Cartilago cordis sizes in differing species

Species	Size	References
Mammals		
Chimpanzee (*Pan troglodytes*)	1.5 mm	[15, 37]
Rabbit (*Oryctolagus cuniculus*)	Varied between 1.67 × 0.8 mm and 0.32 × 0.25 mm	[39]
White rat (*Rattus norvegicus albus*)	Varied between 0.11 × 0.07 and 0.85 × 0.13 mm	[42]
Horse (*Equus caballus*)	25–35 mm in length, cherry-sized cartilago cordis (in the left trigone) with apple-seed sized accessory cardiac cartilage, and 2683.9 × 1109.5 μm[a,b]	[36, 66–68]
Pig (*Sus scrofa domesticus*)	2,293.3 × 735.2 μm[a,b]	[36]
Siberian roe deer (*Capreolus pygargus*)	4.2 × 3.8 × 1.0 mm	[39]
Leopard cat (*Prionailurus bengalensis*)	1.28 × 0.56 mm	[39]
Lion (*Panthera leo*)	2.519 × 0.963 mm (right trigone), 2.148 × 0.815 mm (left)	[39]
Sea lion (*Eumetopias jubatus*)	Pup 0.5 mm × 2.7 mm × 0.2 mm. Adult #1 = 0.6 × 2.5 × 0.2 mm and 0.5 × 1.6 × 0.3 mm, #2 = 1.3 × 3.5 × 0.9 mm and 1.5 × 1.3 × 0.2 mm, #3 = 5 × 3.5 × 0.87 mm	[50]
Japanese weasel (*Mustela itatsi*)	0.35 × 0.34 mm	[39]
Dog (*Canis lupus familiaris*)/	885.3 × 686 μm[a,b]	[36]
Birds		
Daurian redstart (*Phoenicurus auroreus*)	15 × 0.13 mm	[39]
Java sparrow (*Lonchura oryzivora*)	0.09 × 0.074 mm (right trigone), 0.186 × 0.18 mm (left)	[39]
Brambling (*Fringilla montifringilla*)	0.111 × 0.07 mm	[39]
Japanese grosbeck (*Eophona personata*)	Small	[39]
Grey-capped greenfinch (*Chloris sinica ussuriensis*)	0.23 × 0.186 mm (right trigone), 0.372 × 0.05 mm (left)	[39]
Yellow-breasted bunting (*Emberiza aureola*)	Small	[39]
Tristram's bunting (*Emberiza tristrami*)	0.195 × 0.13 mm	[39]
Chestnut bunting (*Emberiza rutile*)	0.24 × 0.074 mm (right trigone), 0.285 × 0.186 mm (left)	[39]
Reptiles		
Snake (general: Mexican python (*Loxocemus bicolor*), Calabar python (*Calabaria reinhardtii*), Green tree python (*Morelia viridis*), Ball python (*Python regius*), Western blind snake (*Rena humilis*), Jamaican blind snake (*Typhlops jamaicensis*), Schlegel's beaked blind snake (*Afrotyphlops schlegelii*), King cobra (*Ophiophagus Hannah*), Russell's viper (*Daboia russelii*), Puff adder (*Bitis arietans*), Sri Lankan green vine snake (*Ahaetulla nasuta*)	Varied 28–574 μm cranial-caudal length	[16]
Little file snake (*Acrochordus granulatus*)	195 μm	[57]
American alligator (*Alligator mississippiensis*)	Three processes of cartilage: 6 mm, 4 mm and 8 mm long	[64]
Amphibians		
Korean salamander (*Hynobius leechii*)	Varied between 0.167 × 0.111 mm and 0.32 × 0.14 mm	[39]
Estimates based on publication figures		
Syrian hamsters (*Mesocricetus auratus*)	300 μm	[17]
Otter (*Lutra lutra*)	1–3 mm	[51]
Spanish terrapin (*Mauremys* leprosa)	500 μm–1.25 mm (increasing with age)	[63]

[a]Measured from photographs. [b]Average. Not all species names were given in publications.

both were males and 1 month old [48]. In a male leopard cat (*Prionailurus bengalensis*), it was found in the right trigone [39] and in cats (*Felis catus*), 58 hearts from 63 (92.1%) individuals had a cartilago cordis located in the central fibrous body [49]. A male lion (*Panthera leo*) had two pieces in the right trigone [39], and it was present in a juvenile male lion aged 10 days old [48]. A female juvenile (3 months old) leopard (*Panthera pardus*) [48], and 3 cases of larger tiger hearts (*Panthera tigris*) also had a cartilago cordis [39].

The sea lion (*Eumetopias jubatus*) had an ossa cordis in one specimen (n = 1/11) and cartilago cordis in four other specimens, located in the right trigones of the heart [50]. In the Musteloidea superfamily, a racoon (*Procyon lotor*) of unknown sex presented with a cartilago cordis [48]. In otters (*Lutra lutra*), the presence of interatrial cartilage, measuring a few millimetres in size, in 3 of the 10 animals was described [79]. A published study also exhibited between one and three cartilage structures per heart, mainly in the fibrous trigones [51]. Ossa cordis and/or early ossification were frequently found in these hearts too in both the animals with and without cartilage. In the Japanese weasel (*Mustela itatsi*), there was a cartilago cordis in the right trigone in one male but not another [39].

Cartilago cordis was present in single cases of the red fox and silver (albino) fox (*Vulpes vulpes*) of unknown sex, a female African wild dog (*Lycaon pictus*) aged 3 years old, and a female wolf (*Canis lupus*), aged about 1.5 years, [48]. In the domesticated dog (*Canis lupus familiaris*), a *trigonum cartilagineum dextrum* and a cartilago cordis septalis have been described within the heart skeleton [66, 67] and a cartilago cordis was located in the central fibrous body of three of five (60%) individuals [36]. Prevalence of a "chondroid metaplasia" within the central fibrous body was 39 of 40 (97.5%) dogs in large breed study [52]. In addition, eight of these 39 (20.5%) hearts had a round mass of cartilage with bone formation and a hollow centre filled with adipose tissue, also within the central fibrous body, a potential ossa cordis [52]. Two Doberman Pinschers who had sudden unexpected death also had cartilaginous cells within the central fibrous body, near to the ossa cordis [53].

Aves

In adult and embryonic quails (*Coturnix coturnix*) and chickens (*Gallus gallus*), cartilage was frequently observed, but it is more appropriate to say this was located in their aortic and pulmonary valves and surrounding tissue including the roots and sinuses [2]. In 71% of these adult chickens and 40% of the adult quails, the cartilage extended from attachments of the aortic leaflets (into supporting sinuses) of the aorta's semi-lunar valve. It should be noted that these were mostly cartilage in the valve, possibly extending into the surrounding tissue rather than a specific cartilago cordis entirely based within the cardiac skeleton. Cardiac cartilage was also stated to be within the cardiac skeleton and aortic ring in chickens, although frequency and age of the birds were not stated [3, 54] and another studying the Japanese silk hen stated it was present from embryonic day 10 into adults [80]. In vivo and in vitro studies in quail-to-chick proepicardial chimeras also indicated cardiac cartilage clusters from proepicardial cells [81]. It is important to note that this study stated previous research showing a cardiac neural crest origin [3] yet show cardiac cartilage clusters arising from proepicardial cells [81]. It is thought that the proepicardium derives from the lateral plate mesoderm and not the neural crest [82]. Therefore, this research indicating proepicardial cells may also play a role in cartilage development [81] is an important addition to the field.

Matumoto [39] investigated a large number of avian species, often without stating numbers or sex, and the following birds had a cartilago cordis specifically within the left trigone only. The wild turkey (*Meleagris gallopavo,* male), Ryukyu Robin (*Larvivora komadori*), Daurian redstart (*Phoenicurus auroreus*), and the yellow-breasted bunting (*Emberiza aureola*), in the female specimen, but not the male, and the Japanese grosbeck (*Eophona personata*, male). In contrast some birds had a cartilago cordis within the right trigone only [39]. In the brambling (*Fringilla montifringilla*), one male of two had one as did a male Tristram's bunting (*Emberiza tristrami*). Some birds in the Matumoto study [39] had cartilago cordis in both the left and right trigones. In the Java sparrow (*Lonchura oryzivora*) one male had one in both the left and right trigones, these were not present in the other specimen. The Japanese grosbeak had one female with a cartilago cordis in both the left and right trigones, whilst it was absent in the other specimen, notable this differed from the anatomical locations observed the male. In the grey-capped greenfinch (*Chloris sinica ussuriensis*) one specimen had one in both trigones, and a male chestnut bunting (*Emberiza rutila*) had them in both trigones, neither were present in the female specimen [39].

The following birds described by Matumoto [39] had cartilage extending from the aortic wall predominantly but extending into the left trigone so not classified as a true cartilago cordis. The grey heron (*Ardea cinerea (jouyi)*), domestic goose (*Anser anser*), black-eared kite (*Milvus migrans*), Eurasian coot (*Fulica atra*), barn swallow (buff-bellied) (*Hirundo rustica gutturalis*), Eurasian tree sparrow (*Passer montanus*), and the yellow-legged buttonquail (*Turnix tanki*) [39].

Table 4. Cartilago cordis shape in different species

Species	Shape	References
Mammals		
Rabbit (*Oryctolagus cuniculus*)	Oval or elliptical or spherical or arched	[39]
Syrian hamster (*Mesocricetus auratus*)	C-shaped with a protuberance, nodular, ellipsoidal or conal	[7, 41]
Brown rat (*Rattus norvegicus*)	Bands or nodules	[39]
White rat (*Rattus norvegicus albus*)	Crescent-shaped	[42]
Mouse (*Mus musculus*)	Bands or nodules	[42]
Donkey (*Equus asinus*)	Two semilunar shaped Cartilago cordis	[43]
Leopard cat (*Prionailurus bengalensis*)	Triangular	[69]
Lion (*Panthera leo*)	Two present, one wedge-shaped and the other oval	[39]
Japanese weasel (*Mustela itatsi*)	Spherical	[39]
Birds		
Ryukyu Robin (*Larvivora komadori*)	Oval	[39]
Daurian redstart (*Phoenicurus auroreus*)	Spherical	[39]
Java sparrow (*Lonchura oryzivora*)	Oval (right trigone) and spherical (left)	[39]
Brambling (*Fringilla montifringilla*)	Oval	[39]
Grey-capped greenfinch (*Chloris sinica ussuriensis*)	Oval (right trigone) and rod-shaped (left)	[39]
Tristram's bunting (*Emberiza tristrami*)	Oval	[39]
Chestnut bunting (*Emberiza rutile*)	Rod-shaped (left trigone) and elliptical (right)	[39]
Reptiles		
Tuatara (*Sphenodon punctatus*)	Likened to a coin	[55]
Iguana (*Iguana iguana*)	Thin nodule	[55]
Snakes (11 species[a], shape not specified in each species)	Three common shapes: disk, rod and block. Most decreased in size at cranial and caudal ends, often splitting into 2+ separate portions	[16]
Oriental rat snake (*Ptyas mucosa*)	Dome-shaped	[58]
Red-eared turtle (*Trachemys scripta elegans*)	Disc-like	[61]
Spanish terrapin (*Mauremys* leprosa)	Elongated bar	[63]
American alligator (*Alligator mississippiensis*)	Main body attached to three processes	[64]
Amphibians		
Korean salamander (*Hynobius leechii*)	Oval	[39]
Shape described from publication figures		
Chimpanzee (*Pan troglodytes*)	Dumbbell-like (two spheres joined)[b]	[15, 37]
Otter (*Lutra lutra*)	Elongated or nodular shape	[51]

[a]Mexican python (*Loxocemus bicolor*), Calabar python (*Calabaria reinhardtii*), Green tree python (*Morelia viridis*), Ball python (*Python regius*), Western blind snake (*Rena humilis*), Jamaican blind snake (*Typhlops jamaicensis*), Schlegel's beaked blind snake (*Afrotyphlops schlegelii*), King cobra (*Ophiophagus Hannah*), Russell's viper (*Daboia russelii*), Puff adder (*Bitis arietans*), Sri Lankan green vine snake (*Ahaetulla nasuta*). [b]The chimpanzee original publication did not describe the shape [15, 37] but personal communications with the authors and Figure 3 (Personal communication, 2024) illustrates the shape which is noted in Table 4.

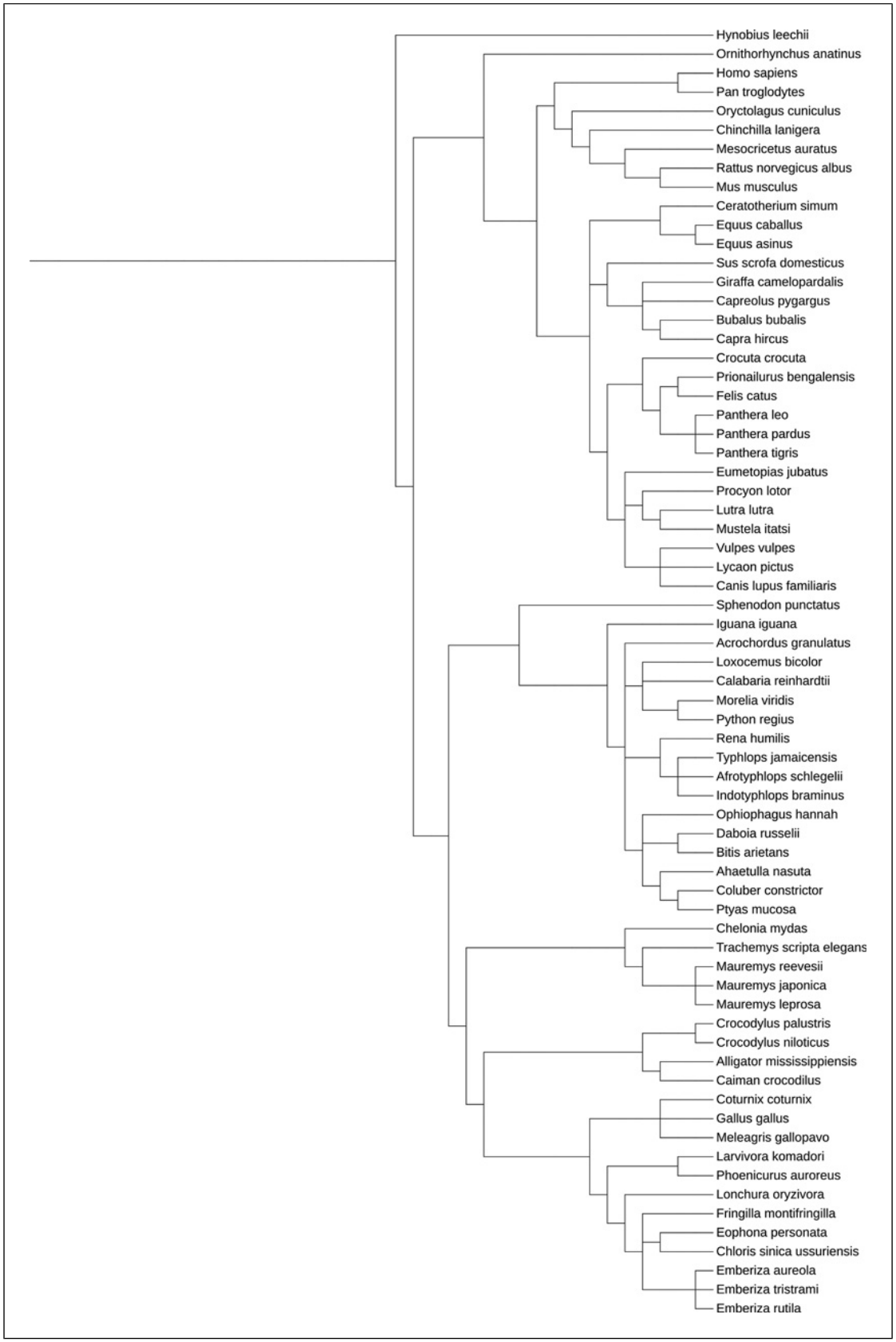
Table 5. Anatomical positions of cartilago cordis in mammals, birds, reptiles, and amphibians

Species	Position	References
Mammals		
Platypus (*Ornithorhynchus anatinus*)	Beside the AV node and bundle	[30]
Human (*Homo sapiens*)	Replacing portions of AV node and bundle of His/ trigone/central fibrous body	[31–36]
Chimpanzee (*Pan troglodytes*)	Right side of the fibrous trigone	[15, 37]
Rabbit (*Oryctolagus cuniculus*)	Border between ventricular and anterior chamber/end of the atrial septum	[38, 39]
Chinchillas (*Chinchilla lanigera*)	Left AV valve and left AV opening	[40]
Hamster (*Mesocricetus auratus*)	Right trigone and/or in the membranous septum (within the ventricular septum)/CFB/fibrous tissue surrounding aortic orifice	[17, 41]
White rat (*Rattus norvegicus albus*)/Brown rat (*Rattus norvegicus*)	Near the aortic ring/right trigones	[39, 42]
Mouse (*Mus musculus*)	Near the aortic ring	[42]
White rhinoceros (*Ceratotherium simum*)	Right trigone	[14]
Horse (*Equus caballus*)	CFB/close proximity to the [atrial] septa/left of the heart at the transition from the upper ventricle into the atrium/ right trigone to the left AV orifice	[36, 39]
Donkey (*Equus asinus*)	Right and left fibrous trigone	[43]
Pig (*Sus scrofa domesticus*)	CFB in/near the AV node/right trigone	[36, 44]
Nubian Giraffe (*Giraffa camelopardalis*)	Left fibrous trigone	[45]
Siberian roe deer (*Capreolus pygargus*)	Extending from the right trigone to the ventricular septum	[39]
Egyptian water buffalo (*Bubalus bubalis*)	CFB	[46]
Goat (*Capra hircus*)	Fibrous body near ostium aortae	[47]
Spotted hyena (*Crocuta Crocuta*)	AV septum	[48]
Leopard cat (*Prionailurus bengalensis*)	Right trigone	[48]
Cat (*Felis catus*)	CFB	[49]
Lion (*Panthera leo*)	Right trigone	[35, 39]
Leopard (*Panthera pardus*)	Caudal to the aortic origin, dorsal to the ventricular septum	[48]
Tiger (*Panthera tigris*)	Caudal to the aortic origin, dorsal to the ventricular septum	[48]
Sea lion (*Eumetopias jubatus*)	Right trigone	[50]
Racoon (*Procyon lotor*)	Caudal to the aortic origin, dorsal to the ventricular septum	[48]
Otter (*Lutra lutra*)	Fibrous trigone	[51]
Japanese weasel (*Mustela itatsi*)	Right trigone	[39]
Red and silver fox (*Vulpes vulpes*)	Caudal to the aortic origin, dorsal to the ventricular septum	[48]
African wild dog (*Lycaon pictus*)	Caudal to the aortic origin, dorsal to the ventricular septum	[48]

Table 5 (continued)

Species	Position	References
Dog (*Canis lupus familiaris*)	CFB	[36, 52, 53]
Wolf (*Canis lupus*)	Caudal to the aortic origin, dorsal to the ventricular septum	[48]
Birds		
Quail (*Coturnix coturnix*)	CFB	[2]
Chicken (*Gallus gallus*)	CFB	[2, 54]
Wild turkey (*Meleagris gallopavo*)	Left trigone	[39]
Ryukyu Robin (*Larvivora komadori*)	Left trigone	[39]
Daurian redstart (*Phoenicurus auroreus*)	Left trigone	[39]
Java sparrow (*Lonchura oryzivora*)	Left and right trigones	[39]
Brambling (*Fringilla montifringilla*)	Right trigone	[39]
Japanese grosbeck (*Eophona personata*)	Left trigone only or left and right trigones	[39]
Grey-capped greenfinch (*Chloris sinica ussuriensis*)	Left and right trigones	[39]
Yellow-breasted bunting (*Emberiza aureola*)	Left trigone	[39]
Tristram's bunting (*Emberiza tristrami*)	Right trigone	[39]
Chestnut bunting (*Emberiza rutile*)	Left and right trigones	[39]
Reptiles		
Tuatara (*Sphenodon punctatus*)	Between pulmonary artery and left aorta	[55]
Iguana (*Iguana iguana*)	Right trigone in the area of the AV junction	[55, 56]
Snakes 11 species with cartilago cordis = Mexican python (*Loxocemus bicolor*), Calabar python (*Calabaria reinhardtii*), Green tree python (*Morelia viridis*), Ball python (*Python regius*), Western blind snake (*Rena humilis*), Jamaican blind snake (*Typhlops jamaicensis*), Schlegel's beaked blind snake (*Afrotyphlops schlegelii*), King cobra (*Ophiophagus Hannah*), Russell's viper (*Daboia russelii*), Puff adder (*Bitis arietans*), Sri Lankan green vine snake (*Ahaetulla nasuta*), Little file snake (*Acrochordus granulatus*), Brahminy blind snake (*Indotyphlops braminus*), Eastern racer (*Coluber constrictor*)	Aorticopulmonary septum/between the pulmonary artery and aorta/dorsal to the anterior edge of the ventricular septum	[16, 57–59]
Green sea turtle (*Chelonia mydas*)	CFB	[60]
Red-eared turtle (*Trachemys scripta elegans*)	Trigone between atria and ventricle	[61]
Chinese pond turtle (*Mauremys reevesii*)	–	[62]
Japanese pond turtle (*Mauremys japonica*)	–	[62]
Spanish terrapin (*Mauremys* leprosa)	CFB	[63]
Mugger crocodile (*Crocodylus palustris*) Nile crocodile (*Crocodylus niloticus*) American alligator (*Alligator mississippiensis*) Spectacled caiman (*Caiman crocodilus*)	Base of the left aorta in crocodilians with one process extending into the ventricular septum/interventricular	[64, 65]
Amphibians		
Korean salamander (*Hynobius leechii*)	Atrial septum	[39]

Anatomical positions as described by the original authors in the published articles. AV, atrioventricular; CFB, central fibrous body.



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Reptilia

In lizards and in a lizard-like rhynchocephalian, the tuatara (*Sphenodon punctatus*; $n = 1$) cartilage was only discovered between the pulmonary artery and left aorta [55]. Three species of lizards ($n = 1$/species), *Varanus indicus*, *Varanus niloticus*, and *Varanus rudicollis* from the Varanidae family (Monitor lizards), were also investigated by Young, but cartilago cordis was not found [16]. In iguanas (*Iguana iguana*), chondroid tissue was found within the fibrous cardiac skeleton in the right trigone in the area of the AV junction of the iguanas [56].

Cartilago cordis has been found in 11 of 42 species of snake (*Serpentes*) investigated. The species investigated

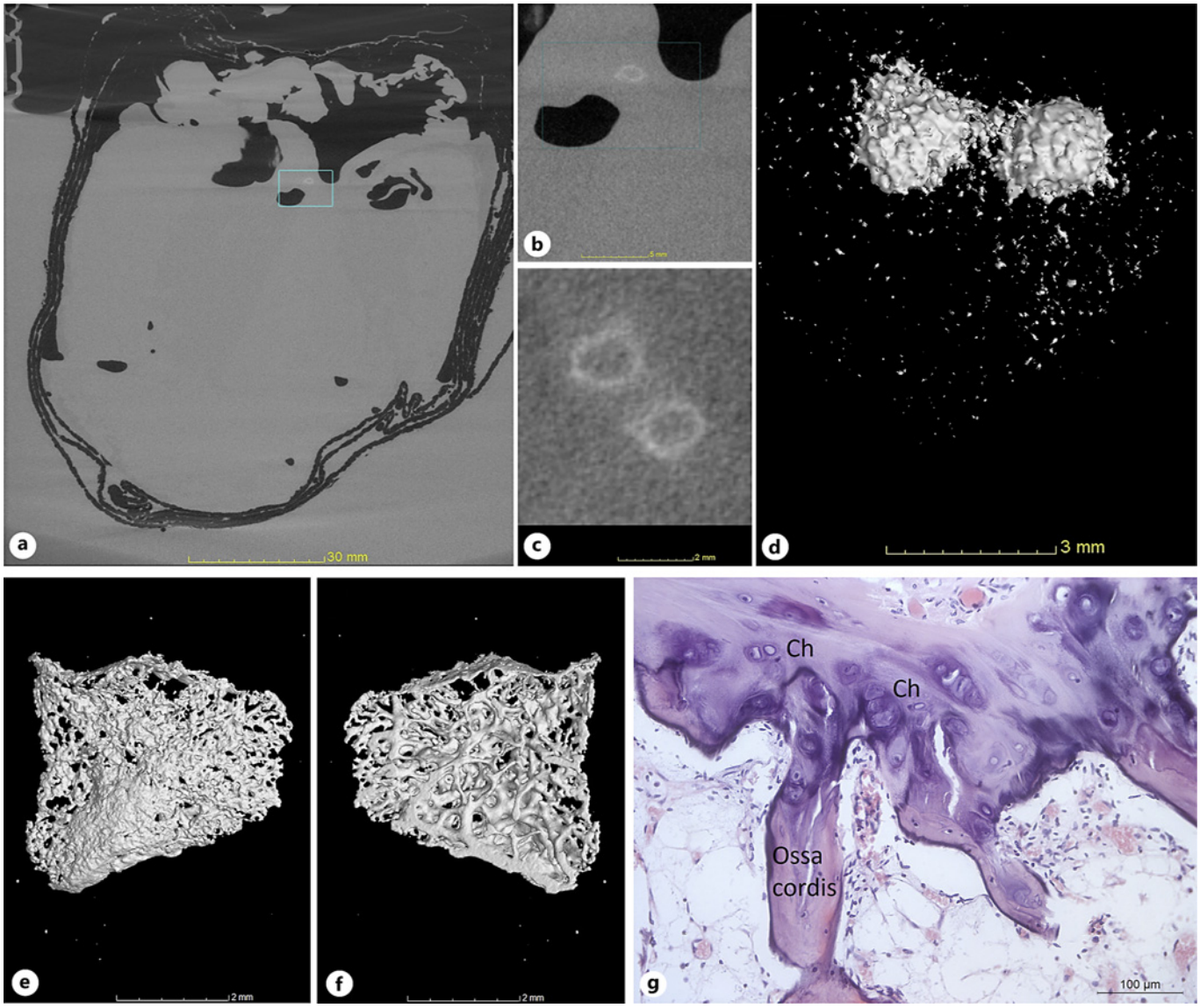
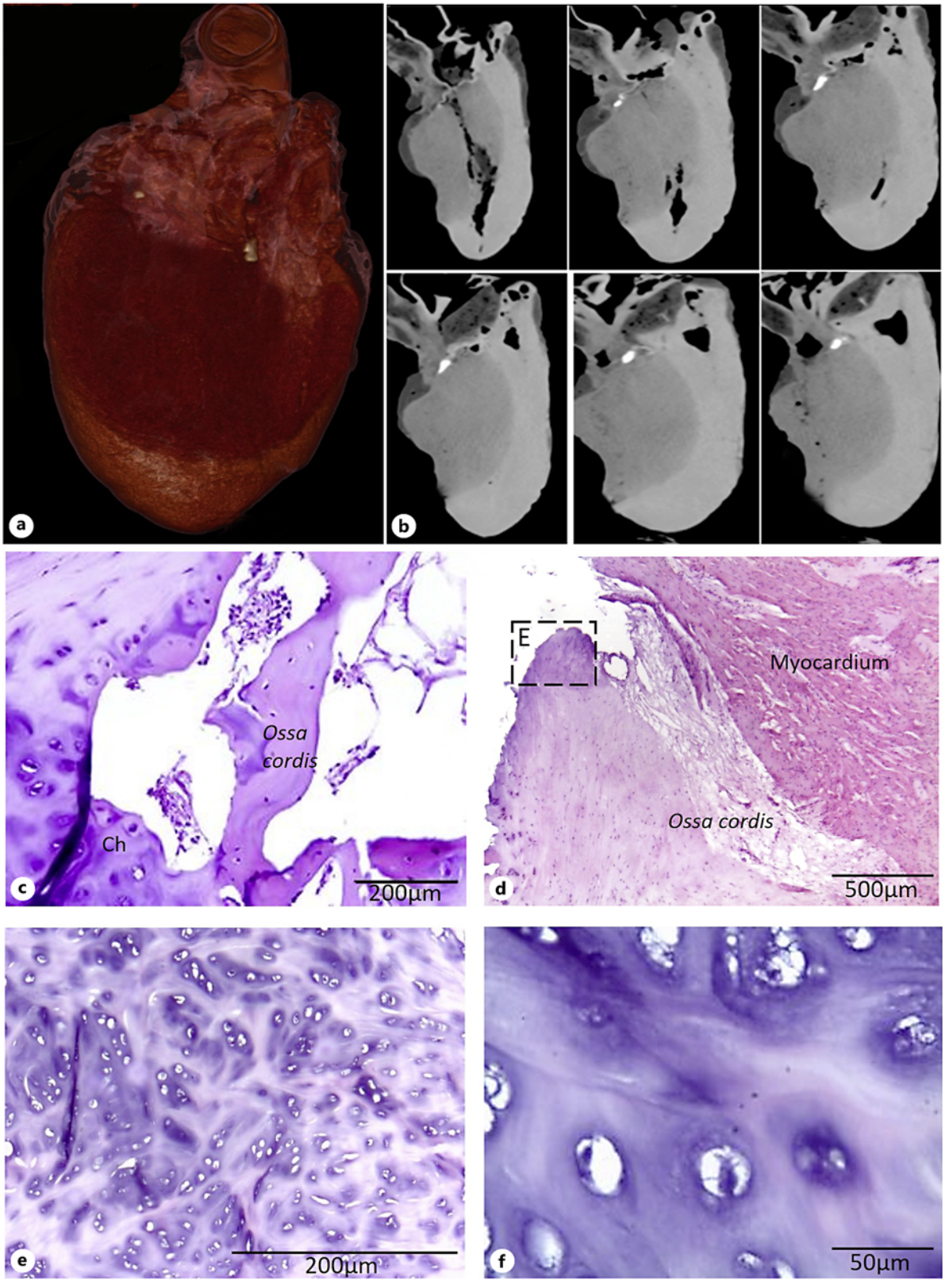


Fig. 3. High-resolution X-ray computed microtomography images of chimpanzee cartilago cordis and ossa cordis with associated cartilage. **a–c** Cartilago cordis within the whole heart. **d** 3D rendered cartilago cordis. **e**, **f** 3D rendered ossa cordis. **g** Haematoxylin and eosin-stained ossa cordis with associated hyaline cartilage. Data visualized using VGStudioMAX v2.2 software (https://www.volumegraphics.com). Samples and methods as described previously [15, 37, 70]. Ch, chondrocytes.

Fig. 2. Evolutionary tree of species with cartilago cordis. Data on species collated from systematic review. Map created using iTOL Interactive Tree of Life (https://itol.embl.de/) and PhyloT v2 (https://phylot.biobyte.de/).



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included were members of the Typhlopidae, Leptotyphlopidae, Aniliidae, Xenopeltidae, Boidae, Pythonidae, Acrochordidae, Colubridae, Crotalidae, Viperidae, Elapidae, and Hydrophiidae families ($n = 55$ total specimens, 1–3 per species [16]. The species with *cartilago cordii* were Jamaican blind snake (*Typhlops jamaicensis*), Schlegel's beaked blind snake (*Afrotyphlops schlegelii*), now western blind snake (*Rena humilis*), Mexican python (*Loxocemus bicolor*), Calabar python (*Calabaria reinhardtii*), green tree python (*Morelia viridi*), ball python (*Python regius*), Sri Lankan green vine snake (*Ahaetulla nasuta*), puff adder (*Bitis arietans*), Russell's viper (*Daboia russelii*), and the king cobra (*Ophiophagus Hannah*). The position of cartilago cordis in snakes varied widely but was found within the aorticopulmonary septum, except for the ball python cartilago cordis which was located in the dorsal wall of the right aorta adjacent to the septum, and therefore not within the aorticopulmonary septum itself [16]. The authors noted that within snakes the cartilago cordis was found in eight species of terrestrial snakes, two arboreal and one aquatic species (from the 32 terrestrial, seven arboreal, and three aquatic species investigated). Cartilago cordis was specifically not present in the three specimens of Oriental rat snake (*Ptyas mucosa*) [16] but another publication reported its presence in this species [58]. In this case, it was reported as a single, fairly large, uniformly thick around its margin but somewhat thinner central region, located dorsal to the anterior edge of the ventricular septum, the number of specimens was not reported [58]. Two papers referenced by other papers [16, 58] reporting cartilago cordis in the Brahminy blind snake (*Typhlops jamaicensis*) where the cartilago cordis was "small and rod-like" [59] and the little file snake (*Acrochordus granulatus*) [57].

In a chelonian, the juvenile green sea turtle (*Chelonia mydas*) fibrocartilage was found within the cardiac skeleton, and hyaline cartilage was also found in the AV valves of the turtle [60]. In the red-eared turtle (*Trachemys scripta elegans*), in a 12-year-old female with necrotic regions of the heart, a prominent hypertrophic cartilago cordis, described as "a very Irregular field of cartilage in the trigone between atria and ventricle" was reported [61]. The cartilago cordis has also been described in two other chelonian species, the Japanese pond turtle and the Chinese pond turtle (*Mauremys japonica* and *M. reevesii*, respectively) as an isolated, homogenous structure provided with a perichondrium (full text was not available for Kashyap [62, 83]). Spanish terrapins (*Mauremys* leprosa) had type II collagen-positive (chondrogenic) cellular condensation remains devoid of perichondrium in the 23 embryos investigated. Later on in development, in all eight post-partum specimens aged 3 months-10 years, there was a cartilago cordis and in the seven specimens aged 18 months or older, cartilage was also found within the right side of the aorta and the authors specified no bone was observed [63].

There were two cartilago cordis structures present in mugger crocodile (*Crocodylus palustris*), American alligator (*Alligator mississippiensis*), and the spectacled caiman (*Caiman crocodilus*) [64]. These were positioned at the base of the left aorta in crocodilians, with one process extending into the ventricular septum [64]. Interventricular cartilage was also observed in 21 hearts from the Nile crocodile (*Crocodylus niloticus*) [65].

Amphibia

The Korean salamander (*Hynobius leechii*) had at least one cartilago cordis taking up almost the entire atrial septum and sometimes another smaller piece was also present in the septum, in all seven specimens examined from both sexes [39].

Fish

Fish generally, like most reptiles generally have no atrial septum, with exceptions such as lungfishes (*Dipnoi*) which are closely related to tetrapods and were the first to develop a small atrial septum and a partial ventricular septum creating two ventricular chambers [84]. It is therefore understandable that cardiac cartilage has not been reported in the context of a true cartilago cordis. It is also interesting that in lungfish, the AV valves are replaced by the AV plug. This structure consists of hyaline cartilage surrounded by fibrous connective tissue in *Protopterus dolloi* and in *Lepidosiren paradoxa*, and fibrous connective tissue alone in *Neoceratodus forsteri* [85–87]. Another study investigated 64 fish species and found no cartilage in the heart except for one specimen (but not others) of *Monacanthas cirrhifer* from the family Monacanthidae (a teleost). This specimen had hyaline cartilage in the valve base of the bulbus cordis so may not considered a true cartilago cordis if not located in the muscular tissue [39]. Cartilage in the valves of other vertebrates is not classified as cartilago cordis, and whether or not these structures in a limited number of fish are true cartilage cordis, given they are in the valve

Fig. 4. Cattle and camel ossa cordis and associated cartilage. **a**, **b** Cattle CT scans showing ossa cordis within the trigones. **c–e** Camel ossa cordis histology exhibiting cartilage surrounding the bone. **f** Individual chondrocytes surrounding camel ossa cordis. Techniques as previously published [74, 75, 78]. Ch, Chondrocytes.

tissue or valve equivalent are debatable. Regardless of the name of the structure, this may indicate an evolutionary lineage for cartilage development in this area of the heart.

There were two studies not included due to the inclusion criteria but they are worth mentioning as they include several vertebrate species. Kojima [88] looked at 142 species and identified that cardiac cartilage was present in a few species but did not specify the anatomical locations within the heart, age, or sex of specimens, or how many specimens were investigated. It is also worth highlighting that the paper also investigated a number of the vertebrate species investigated elsewhere in this review but found no heart cartilage in the specimen(s) investigated. A second study excluded noted "cartilaginous trigones" in several species but did not specifically state whether these were analysed histologically (to confirm cartilaginous material) and also did not specify whether they presented as a specific cartilago cordis structures, individual cells, or foci, and frequently only named the genus [89]. In addition, the ages and sex were not mentioned, but numbers of specimens were provided. Therefore, the species from both of these studies are presented in online supplementary Table S1 but are not included in the rest of the review as it could not be ascertained whether they were definitive *cartilago cordii.*

Cartilago Cordis Presence in Male and Female Specimens

The question over whether males or females specifically have cartilago cordis, and whether there were any differences between the sexes, was difficult to determine, the sexes are shown in Tables 1 and 2 for each study. Unfortunately, sometimes publications did not state the sexes or only had one sex or a single case. Consequently, no direct comparisons or analysis could be drawn between male and female for the studies, but it is clear; it can be present in both males and females in many vertebrate species.

Cartilago Cordis Number, Size, and Shape in Mammals, Reptiles, and Birds

With the exception of the publications that categorically stated that there were two or more cartilago cordis, the remaining publications either stated there was one or assumed a singular structure per heart (Table 1, 2). The exceptions were 2 dogs (aged 5 and 6 years old) which had multiple cartilaginous foci [53] and in another study both a *trigonum cartilagineum dextrum* and a cartilago cordis septalis were described [66, 67]. In contrast, the remaining canine reports indicated one cartilago cordis per dog where present [36, 52]. The otters had between one and three cartilago cordis in individuals with the structure [51]. Furthermore, the number and size of the cartilage pieces increased with age, which implied the "normal" may need to be judged on an individual basis [51]. The Spanish terrapin had one cartilago cordis in the youngest postpartum specimen (aged 3 months) but in all 7 of the older specimens (18 months-10 years), there were two [63]. The mice and rats investigated had two cartilago cordis in the individuals where the structure was present [42]. In the donkey, the left cartilago cordis was larger than the right, but no specific measurements were mentioned [43]. The European rabbit usually had a single one in the right trigone, sometimes two, and sometimes an additional one in the left trigone [39]. A small number of birds also had two cartilago cordis, the Java sparrow, Japanese grosbeak (*Eophona personata personata*), grey-capped greenfinch, and chestnut bunting, and an amphibian, the Korean salamander sometimes had a larger and a smaller cartilago cordis [74]. The sizes and shapes of the cartilago cordis varied between species and within individuals, these are described in Table 3 (size) and Table 4 (shape).

Histological Composition of the Cartilago Cordis

Similarities and differences in structures were reported between vertebrate species. The cartilago cordis can be composed of any type of cartilage, but hyaline was reported in some mammals including: mice [42], giraffe [45], white rats (immature or mature hyaline cartilage) [39], leopard cat (mature) [39], Japanese weasel (mature) [39], dogs [36], and horses (mature hyaline) [36, 39]. In birds, the wild turkey, Ryukyu Robin, yellow-breasted bunting, Daurian redstart brambling had immature hyaline cartilage and Tristram's bunting, Japanese grosbeak and grey-capped greenfinch had mature hyaline cartilage [39]. The Japanese grosbeck, Java sparrow had calcified hyaline cartilage and the chestnut bunting had ossified hyaline cartilage [39]. The amphibian Korean salamander cartilago cordis also consisted of hyaline cartilage (varying between immature/mature and calcified cartilage) [39]. Some reptiles also had hyaline cartilago cordis, including Crocodilia [64], lizards and iguanas [55, 56], and snakes [16].

Some publications went into more detail regarding the hyaline cartilago cordis. In the donkey, the hyaline cartilage had chondrocytes residing in lacunae, surrounded by collagenous fibres [43]. The European rabbit cartilago cordis was mostly immature hyaline cartilage, rarely precartilage, and was a scattered deposition of cartilage cells in one case [39]. In the chinchilla oval or hexagonal,

chondrocytes were present within the central portions of the cartilage, with flattened chondrocytes in the marginal portions, hyaline cartilage with a perichondrium. Hyaluronicacid, proteoglycans, collagen, and elastin protein fibres were present within the extracellular matrix [40]. In iguanas, chondroid tissue consisting of "hyaline like cartilage" with binucleated chondrocytes in big lacunae and extracellular matrix with fibrillar collagen was reported [56]. The little file snake had hyaline cartilage with rounded cells containing spherical nuclei [57]. The general snake species cartilago cordis, particularly the block-shaped ones, had an extracellular matrix (ECM) containing collagen fibres [16]. The Spanish terrapins also had hyaline cartilage and an ECM containing collagen fibres which were further categorised as type II collagen, and a perichondrium surrounding the cartilago cordis composed of collagen fibres and a few layers of flattened cells [63]. In the red-eared turtle, the cartilago cordis, surrounded by connective tissue, was alcianophilic, with no calcification, consisting of hyaline cartilage with a cloudy inter-territorial matrix [61]. The chondrocytes were haphazardly distributed, mostly individual cells within lacuna but some were two cells separated by a thin septum of matrix. A perichondrium could not be identified, but slender chondroprogenitor cells were spread along the perimeter [61].

Fibrocartilage and hyaline cartilago cordis were present in several mammals including the juvenile buffalo [46], and the otters [51], cattle when associated with ossa cordis only [74] and chimpanzees [15, 37] (as exampled in Fig. 3 for chimpanzees and Fig. 4 for camels). The pig "cartilaginous metaplasia" contained hyaline cartilage in one of the four hearts containing a cartilago cordis and an additional one also contained fibrocartilage [36]. In another porcine study, the cartilago cordis consisted of fibrocartilage and hyaline cartilage types, intercellular matrix, lacunar borders and the chondrocytes were relatively numerous, large and spherical [44]. It also showed hyaluronic acid, link proteins, keratan sulphates and subunits, core proteins, clusters of long chains of chondroitin sulphates and short chains ofkeratan sulphates, and their conclusion was that the glycosaminoglycans did not differ significantly in these respects to cartilage elsewhere in the mammalian body [44]. The work on rats also indicated hyaline cartilage with surrounding fibrocartilage surrounded by coarse connective tissue, a perichondrium was not mentioned [42]. The absence of a cartilago cordis perichondrium was, however, noted in otters [51]. Sometimes, a fibrous capsule was seen surrounding the cartilago cordis of Syrian hamsters and the cartilago cordis had an ECM containing collagen fibres which were further categorised as type II collagen [17]. These studies indicated the Syrian hamster cartilago cordis was most commonly composed of hyaline cartilage, but sometimes also fibrocartilage enclosed within dense fibrous connective tissue, collagen fibres and a few layers of flattened cells, with a perichondrium [17, 41]. Interestingly a reptilian species, the green turtle [60] exhibited fibrocartilaginous tissue formation, with hyaline cartilage.

Some papers did not specify the type of cartilage. The dog was variable ranging from chondroblasts and chondrocytes to mature chondrocytes with mineralization [52]. The otter study simply described cartilage [79], as did the sea lion study as one adult had cartilage only and the other two adults and the pup had cartilage within connective tissue [50]. An outlier is the Siberian roe deer its cranial half consists of mature hyaline cartilage and its caudal half was bone with a large medullary cavity [37], arguably an ossa cordis with cartilage.

Cartilago Cordis Formation and Development

Cardiac cartilage is known to be of cardiac neural crest cell origin in birds [3], and this has also been hypothesized in mammals and other animals containing a cartilago cordis. Investigations into cartilago cordis in chinchillas, terrapins, hamsters, chickens, and quails concur with this theory as the scientific publications noted that neural crest cells produce the precursors to cartilage [2, 5, 17, 40, 63]. In addition, cartilage has also been observed developing from epicardial progenitor cells in avian models both in vitro and in vivo [81].

Cartilago cordis has been observed in a wide range of developmental ages. Chondroid tissue has been observed in terrapin embryos but was located in the right aortic valve [63]. Embryological chondrogenesis in terrapins started as a type II collagen-negative prechondrogenic cellular condensation; collagen was produced at embryonic stage 23 (from Yntema's developmental stages [63]). The perichondrium formed postpartum, after which the condensation became hyaline cartilage to form the mature cartilago cordis [63]. Cartilago cordis can also form postpartum, with the ages of presenting animals varying greatly from neonates through to adults (Tables 1, 2). Cartilago cordis in Syrian hamsters was observed from 2 days old all the way up to adults aged 842 days old [17]. In this study, chondrogenesis in the central fibrous body was observed from the 2nd day post-birth, with the highest production of cartilaginous deposits occurred between the ages of 40–80 days, and new deposits were uncommon after this time period. In contrast to the embryonic chondrogenesis observed in terrapins,

postpartum chondrogenesis occurred in the hamster, with a distinct lack of prechondrogenic condensations [17]. Cartilage formation began with cells grouped in a type II collagen-positive ECM, which then multiplied and differentiated to form the mature cartilago cordis. The presence or lack of prechondrogenic cellular groups may result from the differences in timing of chondrogenesis before or after birth; however, the reason for these differences is unknown. It has also been suggested that early cartilago cordis formation is not a result of ageing [17]. Similarly, another study suggested formation within a month after birth in the hamster [5], and many other animals also have later development of the cartilago cordis (Table 1). In dogs, a cartilago cordis was found from 2.5 months to 12 years old, the horses with a cartilago cordis were aged between 2.5 and 3.5 years old, the pigs were 5 months old on average [36]. One limitation on understanding age of development is that many studies do not state ages or have looked at adults but not embryos/neonates/young, therefore understanding formation is difficult from the present literature.

Regarding development it is also worth addressing whether cartilago cordis develops into the bone ossa cordis. Whilst proposals have indicated that ossa cordis may develop from cartilage tissue, via endochondrial ossification [37], it is evident that not all species with cartilago cordis also develop ossa cordis. The present review found 68 vertebrate species have reported findings of cartilago cordis. A recent review [27], plus interim literature searches also by our group, has identified just 15 species with ossa cordis [37, 74, 75]. It is very interesting that many of these vertebrate species show cartilage associated with the ossa cordis. It is also important that the only 10 of these species (cat, chimpanzee, dog, giraffe, goat, horse, otter, sea lion, buffalo, sheep) have also had cartilago cordis recorded. In contrast the camel, deer (both white-tailed and unspecified species), cattle, elephant have not yet had published sightings of cartilago cordis. It is also essential to highlight that investigating the potential development of cartilago cordis into ossa cordis is hindered by the scientific techniques and literature presently available. Imaging such as X-ray, computed tomography, and computed microtomography can visualise higher density tissue within the heart, but histological techniques must then be used to identify whether these high-density structures are bone or cartilage. To follow an individual animal as it ages is presently not possible as removing histological samples from the heart would be complex and X-ray computed microtomography can only be conducted on cadaver material. Looking at younger versus older animals would not confirm whether an ossa cordis observed developed directly from a cartilago cordis. Although, in general, in the 10 species where both have been observed, it is often the younger animals that contain cartilago cordis and the older ones which have an ossa cordis. Prevalence of either cartilago cordis or *ossa cordis* is also not 100% in the majority of species, and the published information relating to many of the species regarding both cardiac structures remains limited to just a few individuals of each species.

Proposed Functions of Cartilago Cordis

The trigger for cartilage development in the heart is unknown but one theory proposed [40] relates to high mechanical forces. The authors indicate this is unlikely to be the sole reason for development, but it may trigger cartilage formation to counteract high mechanical stress in specific areas of the heart such as the AV valves. Mechanical stress is deemed a potentially important factor contributing to the development and function of cardiac cartilage in general [4, 6, 15, 17, 39, 42, 72] and the cartilaginous foci are often observed in cardiac anatomical locations that experience particularly intense mechanical stress [6]. Therefore, mechanical stress is presumed to be essential for the differentiation of cells and tissue into cartilage [17], especially hyaline cartilage [4]. In addition to aiding contraction, it has been suggested that in chinchillas, chickens, quails, snakes, terrapins and hamsters the cartilago cordis helps support the heart in areas of high mechanical stress, a similar function to that proposed with ossa cordis [2, 16, 17, 27, 40, 63]. For example, the leaflets of the AV valves experience high levels of mechanical stress during contraction, and this region is where cartilaginous tissue was present in chinchillas [40]. Green sea turtles have hyaline cartilage in the AV valves too, leading the authors to hypothesis that the cartilage plays a role in resisting mechanical forces against those valves during pressure changes as the turtle dives below sea level [60]. The aorticopulmonary septum in the hearts of chickens and quails is a region of high mechanical stress and it was suggested that cartilago cordis acts to support the heart against these forces [2]. Despite the cartilago cordis presenting in a different position, similar findings were reported in hamsters where the cartilaginous tissue was noted to support the heart under mechanical stress. In addition, cartilage in hamsters exhibited more calcification when exposed to greater mechanical stresses; these findings were similarly supported in an investigation of cartilage found in otter hearts [17, 51].

Some researchers have theorised that cartilago cordis provides rigidity within the heart and therefore stabilises the heart during its cardiac cycle, as it undergoes systole and diastole [13, 17]. In snakes and hamsters, it has been suggested that the cartilago cordis is present to aid myocardial contraction [16, 17]. In the hamster, the cartilago cordis was strongly linked to a role within myocardial contraction due to its position near the ventricular and atrial septa, a similar position to ossa cordis and the human fulcrum [17, 31]. The cartilago cordis may also provide protection to the heart's vital structures. Observations made in platypus show that the cartilago cordis resides in contact with the AVN suggesting it may form to protect this vital tissue [30]. In snakes, it has also been suggested that due to the vast variation in the shape and size of cartilago cordis across species it was difficult to interpret function but it likely supports the roots of the aorta and pulmonary vein [16]. Likewise, the cellular and intracellular characteristics of the cartilago cordis in Syrian hamsters were the same as its tracheal cartilages, and therefore the authors of these studies also indicated its function may be similar, to keep aortic orifices open, preventing constriction or collapse [13, 41]. Little has been published on cartilago cordis development during ageing of pigs, horses, or dogs, but the individuals with a cartilago cordis/cartilaginous metaplasia did have increased levels of connective tissue production both inside and outside of the AVN alongside the observed cartilago cordis [36].

Pathological and Disease Status in Relation to Cartilago Cordis Presence

To further explore where cartilago cordis is a present in normal physiology, the health status of the animals should be explored, and this differed between the published studies. In cardiomyopathy affected cats, compared to healthy controls, it was suggested that the presence of the cartilago cordis in the central fibrous body compressed the adjacent elements of the cardiac conduction system leading to degeneration and atrophy; and hence, potentially initiated conduction disturbances [49]. It was thought that non-suppurative endocarditis and myocarditis led to fibrosis of the heart – notably the endocardium, myocardium and cardiac conduction system, before finally resulting in the formation of cartilage and bone [49]. The chimpanzee with a cartilago cordis had marked idiopathic myocardial fibrosis (IMF), as did three others which had ossa cordis, whereas those with no IMF or lower levels of IMF did not have cartilago cordis or ossa cordis present [15, 37]. In the case study of an adult red-eared turtle, the authors noted the cartilago cordis may have disturbed the flow of blood leading to local atrophies as acute cell death in a larger part of the ventricle was also observed [53, 61]. The study of 2 dogs who had sudden exhibited cartilaginous cells within the central fibrous body [53], but other studies of apparently healthy dogs also exhibited a cartilago cordis [36, 52]. The study analysing the AVN of healthy horses, dogs, and pigs did show that when cartilage was present within cardiac skeleton the AVN was reduced in size, there was a reduction in the size and number of the nodes functioning P-cells (nodal pacemaker cells), and the levels of collagen increased within the AVN [36]. Collectively, the changes were thought to result in a declined transmission of electrical impulses through the AVN and therefore prevented efficient cardiac conduction [36]. Therefore, although the majority of studies included in this review either specified a healthy heart or did not mention a pathology, indicating that a healthy heart can contain cartilago cordis, it has been suggested that cartilago cordis formation/presence may have detrimental effects or may be present in some pathological conditions.

Discussion

This review revealed that cartilago cordis were present in a limited number of mammals, reptiles, an amphibian, and some birds. A single cartilago cordis per heart was most common, however, up to three cartilage structures have been identified some individuals, whilst in others, especially younger specimens, cartilaginous foci were described (Table 1, 2). For some species, the prevalence appeared to be 100% (Table 1, 2), but importantly these were often in single animal case studies; therefore, the results may not be representative of the entire species. Naturally cartilago cordis has also not been reported in an extremely large number of species with a potential prevalences of anywhere between 0 and 100%. Some species had a fibrocartilage cartilago cordis, others hyaline, and some were unspecified cartilage types or chondrocytes. In some cases, such as the Spanish terrapin, it was clear that composition differed with age, as did the number of cartilago cordis observed.

The cartilago cordis structure shapes ranged from spheres, discs, nodules and through to elongated structures (Table 4) with sizes ranging from 195 μm [57] to over 8 mm [64] (Table 3). Its presence in differing anatomical locations in the trigones (as discussed previously for each species where specified and Table 5) depended on factors such as age, species and was often different in differing specimens from within the same species. These

intraspecies and interspecies differences may result from varying functions, for example, specific shapes/sizes depending on the support required, or due to discovery at different stages of formation/age or be due to differences between different individuals. In 1938, Matumoto [39] theorised that cartilago cordis only developed postpartum. By 2008, this was disproved in the people and the Spanish terrapin, with chondrogenesis present in embryos [31, 32, 63].

Few studies have directly investigated the formation or function of cartilago cordis; therefore, the definitive function of the cartilago cordis is presently unknown. Researchers have agreed that its presence was not required for normal cardiac function, given that it is not present in all vertebrate species or even within all individuals in every species, and suggested it simply reflected the potential for chondrogenesis in this area of the heart [2, 16, 63]. Alternatively, it may be a supportive mechanism to reinforce the aorta and pulmonary artery roots [16], the semilunar valves [64], or other cardiac tissue [17, 55, 63]. It has been suggested that cartilage may help maintain the diameter of the aorta in hamsters or the foramen of Panizzae in Crocodilia [41, 64]. Functional theories have also centred around it forming as a response to mechanical stress [17, 42, 51]. Stress may cause increased collagen synthesis [90], therefore as the cartilago cordis contains type II collagen, increased synthesis could result in its formation [15]. Cardiac ageing and disease [15] have also been proposed as contributing factors but again this differs between species as many hearts in the studies conducted were not affected by heart disease and some species exhibited embryonic or early postpartum formation of the structure. In contrast some species only had cartilago cordis in older specimens, which could also suggest a supportive function to assist with cardiac ageing [51]. It is likely that mechanical stress and ageing are related; therefore, differences in timing of postpartum/ageing formation could depend on which is the greater stimulant, pathological stress or natural ageing. Some researchers also proposed that cardiac chondrogenesis is controlled by a genetic influence [17]. Sox9 encodes a transcription factor required for chondrogenesis, especially within heart valves [91, 92], and it was discovered that BMP2 activates Sox9 in cells destined to form cartilage and the valves themselves [93]. Due to these similarities in regulatory mechanisms, it is possible that the cartilago cordis develops as an indirect consequence of valvular epigenetics. For animals in which embryological chondrogenesis occurs, this may be more likely. A further formation and function theory is that cartilago cordis may be an intermediate stage in the formation of an ossa cordis especially as many ossa cordis have cartilage associated, surrounding, or even within them (Fig. 3, 4) [13, 15, 74, 78].

In conclusion, the cartilago cordis, located within the cardiac skeleton, is commonly composed of hyaline and/or fibrocartilage cartilage. The shape, size, number of structures and prevalence varied within and between vertebrate species, and little is known about its development or function within the heart. Given the complexity of finding cells or potentially small cartilago cordis in this region of the heart, there may be many more vertebrate species, or individuals within a species, which may have this structure present. Naturally overarching questions remain about cartilago cordis. Why do some species have cartilago cordis whereas others do not? Which pathways and mechanisms enable (or disable) cartilago cordis formation and degradation? Are pathways and mechanisms activated or prevented in these species which do not have a cartilago cordis or in species which develop them later in life? Why do some vertebrate species with cartilago cordis never exhibit an ossa cordis? Future work may include cases studies but would benefit from some larger, more detailed studies focussing on genetics, age, development and origin, function, and disease status, which may help us understand development and function and may also result in use of the cartilago cordis as a pathological indicator in veterinary medicine.

Statement of Ethics

As stated in the citations, Figures 3 and 4 were created with ethical permission given by the University of Nottingham, School of Veterinary Medicine and Science Ethics Committee (ethics No. 1843 160905, 3186 200604, 1843 160905, 3524 211209) and/or by the Animal Welfare and Ethics Committees, in the Faculty of Veterinary Medicine, Alexandria University (No. 02/13/2023/02/01/212 and 013/2023/03/11/224), in adherence to institutional and national guidelines. For the remainder of the study, a Statement of Ethics is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

V.K., A.B., S.M., T.S., A.A., W.P., and C.S.R. undertook the literature searches. A.A., S.A.A.E.-G., M.A.M.A., C.J.S., and C.S.R. created the figures. V.K., C.J.S., and C.S.R. received funding. C.S.R. supervised the students and project managed the concepts, research, and data. V.K., A.B., C.J.S., W.P., and C.S.R. wrote the manuscript. S.M., T.S., A.A., S.A.A.E.-G., and M.A.M.A. added intellectual input to the manuscript. V.K., A.B., S.M., T.S., A.A., W.P., S.A.A.E.-G., M.A.M.A., C.J.S., and C.S.R. approved the final manuscript.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article (and/or) its supplementary materials. Further enquiries can be directed to the corresponding author Catrin.rutland@nottingham.ac.uk, catrin.rutland@nottingham.ac.uk.

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