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OBSERVATIONS ON THE REPRODUCTIVE BIOLOGY OF *CANDOIA CARINATA* (SERPENTES, BOIDAE)

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Abstract: *Candoia carinata* reaches sexual maturity at about 350 mm snout-vent length. Adult females are typically larger than males, suggesting a strong sexual dimorphism; further, adult females are more numerous than males. Gametogenesis in males appears cyclic and seasonal with spermiogenesis in May and June; in females, there appears to be no seasonality of vitellogenesis and ovulation.

Introduction

The Pacific keeled boa, *Candoia carinata,* is a slender bodied, moderate sized (snout-vent length to 1200 mm) snake of the islands of the Southwest Pacific. It is predominantly a denizen of lowland forest from Sulawesi eastward through the Moluccas and New Guinea to and including the Solomon Islands. Where it is sympatric with the terrestrial *Candoia aspera, C. carinata* is arboreal, although not exclusively so, and, where sympatric with the arboreal *C. bibroni,* it is terrestrial (McDowell, 1979:50).

Unlike their python relatives of Australasia, *Candoia* are viviparous. However, the reproductive biology of both boid groups from this area is poorly known. Only the green tree python, *Chondropython viridis,* has been examined in detail (e.g., Walsh, 1977) owing to its popularity as a zoo and snake-fancier's display item. Our goal has been to document as many aspects of *Candoia carinata* reproduction as possible through an examination of museum specimens.

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Materials and Methods

The analyses are based on 82 immature and adult *Candoia carinata* from the herpetological collection of the United States National Museum of Natural History (USNM); specific locality data are available from the authors. The specimens derive from much of this species' geographic range and can be subdivided into four general geographic samples: Halmahera; New Guinea; Solomon Islands; Palau Islands. Their chronological span is equally as broad, extending from 1943 to 1981.

The data obtained for each specimen are: snout-vent length; tail length; gonad or sex cell size; and collecting date. The manner of measurement is defined in Zug *et al.,* (1979). The sex of pre- and postnatal snakes was determined by the presence and size of pelvic spurs (Stickel and Stickel, 1946).

The reproductive state of females was determined by measuring follicle size. The ovarian follicles were divided into three size classes (<1.0 mm diameter, 1.0-4.9 mm, and >5.0 mm), and the number of follicles in each class and in each ovary was recorded. The approximate stage of fetal

development was noted with particular attention to near-term fetuses using Zehr's (1962) normal stages. The reproductive state of males was determined through histological examination of the seminiferous tubules and epididymis. The anterior half of the left testis was removed from each male with well-developed testes. Preparation followed standard histological procedures; sections were cut at 6 μm and stained with Harris' hematoxylin and eosin and Berg's methylene blue. The testes were scored for stage of spermatogenesis and for presence of spermatozoa in the ductus epididymidis. The external diameters of five seminiferous tubules were measured by ocular micrometer for each specimen. Portions of ovaries were also prepared histologically (H&E; 10 μm) in order to verify the presence of corpora lutea and atretic follicles.

Body Dimensions

Pre- and postnatal.—Near-term fetuses (stage 37, Zehr 1962) occur in two females and are assumed to have been within a few days to few weeks of birth. All show a very restrictive size range (Fig. 1). The average snout-vent length of the prenatal snakes is 194.2 mm (stand. deviat.=9.04; N=15). There is no apparent positive correlation between average prenatal size and female size, for the smallest female (620 mm SVL) does not have the smallest fetuses (average 201.9 mm SVL; N=7) nor the largest female (1035 mm SVL) the largest fetuses (average 187.5 mm SVL; N=8).

Postnatal snakes were defined as those possessing yolk sac scars. They range in snout-vent length from 126 to 250 mm ($\bar{X}$ =190.1 mm; s=27.18; N=14) and possess a greater size variability than the prenatal snakes (Fig. 1); one female with near-term fetuses was captured with four, presumably

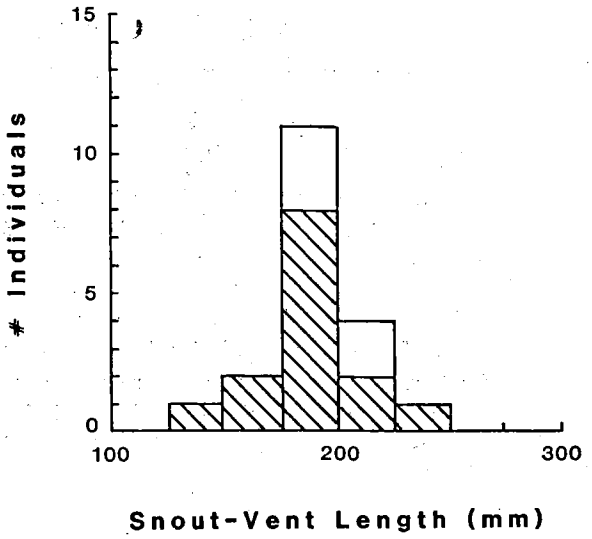

Fig. 1. The frequency of prenatal (unshaded) and postnatal (shaded) snakes by body length (snout-vent length in mm).

recent born young ($\bar{X}$=192.5 mm SVL). Some postnatal snakes are smaller than the prenatal ones. Evidence of the yolk sac scar (i.e. posteriorly notched ventrals) was observed up to 385 mm SVL, indicating that yolk sac scars persist for a long time in some *C. carinata*. The general pattern for the disappearance of the scar is: At birth, the scar possesses a blood scab, and the scab and flesh usually divide two ventral scales. A third scale anterior to these two is often partially divided or indented, and a fourth scale posterior to these is sometimes partially or completely divided. As the flesh heals, the medial edges of the ventral scales grow together (first with anterior scales and on the anterior edge of each scale) until the divided scales disappear, leaving only a narrow cleft visible on about three scales. Immediately prior to its disappearance, the scar remains only as tiny median notches on the posterior border of two or three ventral scales.

Smith (1947) and Neill (1962) indicate that young snakes may be tentatively aged by the rate of healing of the yolk sac or

umbilicus scar. Smith's data on *Thamnophis radix* indicates that 72 to 110 hours are required to close the scar from the rupture of the umbilicus, hence the date of birth can be accurately estimated in recent postnatal individuals. Neill proposes an ability to age both oviparous and viviparous snakes from hatching/birth to the snakes' fourth or fifth month of activity. Aside from a statement that the scar closes in about 4 days, Neill provides no other age specific characteristics for the process of healing and disappearance of the scar. Our sample of 14 postnatal snakes with distinct scars derives from February, March, May, August, and September. Several August and September specimens are likely to be within a week or two of birth; however, the wide temporal occurrence of yolk sac scars suggests an extended period of parturition or that scar closure in *Candoia* differs greatly from that in *Thamnophis*.

Early in our study with about eight postnates examined, a positive correlation occurred between body length and number of ventrals between scar and vent. Such a correlation would imply that the scar shifts anteriorly as the snake grows and eventually disappears. Such mobility of the scar would require: 1) the addition of new ventral scales between the scar and the vent, or 2) the progressive cleavage and fusion of ventral scales as the scar moves forward. Neither of these developmental features seems likely. To test for such scar mobility, the sample size was increased, and the correlation (Fig. 2) disappeared.

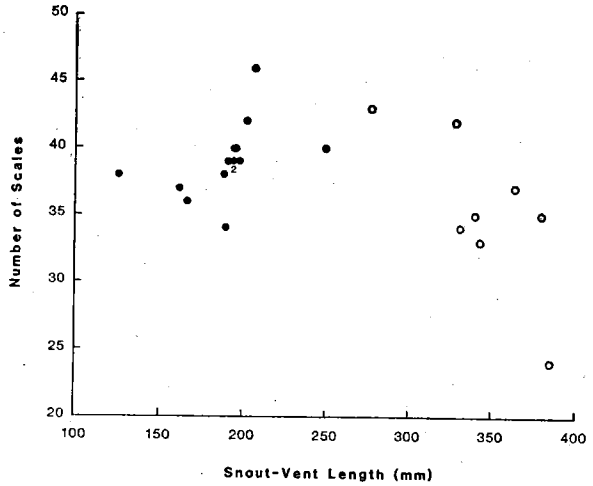

Fig. 2. The relationship of body length (mm) to the position of the yolk sac scar (number of ventral scales craniad of vent) in young *Candoia carinata*. Postnatal snakes with distinct scar are indicated by solid circles, juveniles with faint scars by open circles.

Sexual maturity.—Sexual maturity is determined by the presence of yolking follicles (i.e., follicles >5.0 mm diameter; histologically, all such follicles possess yolk platelets) in the ovary or ova/fetuses in the oviducts of the females, and the presence of spermatozoa in the ductus epididymidis or spermiogenesis in the seminiferous tubules of the males. Two females with oviducal eggs have snout-vent lengths of 315 mm and 412 mm. Thus, females can mature by 315 mm SVL. Not all females are, however, mature at this length; another female (328 mm SVL) has undeveloped ovaries with no visible follicles. The smallest male undergoing spermiogenesis and with spermatozoa in the epididymis is 340 mm SVL, and all larger males possess the characteristics of sexual maturity. In general, female and male *C. carinata* are sexually mature by about 350 mm SVL. Perhaps, some populations reach maturity at a significantly smaller size, but our limited sample and its wide geographic coverage prevent our differentiation of such a phenomenon.

Sexual dimorphism.—In the postnatal snakes, snout-vent length of males averages 200.0 mm (range 162–250 mm; s= 24.30; N=8) and of females 176.8 mm (range 126–195 mm; s=26.93; N=6). These

means are not significantly different ($t = 1.92$, $p > 0.05$). In juveniles and adults, females tend to be larger, the reverse of the situation for postnatal snakes. The average snout-vent length for juvenile and adult females is 597.6 mm (range 277–1160 mm; $s = 183.15$; $N = 55$) and 449.2 mm (range 340–605; $s = 78.50$; $N = 13$) for males. The difference of these means is statistically significant (t test for unequal variance, $t = 4.81$; $p < 0.0001$) and is strongly suggestive of sexual dimorphism in body size.

A comparison of tail length in adult snakes shows a faint trend towards sexual dimorphism. The slope of a linear regression (Y, tail length; X, SVL) for males is 0.1520 and 0.1146 for females. The tail of an adult male is, thus, slightly longer than that of a female of equivalent body length. McDowell (1979) recognizes the presence of two, largely allopatric, morphs in *Candoia carinata*: "Short-tails" and "Long-tails". The morphs are characterized by the proportion of subcaudal scales to ventral scales and not the actual or proportional tail length. Parker (1982) confirms the existence of these two morphs in the Western Province, Papua New Guinea. Our samples derive largely from the range of the "Short-tails" and our data are composed of body and tail length measurements rather than ventral and subcaudal scale counts and, hence, are inappropriate for testing the existence of the two morphs in our samples. Nonetheless, we wish to note that the difference between the slopes of the tail length to body length regression in females and males requires an analysis of subcaudal and ventral scale counts segregated by sex.

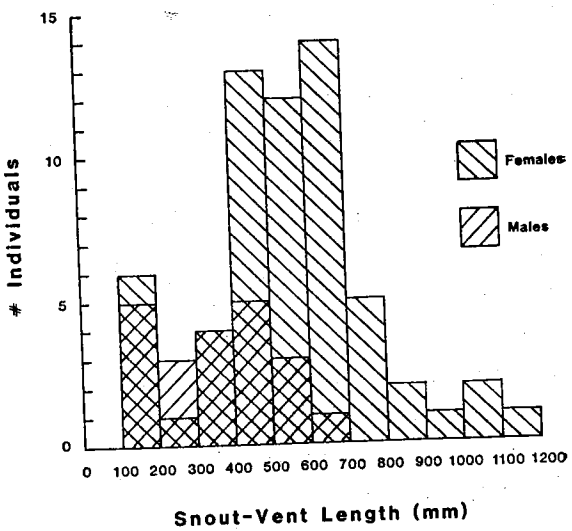

Fig. 3. Changes in sex ratio with increasing body size (age) in *Candoia carinata*. Note male and female columns are overlaid.

Reproductive Characteristics

Sex ratio.—Among immature and postnatal *C. carinata*, the sex ratio is approximately equal. Seventeen late-term embryos were removed from one pregnant female, and the sex ascertained by presence or absence of well-formed pelvic spurs on both sides of the vent. Nine of these embryos were male, seven were female, and one could not be sexed owing to poor preservation. Seven embryos were removed from another female; three embryos were males, four females. Five of seven litters born to captive *C. carinata* had nearly equal numbers of males and females; in the two other litters, males were more numerous in one litter, females in the other one (Fauci, 1981). In our sample (Fig. 3), the sex ratio is approximately equal for *C. carinata* up to a snout-vent length of about 400 mm (12 males and 11 females are less than 400 mm); thereafter, the proportion of males to females decreases. In the 500–600 mm and 600–700 mm classes, the ratios are significantly different ($\chi^2 = 5.47$, $P < .05$; $\chi^2 = 11.27$, $P < .005$, respectively). McDowell's samples (1979) also deviate significantly from a 1:1 sex ratio, although he observed geographic variation for sex ratios

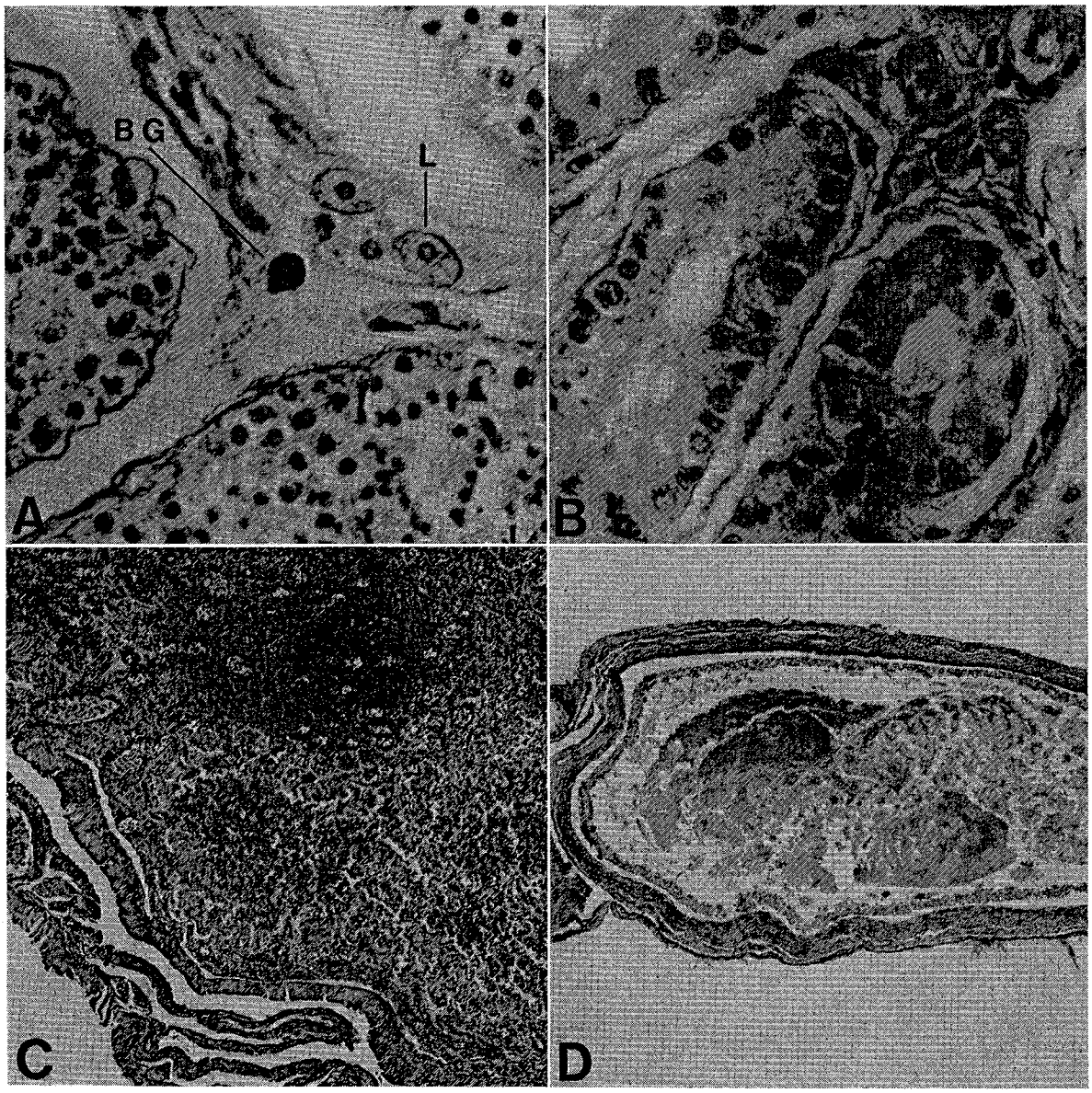

Fig. 4. Gonad histology of adult *Candoia carinata*. (A) Interstitial tissue with basophilic granulocyte (BG) and Leydig cells (L) lying between seminiferous tubules; USNM 122593 (500×). (B) Seminiferous tubule wall during regression; USNM 120058 (500×). (C) Wall of a corpus luteum; USNM 120233 (100×). (D) Atretic follicle; USNM 120060 (50×).

and had several individual samples with a 1:1 ratio. The proportional reduction in number of males cannot be attributed to a slower growth rate or to a smaller maximum size for males, because these two factors would cause an accumulation of males in the sexually mature size classes. The proportional difference may reflect a higher mortality in males or greater mobility of the females, hence their higher visibility.

Male aspects.—The testes of *C. carinata* are yellow to white, ellipsoidal organs lying within the mesorchium parasagittally along the dorsal body wall immediately anterior to the lobular kidneys. The right testis lies anterior to the left one and is usually longer. The testes have the typical amniote

Table 1. Monthly Distribution of Spermatogenic Stages for Three Samples of *Candoia carinata*. E denotes presence of spermatozoa in epididymis; the integers are the the stages of Goldberg and Parker (1975) as described in the adjacent text

	Feb	Mar	Apr	May	Jun	Sep	Oct	Dec
Halmahera	2		3 E	4 E	4/5 E		1 E	
New Guinea		2						1
Palau				4 E		5		

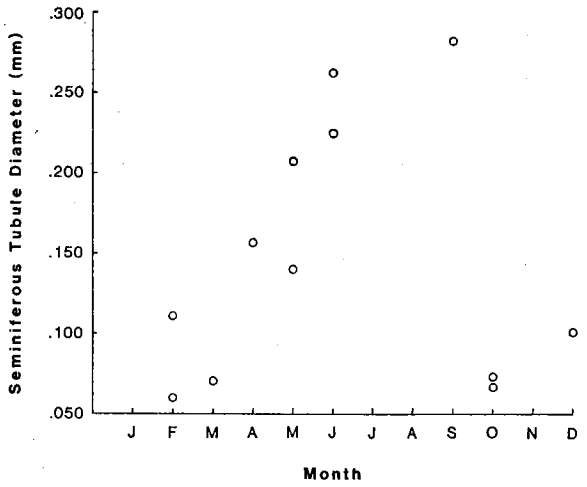

Fig. 5. Monthly variation in seminiferous tubule diameters in *Candoia carinata*. Each circle represents the mean external diameter of five tubules for one individual.

structure, composed of a mass of convoluted seminiferous tubules surrounded by a loose network of interstitial tissue and encased in a smooth connective tissue capsule. The interstitial tissue (Fig. 4A) is composed of Leydig cells (singly or in small clusters), small blood vessels, and basophilic granulocytes in a thin web of connective tissue. No segmentation is apparent within the testes; neither is zonation apparent within individual tubules. Spermatogenesis appears to progress at the same stage throughout the length of a tubule.

The spermatogenic cycle follows the general pattern described by Volsøe (1944) and can be divided into stages as proposed by Goldberg and Parker (1975). Testes showing active spermatogenesis (stages 4 and 5) contain a predominance of secondary spermatocytes and spermatids in various stages of metamorphosis. During spermiogenesis, the seminiferous tubules reach their greatest diameter with the greatest thickness of the germinal epithelium. The ductus and ductuli epididymides, lined by cuboidal or low columnar epithelium, are filled with spermatozoa. Upon the completion of spermiogenesis, the thickness of the germinal epithelium reduces (regression, stages 6 and 1) to a single layer of spermatogonia and Sertoli cells (Fig. 4B), and there is a corresponding shrinkage of the seminiferous tubule diameter. Of the specimens showing regression, only one had spermatozoa in the epididymis. With the renewal of spermatogenesis (recrudescence), the spermatogonia become more numerous and primary spermatocytes appear (stage 2). The germinal epithelium, thus, slowly increases in thickness, primary spermatocytes divide into secondaries, and secondary spermatocytes, in turn, divide into spermatids (stage 3). The increasing thickness in the epithelium is accompanied by gradual enlargement of seminiferous tubule diameter.

Although our sample size is small and geographically widespread, both the monthly distribution of spermatogenesis (Table 1) and that of seminiferous tubule diameter (Fig. 5) indicate an annual cycle. Excluding the Palau sample for September,

tubule diameter shows recrudescence from February through June and regression from August through February. This pattern is more explicit in the spermatogenic stages (Table 1). If *Candoia carinata* follows the general pattern of temperate snakes, males court and copulate in the regression months, August-February.

Female aspects.—The ovaries are sac-like structures within the mesovarium. Each wall is composed of a layer of dense irregular connective tissue covered on the inside and outside by an epithelial covering. The follicles form within the ovarian wall, and project into the central lumen as they grow. The left ovary lies posterior to the right, and length varies with the number of developing follicles.

Primordial follicles occur in isolated clumps near the surface of the ovary. In follicles less than 5.0 mm diameter, a refractile zona pellucida surrounds the oocyte. Clear divison into a zona radiata and an outer zone is not apparent. A layer of large pyriform cells surrounded internally and externally by a layer of smaller cells forms the granulosa layer. In follicles greater than 5.0 mm, the large pyriform cells in the granulosa layer are much reduced in size; a zona radiata and outer zone are clearly seen in the zona pellucida, and numerous droplets, possibly lipid in content, fill the peripheral ooplasm. The theca is always thin and not well differentiated into a theca interna and externa. In atretic follicles, however, the theca interna appears as a thin, less collagenous layer, and in several corpora lutea, the theca externa appears as a wavy, collagenous layer, much less vascularized than the highly vascular theca interna (Fig. 4c & d).

In all females with evidence of past ovulation (e.g., oviducal eggs, embryos, or, in one specimen, a distended oviduct) flattened, dark-colored corpora lutea are visible upon gross examination. They contrast distinctly to the usually rounded, cream-colored follicles and occur in a one-to-one correspondence to the number of oviducal eggs or embyros in the female.

A corpus luteum from a specimen with late-term embyros had a distinct theca externa, composed of a thick layer of wavy, collagenous fibers with nuclei concentrated near the peripheral edge, and a theca interna, also thickened but less collagenous and highly vascular. The luteal cells have a flocculent cytoplasm and pigment granules, and are scattered through a stroma of connective tissue. Numerous blood vessels are also present (Fig. 4c). Post-parturition corpora lutea have less pigment in the luteal cells and a much greater abundance of connective tissue.

Most ovaries also possess discolored and abnormally shaped follicles. These are usually milky-white in appearance, but sometimes are darkly colored much like corpora lutea. Histological examination shows these to be atretic follicles. Although the appearance of atretic follicles depends on the degree of development before the onset of atresia and on the stage of atresia, the observed follicles share a number of characteristics. Both the theca externa and interna are thicker and clearly differentiated. In earlier stages of atresia, the granulosa layer is reduced in thickness and no longer clearly polymorphic. The ooplasm is disrupted and sometimes darker in color (Fig. 4d). In later stages of atresia, the granulosa cells coalesce in the central lumen and remnants of yolk remain. Remnants of the zona pellucida also persist at this stage.

Since our sample size is small, we recognized only three follicular classes:

Table 2. Monthly Distribution of Oogenic, Oviducal, and Postnatal Stages for Four Samples of *Candoia carinata*. Abbreviations: Oogenesis—G germination, H hydration, V vitellogenesis; E embryos; O oviducal eggs, embryos not apparent; P postnatals or hatchlings. The integers denote the number of individuals

	Jan	Feb	Apr	May	Jun	Jul	Aug	Sep	Oct	Dec
Halmahera			G H/3	G H/2	H/2 V/2		G H/2		G	G/2 H
New Guinea				V	G H/3 V O				H	
		P		P						
Solomons	H					H	H/3 V/3 E P/4	G H/2 V P/5	H V E	
Palau						O		G H		
		P								

<1.0 mm diameter, germination; 1.0–4.9 mm, hydration; >5.0, vitellogenic. Table 2 shows the largest follicle class recorded in each female, by locality, for month collected. This monthly distribution provides no strong indication of seasonality for ovulation. Vitellogenic follicles and embryos in females occur from May through October; however, their absence in other monthly samples may be due to small sample size.

Actual litter size is limited to two females with advanced fetuses (Zehr stage 37); these fetal litters were 7 and 17. In captivity, one female gave birth to 64 young (Murphy *et al.*, 1978); four other captive females had litters of 16 to 27 young (Fauci, 1981). Litter size estimated from vitellogenic follicles ranges from 1 to 30. Many of these latter estimates exceed the actual oviducal litter data. Such disparity occurs in other snakes, e.g., *Dipsas catesbyi* (Zug. *et al.*, 1979), and is reinforced by the presence of atretic follicles in *Candoia carinata*.

Discussion and Summary

The data from the female sample is equivocal and can be treated as evidence for a cyclical or non-cyclical pattern. Snakes with embryos, oviducal eggs, or vitellogenic follicles (diameter >5 mm) occur in the months of May through October. If only embryos and oviducal eggs are considered, the monthly distribution is June through October. This ignores, of course, regional variation; our monthly sample is too small to be more than suggestive for individual localities. In the Solomons, it must occur for a period of at least three months, because two specimens (August and October) had late-term embryos.

One interpretation of these data is the

presence of continuous reproduction. Samples from the months where no vitellogenic follicles (>5 mm), oviducal eggs, or embryos were found (November-April) are small or nonexistent. Only in the months of June, August, and September do the samples consist of more than five animals. Failure to find evidence of continual reproduction may be more a product of sample size than a real pattern.

The evidence for a cyclical pattern does, however, appear in the male reproductive data. Seminiferous tubule diameter was lowest during the months of February, March, October, and December, and highest in April, May, and June (see Fig. 5). The seminiferous epithelium appears to go through an annual cycle in which the epithelium is low and only contains spermatogonia and Sertoli cells in the October and December specimens (subadults also possess this stage), through a stage in which it thickens with the addition of layers of primary spermatocytes in the February and March specimens, to the stage dominated by spermatocytes (particularly secondaries) in April and May, and full spermiogenesis in June and September. Regression occurs in October and December specimens. These data would support an interpretation that copulation occurs during the late dry season. Only a September specimen does not fit this pattern, and this adult male is from the Palau Islands, considerably to the north of the other localities and perhaps has a later breeding period.

Both sexes reach sexual maturity by about 350 mm SVL. The occurrence of mature females at 315 and 412 mm SVL suggests that sexual maturity may occur at different sizes (perhaps different ages) in different populations. In fact, the data herein are more suggestive than absolute owing to the wide geographic and temporal composition of our samples. Our data suggest a striking size dimorphism with females attaining body lengths nearly double that of the largest males. Similarly adult females are more numerous in the USNM collection, thereby producing strongly skewed sex ratios for adults. These data and other data such as clutches of 1–30 follicles must serve as preliminary hypotheses to be tested by larger samples of limited geographic range collected on a monthly schedule.

Literature Cited

Fauci, G. (1981) Breeding and rearing of captive Solomon Island ground boas, *Candoia carinata paulseni*. Herpetol. Rev., **12**, 60–61.

Goldberg, S. R. and Parker, W. S. (1975) Seasonal testicular histology of the colubrid snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. Herpetologica, **31**, 317–322.

McDowell, S. B. (1979) A catalogue of the snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum. Part III. Boinae and Acrochordoidea (Reptilia: Serpentes). J. Herpetol., **13**, 1–92.

Murphy, J. B., Barker, D. G. and Tryon, B. W. (1978) Miscellaneous notes on the reproductive biology of reptiles. 2. Eleven species of the family Boidae, genera *Candoia, Corallus, Epicrates* and *Python*. J. Herpetol., **12**, 385–390.

Neill, W. T. (1962) The reproductive cycle of snakes in a tropical region, British Honduras. Quart. J. Florida Acad. Sci., **25**, 234–253.

Parker, F. (1982) The snakes of Western Province. Wildl. Papua New Guinea, **82/1**, 1–78.

Smith, A. G. (1947) Navel closure time and age in the young of *Thamnophis radix*. Herpetologica, **3**, 153–154.

Stickel, W. H. and Stickel, L. F. (1946) Sexual dimorphism in the pelvic spurs of *Enygrus*. Copeia, **1946**, 10–12.

Volsøe, H. (1944) Structure and seasonal variation of the male reproductive organs of *Vipera berus* (L.). Spolia Zool. Mus. Hauniensis, **5**, 7–157.

Walsh, T. (1977) Husbandry and breeding of *Chondropython viridis*. Nat. Assoc. Sound Wildl. Prog. Newsl., **1**, 10–22.

Zehr, D. R. (1962) Stages in the normal development of the common garter snake, *Thamnophis*

sirtalis sirtalis. Copeia, **1962**, 322–329.

Zug, G. R., Hedges, S. B. and Kunkel, S. (1979) Variation in reproductive parameters of three neotropical snakes, *Coniophanes fissidens, Dipsas catesbyi*, and *Imantodes cenchoa*. Smiths. Contrib. Zool., **300**, 1–20.

要　　約

Candoia carinata の繁殖についての観察

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Candoia carinata は頭胴長 350 mm で性成熟に達する。メスの成蛇はオスよりも大きく，性的二型を示唆しており，また数もメスの成蛇の方が多い。配偶子形成はオスでは周期的かつ季節的で，精子形成は 5～6 月に起こる。一方，メスでは卵黄形成や排卵に季節性はない。

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