



## ***Schistosoma ovuncatum* n. sp. (Digenea: Schistosomatidae) from northwest Thailand and the historical biogeography of Southeast Asian *Schistosoma* Weinland, 1858**

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### **Abstract**

*Schistosoma sinensium* Bao, 1958 was first isolated from an unidentified snail in Sichuan Province, PR China. This species was apparently rediscovered in Chiang Mai Province, northwest Thailand (Baidikul et al., 1984); the definitive host was the rat *Rattus rattus* and the intermediate host was the snail *Tricula bollingi*. In this paper *S. sinensium* is rediscovered in Sichuan Province and compared with worms recovered from experimentally infected mice, which had been exposed to cercariae shed by *T. bollingi* from Chiang Mai. Evidence is presented suggesting that the schistosome collected by Baidikul was not *S. sinensium* and that a new species is involved. The new species, named *Schistosoma ovuncatum* (etymology: *ovum* (egg) + *uncatus* (hooked)), is described and compared with related taxa. All previous papers on the Thai schistosome have used worms recovered from field-collected rodents only; this is the first account in which the life-cycle has been completed in the laboratory, using cercariae shed by *T. bollingi*, and the resulting worms described. *S. ovuncatum* differs from *S. sinensium* in terms of size and shape of body and egg, number of testes, size of ovary, length of vitellarium, intermediate host and biogeographical distribution. The relationships of the two taxa and their position with respect to the *Schistosoma indicum*- and *S. japonicum*-groups are discussed. The implications of the findings for the evolution of human schistosomiasis in the region are also commented upon.

### **Introduction**

In 1956 schistosome cercariae were observed emanating from a caenogastropod snail resembling *Oncomelania* (Gastropoda: Pomatiopsidae) and collected in Mianzhu County, Sichuan Province, PR China (Figure 1). Laboratory infections using these cercariae led to the discovery of a new species, namely *Schistosoma sinensium* Bao, 1958. The morphology of the adult worm, cercariae and ova, as well as the pomatiopsid snail intermediate host, serve to distinguish this species from others with a conspicuously spined egg. The lack of eye bulges, suprapedal fold or elongated spermathecal duct and the mode of progression suggest that the Mianzhu snail (Figure 2A) is not *On-*

*comelania*. Indeed, Sun (1959) suggested that the snail involved was a species of *Tricula* (Gastropoda: Pomatiopsidae), such as *T. humida* or *T. gregoriana*. Greer et al. (1989) cited a later paper (Pao, 1959) as the first use of the name *S. sinensium*. However, in this paper Bao (1958) is given as the authority for this taxon, as first adopted by Baidikul et al. (1984). It has since become apparent that Pao is simply a different transliteration of Bao.

In 1983 field rats trapped around a stream at Wat Tam-Tab-Tao, Fang District, Chiang Mai Province, northwest Thailand, were examined (Baidikul et al., 1984) and their livers found to contain eggs bearing a subterminal spine and resembling those described by Bao (1958). Of the snails collected from the stream,

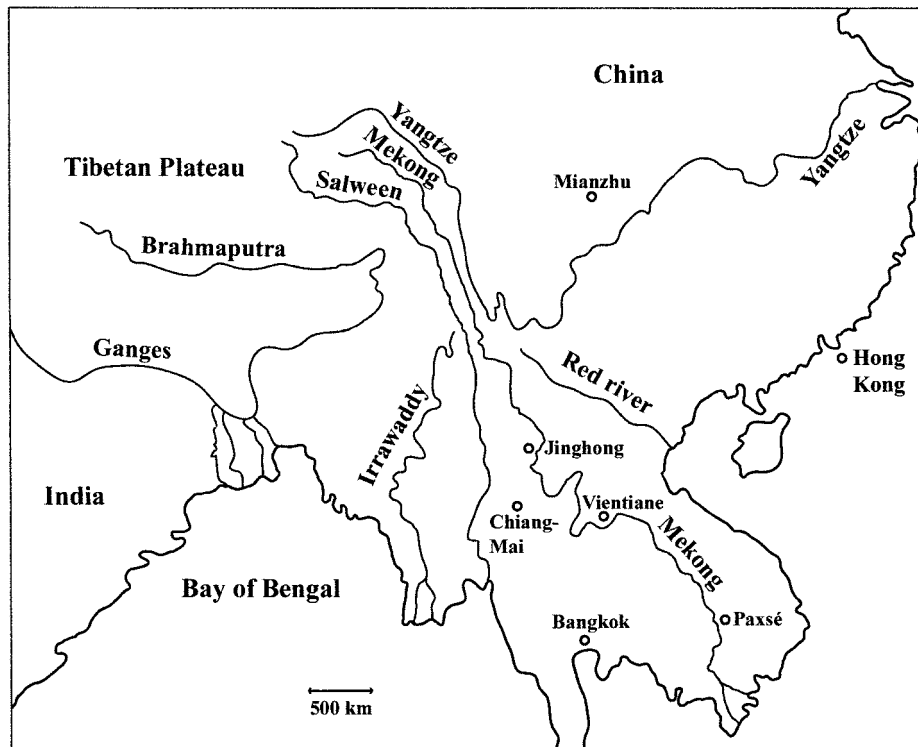


Figure 1. The major rivers draining the eastern margins of the Tibetan Plateau and the type localities for *Schistosoma ovuncatum* n. sp. and *S. sinensium* near Chiang Mai, northwest Thailand, and Mianzhu, Sichuan Province (PR China), respectively. Scale approximate.

*Tricula bollingi* Davis, 1968 (Figure 2B) was found to shed furcocercous cercariae that lacked eye-spots. The natural infection rate in *T. bollingi* (no. snails shedding cercariae  $\times 100/\text{no. examined}$ ) was found to be 0.62%, comparable with Bao's (1958) figure of 0.55%. Baidikul et al. (1984) did not provide a description of the worms involved, which they assumed to be *S. sinensium*. Like Bao (1958), Baidikul and his co-workers failed to detect the eggs of this schistosome in stool samples taken from local villagers. The definitive hosts of both schistosomes were thus assumed to be *Rattus rattus* and other species of that genus. In 1981 the same (Thai) *T. bollingi* population was sampled and 2.7% of snails found to shed cercariae (Greer et al., 1989). Again, eggs with a conspicuous subterminal spine were reported from perfused field-collected rats and from laboratory animals exposed to cercariae arising from *T. bollingi*. In this case the life-cycle of the schistosome was completed in the laboratory; however, these authors obtained eggs from laboratory infections only and again based their anatomical descriptions on specimens collected from trapped field rats. *S. sinensium*-like schistosomes, and relevant snails, have proven difficult to collect and

maintain; this has often limited the scope of past studies despite considerable effort on the part of the researchers. The worms described by Greer et al. (1989) differed from those of Bao (1958) in that the ventral sucker of the male was smaller and positioned more anteriorly, reunion of the gut caeca occurred in a more anterior position, there were fewer testes, the worms were spined, and in the female Thai worm the ovary was longer (see Table 1). In view of these differences, the missing data in Bao's (1958) account, and uncertainty regarding the origin of eggs observed in earlier studies, a taxonomic reappraisal of these taxa was called for.

*Tricula bollingi* is assigned to the Pomatiopsidae, a family of conservative rissocean snails comprising two subfamilies: the Pomatiopsinae, to which the intermediate hosts of *Schistosoma japonicum* Katsurada, 1904 (i.e. *Oncomelania hupensis* sub-spp. Gredler, 1881) are assigned, and the Triculinae, which includes *Neotricula aperta* (tribe Pachydrobiini) the intermediate host of *Schistosoma mekongi* Voge, Bruckner & Bruce, 1978 in Laos and Cambodia. *T. bollingi* is assigned to the Triculinae, tribe Triculini. The modern Triculinae are entirely Southeast Asian and southern

Table 1. Measurements from *Schistosoma sinensium* and *S. ovuncatum* n. sp. Data are means followed by the range and sample size in parentheses. Precision  $\pm 5 \mu\text{m}$ , except for proportional data or quotients, which are given to two significant figures. Counts (e.g. number of testes) are given as modal values. Data in the right-most column are taken from the paper by Bao (1958) and significant differences from *S. ovuncatum* (i.e. mean lies outside the central 90% of range for the latter) are highlighted. Ranges reported by Bao (1958) for widths may not be equivalent to those in the present study, as a result of differences in measurement protocol.

Species	<i>S. sinensium</i>	<i>S. ovuncatum</i>	<i>S. sinensium</i>
Source	Present data	Present data	Bao (1958)
Body l. (M) L	2,235 (1,425-2,985, 5)	4,970 (4,210-6,090, 13)	<b>3,425</b> (3,200-3,650, 6)
Body l. (F) L	3,940 (3,835-4,040, 2)	4,230 (3,240-4,725, 9)	3,550 (3,300-3,800, 4)
Max. body w. (M) W	210 (170-275, 5)	355 (260-470, 12)	<b>270</b> (230-270, 6)
Max. body w. (F) W	120 (110-130, 2)	260 (155-300, 9)	<b>150</b> (130-150, 4)
Width at vs (M) w	95 (70-130, 5)	215 (150-260, 12)	-
Width at vs (F) w	20 (15-20, 2)	75 (60-100, 9)	-
W/w (M)	2.3 (1.9-2.5, 5)	1.7 (1.3-3.0, 12)	-
W/w (F)	6.1 (6.0-6.2, 2)	3.4 (2.3-4.8, 9)	-
L/W (M)	11 (8.5-17, 5)	14 (11-18, 12)	-
L/W (F)	33 (30-37, 2)	17 (14-21, 9)	-
Diam. oral sucker (M)	70 (55-90, 5)	180 (140-220, 12)	<b>140</b> (130-150, 6)
Diam. oral sucker (F)	20 (21-24, 2)	60 (45-80, 8)	-
Diam. ventral sucker (M)	190 (150-250, 5)	270 (215-315, 12)	250 (240-265, 6)
Diam. ventral sucker (F)	45 (45-45, 2)	65 (50-80, 8)	-
Forebody (M)	255 (200-360, 5)	405 (275-560, 11)	<b>600</b> (? , 6)
Forebody (F)	85 (85-90, 2)	160 (120-225, 6)	-
Ventral sucker position, anterior fraction of worm:			
M	0.2 (0.1-0.2, 5)	0.1 (0.1-0.3, 11)	-
F	0.0 (0.0-0.0, 2)	0.1 (0.0-0.1, 6)	-
Caeca union, posterior fraction of worm:			
M	0.1 (0.0-0.2, 5)	0.1 (0.1-0.2, 10)	<b>0.3</b> (? , 6)
F	0.3 (N = 1)	0.4 (0.4-0.4, 6)	-
Caeca l. (M)	1,775 (1,035-2,570, 5)	3,350 (2,525-4,120, 10)	-
Caeca l. (F)	3,910 (3,905-3,910, 2)	3,850 (2,885-4,390, 6)	-
Testes l.	55 (40-70, 5)	105 (70-125, 11)	-
Testes w.	30 (20-40, 5)	55 (35-70, 12)	-
Length of testicular chain	195 (160-245, 5)	260 (185-385, 12)	-
Number of testes	9 (8-9, 5)	7 (6-7, 12)	<b>8</b> (N = 6)
Extent of vitellarium	0.6 (0.5-0.7, 2)	0.4 (0.4-0.4, 7)	<b>0.6</b> (? , 4)
Ovary l.	300 (280-315, 2)	315 (215-450, 9)	300 (? , 4)
Ovary w.	55 (55-55, 2)	140 (100-175, 9)	<b>80</b> (? , 4)
Eggs <i>in utero</i>	(0-1, 2)	15 (1-20, 8)	<b>1</b> (1-1, 4)

The abbreviations M and F refer to male and female (respectively), diam. to diameter, l. to length, w. to width and vs to ventral sucker. Extent of vitellarium refers to the proportion of the body occupied by the vitelline gland (measured from the posterior).

Chinese in distribution. In this paper the Mianzhu snail is referred to as a species of *Tricula*; however, this is unlikely to be *T. gregoriana* or *T. humida* as there are differences in radular characteristics and marked conchological differences (e.g. number of whorls, depth of suture, etc.). In addition to *N. aperta* and *O. hupensis*, several taxa allied to *T. bollingi* are known to act as intermediate host for *Schistosoma* spp. The snail

*Robertsia kaporensis* Davis & Greer, 1980 (Triculiinae: Pachydrobiini) transmits *Schistosoma malayensis* Greer, Ow-Yang & Yong, 1988 in peninsular Malaysia, mainly affecting the aboriginal peoples of the region. *Jinhongia jinhongensis* (Guo & Gu, 1985) (Pachydrobiini) is also reported to transmit a species of *Schistosoma* in tributaries of the Mekong River in Yunnan Province, PR China. Clearly, several lineages

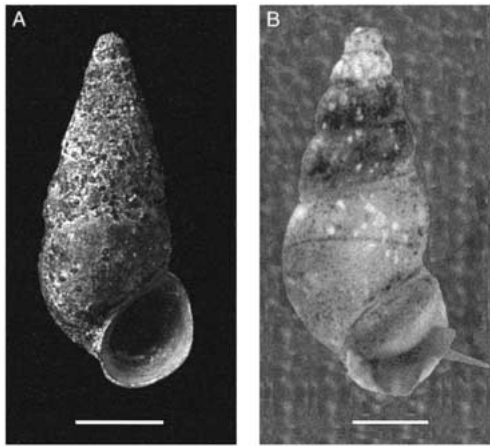


Figure 2. A. The Mianzhu snail, intermediate host for *Schistosoma sinensium*, a species of *Tricula*. B. *Tricula bollingi*, the intermediate host for *Schistosoma ovuncatum* n. sp. in northwest Thailand. Scale-bars: 1 mm.

within the Pomatiopsidae are characterised by the ability to act as host for *S. japonicum*-group species, and reciprocal illumination between snail and schistosome phylogenies can strengthen our understanding of schistosome phylogenetics in Asia.

Earlier biogeographical data, such as those employed by Davis (1980) and Davis et al. (1992) indicated that the major isolating events affecting pomatiopsid taxa occurred around 12 million years ago (mya). Consequently, the mid-Miocene was regarded as the point at which precursors of present day *S. japonicum*-group species became isolated in the different rivers draining the Tibetan Plateau. These rivers drained into and across southern China and Southeast Asia (Figure 1). For example, pre-*S. japonicum* may have become isolated in amphibious Pomatiopsidae of the Yangtze River drainage (southern China), and pre-*S. mekongimalayensis* in Triculinae of the evolving Mekong, Salween and/or Irrawaddy River systems. However, more recent palaeogeographic models suggest a later separation of these drainage systems (see Xu, 1981; Hutchinson, 1989; Hall, 1998) and diversification of the *S. japonicum*-group during the Pliocene. Furthermore, such models suggest that *S. sinensium* and the Thai schistosome are likely to be different species, having been separated over the last 5 million years. *S. sinensium* and similar taxa in southern China and northern Thailand, on the eastern margin of the Tibetan Plateau, are key taxa in our models of the origin and evolution of *Schistosoma* in Southeast Asia. Consequently, the present study was also moti-

vated by a need to test the predictions of new historical biogeographical models for the *S. japonicum*-group.

## Materials and methods

### Collection of specimens

Schistosomes were collected from the intermediate host as follows. *Tricula bollingi* were collected on 30.v.2000 from a small, clear stream (maximum depth about 80 cm) at Wat Tam-Tab-Tao, 120 km north of Chiang Mai, Fang District, Chiang Mai Province, northwest Thailand (19°39'45"N; 99°7'00"E) (Figures 1, 2). This site lies in the Ping River valley near the Thai-Burmese border. The snails were then returned live to the Faculty of Science, Mahidol University, Bangkok. Screening on 1.vi.2000 revealed an infection rate of 0.45% among the 880 snails collected. The period of cercarial emergence was between 16:30 and 23:00, with the peak around 21:00. Cercariae emerged after the snails had been kept in the dark for 2 h. The life-cycle was completed in the laboratory using an ICR strain of white mice. Mice were anaesthetised using sodium pentobarbital and exposed by transferring cercariae to their shaved abdomens using a small wire loop. Mice were killed 8 weeks after exposure and adult worms recovered by perfusion. Worms were fixed in 10% neutral-formalin. Laboratory-reared *T. bollingi* were individually exposed to 2-3 miracidia hatched from eggs recovered from infected mouse liver. Cercariae began to emerge 7 weeks after exposure.

*Schistosoma sinensium* was collected from the intermediate host, a previously undescribed species of *Tricula*, on 12.iv.2000 in Han-wang District, Mianzhu County, Sichuan Province, PR China (30°4'10"N; 104°8'12"E). The snails were found in irrigation channels serving small market gardens and fed by spring water arising in the foot-hills of Mount Chiu-ting. Cercarial emergence was again in the scotophase. Mice were exposed to cercariae in the laboratory of the Mianzhu anti-schistosomiasis brigade following the procedure above. The infection rate among the snails sampled at Han-wang was 0.42%. As with the Thai material, worms were recovered 8 weeks after exposure. Voucher specimens of the *S. sinensium* material are housed in The Natural History Museum, London (NHM), Reg. No. 2001.1.8. 14-17 (spirit) and 2001.1.8. 12-13 (mounted permanent slides).

### Preparation and examination of specimens

The worms were stained with acetocarmine, counter-stained with fast green and morphometric data were then recorded. They were subsequently mounted in Permount and photographed. Measurement of unmounted worms allowed manipulation during measuring and avoided distortion due to squashing. Eggs and cercariae were measured live (the eggs in normal saline), after the latter had been immobilised by cooling (2-4 °C). Two eggs were dissected from one of the female worms of each species and were found to be of the same shape as those recovered from the liver samples. The eggs dissected from the worms were smaller (by about 7%) than those recovered from liver samples. The data in Table 2 refer to measurements of eggs from liver preparations only. Many of the eggs in the preparations were observed to hatch and the miracidia were used to complete the life-cycle in the laboratory. Consequently, the eggs measured were probably mature or nearly mature. Of the 13 male Thai specimens measured, 11 were paired with a female worm; the unpaired males did not show significant morphometric differences from the other male worms (possibly having been paired *in vivo*). In the case of *S. sinensium* two males were paired with whole female worms and one with a female worm fragment. Again, the two apparently unpaired male *S. sinensium* did not show morphometric differences sufficient to warrant division of the data-set to distinguish paired and unpaired worms. In both taxa a number of worms was recovered that had suffered marked damage and these were excluded from the measurements. All measurements are given in micrometres; these are maximum values unless otherwise specified. For example, the testes width is the maximum width of the first or last testis (whichever was the larger).

Measurements were taken from the lateral aspect of the specimens. Forebody length refers to the distance from the anterior margin of the ventral sucker to the anterior extremity of the worm. The body width at the ventral sucker was measured just posterior to the ventral sucker peduncle. Table 1 also gives the fraction of the total body length lying anterior to the ventral sucker (rows 18, 19) and the fraction lying posterior to the reunion of the gut caeca (rows 21, 22). In the male, measurements of body width exclude the folds of the gynaecophoric canal. In the female the proportion of the total body length occupied by the vitellarium, lying in the posterior part of the worm, was also recorded (Table 1). Measurements of cercarial furcae are given as the length of a single furca of the pair. The 8 week-

Table 2. Measurements of eggs and cercariae for *Schistosoma sinensium* and *S. ovuncatum* n. sp. Data are mean followed by the range and are presented with an accuracy of  $\pm 5 \mu\text{m}$ . Data in the right-most column are taken from the paper by Bao (1958) and significant differences from *S. ovuncatum* are highlighted (i.e. mean lies outside the central 90% of the range for the latter).

Species	<i>S. sinensium</i>	<i>S. ovuncatum</i>	<i>S. sinensium</i>
Source	Present data	Present data	Bao (1958)
<i>Egg</i>			
N	5	5	100
Length	105 (95-115)	70 (65-80)	<b>105</b> (95-115)
Width	40 (40-40)	45 (40-45)	45 (40-50)
Spine length	15 (10-20)	5 (5-5)	<b>20</b> (15-20)
<i>Cercaria</i>			
N	5	5	?
Body length	160 (160-165)	125 (110-145)	<b>160</b> (?)
Body width	65 (60-65)	75 (70-80)	<b>70</b> (?)
Tail length	140 (140-145)	140 (140-145)	140 (?)
Tail width	35 (35-40)	30 (25-30)	<b>35</b> (?)
Furcal length	90 (85-90)	70 (50-110)	90 (?)

old worms were adults, and gravid females were found in both the Thai and Sichuan samples; Bao (1958) also reported the onset of oviposition to be between 5.5 and 10 weeks. Bao's worms were 10 weeks old when examined; however, animals were killed at 8 weeks in the present study to avoid suffering and morbidity in experimental hosts. Collection of *T. bollingi* was difficult, as the snail was rare and patchily distributed; the infection rates were also low. Access to material and collection sites in China was restricted and facilities for the collection and examination of worms were limited. Consequently, the sample sizes are smaller than would be possible with more accessible taxa. The methods employed were developed in keeping with the facilities available in the endemic areas.

### *Schistosoma ovuncatum* n. sp.

*Type-host*: *Tricola bollingi* Davis, 1968 (intermediate host).

*Definitive host*: Unknown. Possibly *Rattus rattus*, according to Baidikul et al. (1984).

*Laboratory host*: *Mus musculus*.

*Type-locality*: Wat Tam-Tab-Tao, Fang District, Chiang Mai Province, Northwest Thailand.

*Type-specimens*: Holotype (female) and allotype (male) collected *in copula*, NHM Reg. No. 2001.1.8.1

(mounted, permanent slide); Paratypes, NHM Reg. No. 2001.1.8. 2-11 (spirit). 13 male and 9 female worms were examined.

*Etymology*: The egg of this species bears a hook-like spine, hence the name from *ovum* (egg) and *uncatus* (hooked).

#### *Description* (Figures 3-5)

##### *Female (holotype)* (Table 1, Figure 3)

Body filiform but posterior half expanded; posterior extremity tapered to point; anterior terminates in small round oral sucker. Length 4,725 holotype (range of all specimens 3,240-4,725); maximum body width 280 (260-470) at level of middle of vitelline field. Body width just anterior to uterus 60 (60-100); marked constriction present in female just posterior to ventral sucker. Tegument smooth, lacking tubercles or spines. Diameter of oral sucker 60 (45-80). Ventral sucker round, supported by fleshy peduncle, protruberant in lateral view, diameter 65 (50-80), arises in anterior 5% of worm. Forebody 160 (155-265) (Table 1). Pharynx absent. Oesophagus long, sigmoid, with pronounced glands (oesophageal bulb) in forebody. Caecal bifurcation just anterior to ventral sucker, reunion at c. 60% of body length (Table 1), just posterior to ovary. Length of caeca in hindbody 4,390 (2,885-4,390), terminating blindly 180 from posterior extremity of worm.

Vitellarium follicular; field extensive, occupies posterior 45% of worm (Table 1), being 2,100 long; follicles flask-shaped, present either side of gut caeca. Immediately anterior to vitelline field lies swollen section of oviduct leading from ovary (presumably this functions as seminal receptacle; other forms of seminal receptacle absent). Ovary position equatorial, coiled and tapered anteriorly, length 315 (215-450), width 135 (100-175) (Figure 3B). Uterus elongate, coiled intercaecal, extending between ovary and ventral sucker, contains > 15 eggs. Egg suboval, with one side having less curvature (Figure 4A); short, hook-shaped, subterminal spine arises from adapical edge of flattened region at base of side of least curvature; axis of spine either parallel to side of least curvature or tilted at 45° so that spine is flattened against base of egg (unembryonated state); of > 50 eggs observed from livers of 2 mice, all bore hook-shaped spine as described above – none resembled egg of *S. sinensium* described by Bao (1958) or Greer et al. (1989).

##### *Male (allotype)* (Table 1, Figures 3A, 5)

Body elongate, scuteiform at posterior limit and ter-

minating anteriorly with oral sucker. Length 4,480 (4,210-6,090); maximum body width of 390 (260-470) occurs at mid-body. Constriction is present at anterior limit of hindbody; body width at this point 255 (150-260). Tegument smooth and lacking tubercles or spines. Ventral sucker as in female but diameter much greater being 270 (215-315), arises at 10% of body length (Figure 5A). Forebody 270 (275-560). Diameter of oral sucker 160 (140-220). Oesophagus as female. Caeca appear to reunite at 85% of body length, with length in hindbody 3,285 (2,525-4,120), terminating c. 100 from posterior extremity.

Testes 7 (6-7), ovoid, contiguous in single row arising in anterior hindbody, just posterior to origin of gynaecophoric canal (Figure 5A), form chain, with first and last testes generally larger than others (testes rarely overlap), length 115 (70-125), width 70 (35-70); length of testes chain 290 (185-385). Vas deferens runs along ventral margin of anterior testes chain into intercaecal cirrus-sac. Male genital pore opens into anterior margin of gynaecophoric canal. Cirrus-sac length 65  $\mu\text{m}$ , width 55  $\mu\text{m}$  (Figure 5B). Gynaecophoric canal well developed (Figure 3B); canal reaches almost to posterior extremity.

##### *Cercaria* (Figure 4B)

*Cercaria furcocercous*, apharyngeate and spined. Resting cercaria found floating at water's surface with tail curved downward. Cercariae resemble those of *S. japonicum* and *S. mekongi* in that they swim only at water's surface and not throughout water column.

#### *Comparison with new material of Schistosoma sinensium*

##### *Male* (N = 5) (Table 1, Figure 6)

The average length of the male *S. sinensium* after 8 weeks was 2,235  $\mu\text{m}$ , ranging from 1,425 to 2,985; this is slightly less than the 3,200 to 3,650  $\mu\text{m}$  reported by Bao (1958); however, the worms were some 10 weeks old in Bao's study. The average length of male *S. ovuncatum* n. sp. (4,970  $\mu\text{m}$ ) exceeds that of *S. sinensium* in both the present study and in Bao's original description. Similarly, the maximum body width of 355  $\mu\text{m}$  recorded in the present study for *S. ovuncatum* exceeds that recorded for *S. sinensium* by Bao (270  $\mu\text{m}$ ) and by the present authors (210  $\mu\text{m}$ ). The body width, immediately posterior to the ventral sucker, recorded for *S. sinensium* was also less than that for *S. ovuncatum* (Table 1); this again suggests that male *S. sinensium* tends to be a smaller

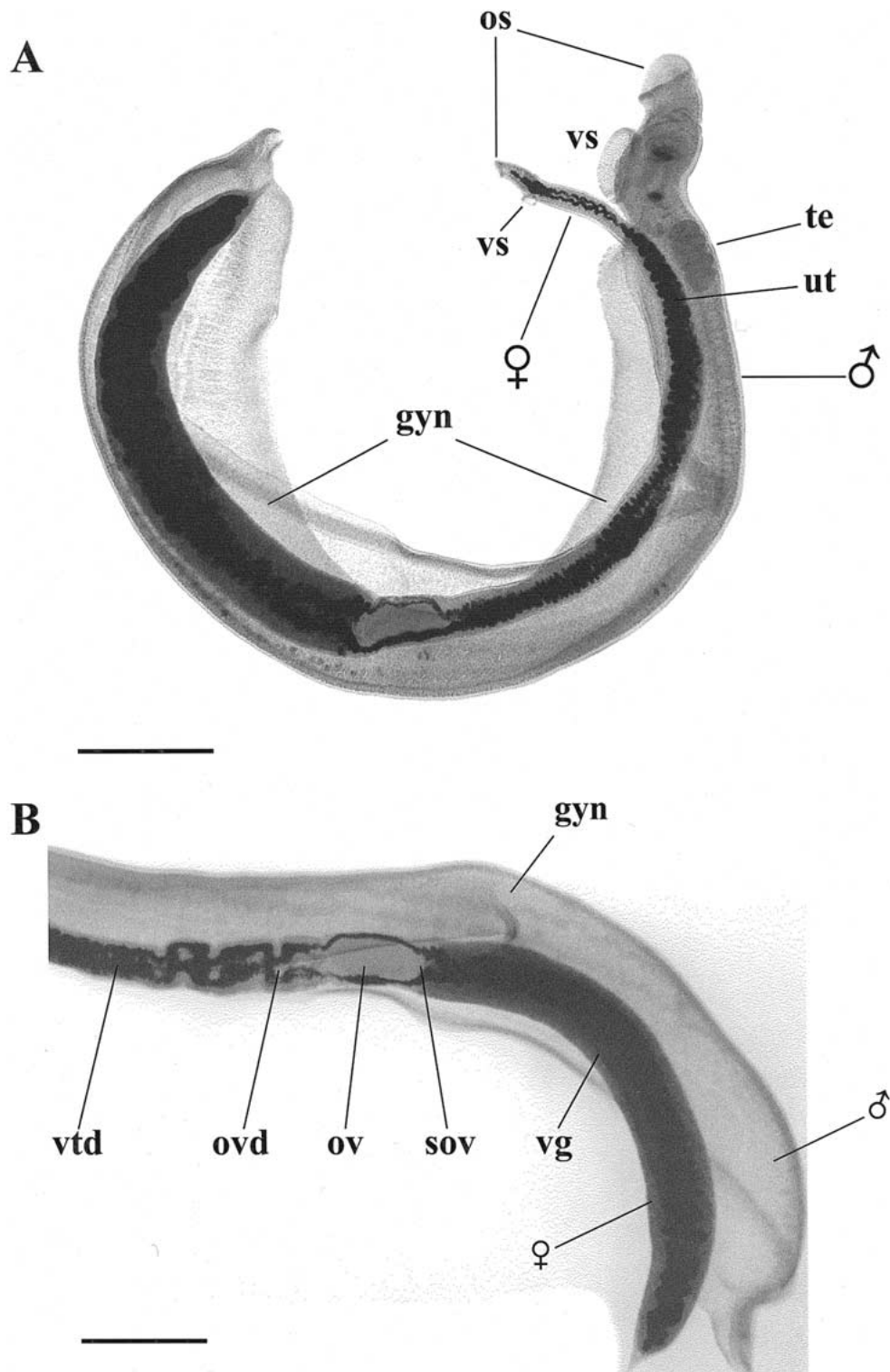


Figure 3. *Schistosoma ovuncatum* n. sp. A. Male and female in copula (holotype and allotype). B. Posterior region of female in gynaecophoric canal of male. Abbreviations: gyn, gynaecophoric canal; os, oral sucker; ov, ovary; ovd, oviduct; sov, swollen section of oviduct; te, testes; ut, uterus; vg, vitelline gland; vs, ventral sucker; vtd, vitelline duct. Scale-bar: 500  $\mu$ m.

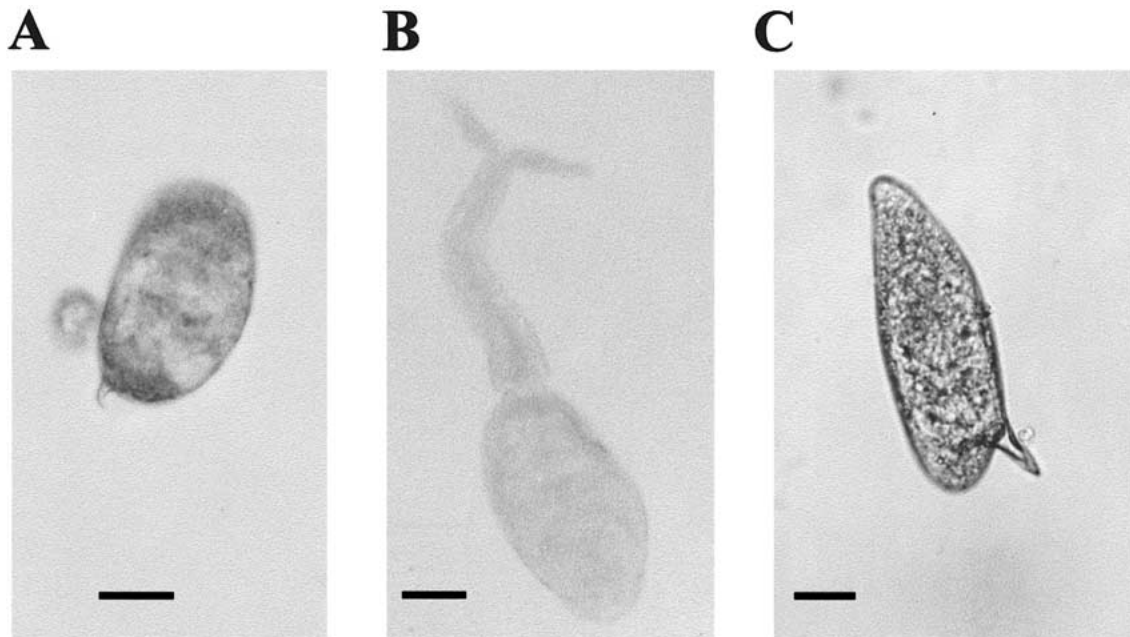


Figure 4. A. The living egg of *Schistosoma ovuncatum* n. sp. from a laboratory infection of the mouse. B. The resting cercaria of *S. ovuncatum*. C. The living egg of *Schistosoma sinensium* from the liver of a laboratory infected mouse. Scale-bars: A,C, 20  $\mu\text{m}$ ; B, 40  $\mu\text{m}$ .

worm. In addition, the constriction just posterior to the ventral sucker (Figure 6) is most pronounced in *S. sinensium* on average, although there is considerable variation in the values for *S. ovuncatum* (Table 1, *W/w*). Like *S. ovuncatum*, *S. sinensium* appeared to possess a smooth tegument which lacked tubercles or spines; this also agrees with Bao's (1958) observations for *S. sinensium*. The diameters of the oral and ventral suckers were again greatest in *S. ovuncatum*. The forebody length of *S. sinensium* was recorded as 600  $\mu\text{m}$  by Bao (1958); this is much greater than the average of 255  $\mu\text{m}$  recorded in the present study. The forebody length of 405  $\mu\text{m}$  observed for *S. ovuncatum* lies between that reported by Bao for *S. sinensium* and that observed for *S. sinensium* here. The position of the ventral sucker appeared to be in a similar position in both the present study and in Bao's (1958) account for *S. sinensium*.

The intestinal bifurcation in *S. sinensium* occurred just anterior to the ventral sucker (Figure 6B); this agrees with the findings of Bao (1958) and is also the condition seen in *S. ovuncatum*. In the present study the caeca of *S. sinensium* were observed to reunite at about 95-97% of body length. However, Bao (1958) reported that the caeca rejoined at about 40% of body length; this difference could be due to problems in ob-

serving the gut where there are heavy deposits of dark pigment.

The testes were 8-9 in number (usually 9) in *S. sinensium* (Figure 6B); the number observed by Bao was 8. This is in contrast to the (maximum of) 7 testes found in *S. ovuncatum*. The testes of *S. sinensium* were often found to overlap to some degree; however, this was rarely the case with *S. ovuncatum*. In this study the testes of *S. sinensium* were found to be significantly smaller, in terms of both length and width, than those of *S. ovuncatum*. The length of the testes chain was also considerably less in *S. sinensium*.

*Female* ( $N = 2$ ) (Table 1, Figures 6A, 7)

The average length of the female worm after 8 weeks was 3,940  $\mu\text{m}$ ; this is little different from the 4,230  $\mu\text{m}$  recorded for *S. ovuncatum* and the 3,550  $\mu\text{m}$  reported by Bao (1958). However, the mean maximum body width (mid-vitellarium) of *S. sinensium* was found to be 120  $\mu\text{m}$ , significantly less than that of *S. ovuncatum*. Bao (1958) recorded a maximum body width of 150  $\mu\text{m}$  for *S. sinensium*, similar to that observed here but again much less than that of *S. ovuncatum*. The ratio of length to maximum width (*L/W*, Table 1) indicates that the *S. ovuncatum* female is a significantly broader worm in relation to its body length; this is in contrast to the findings for the male



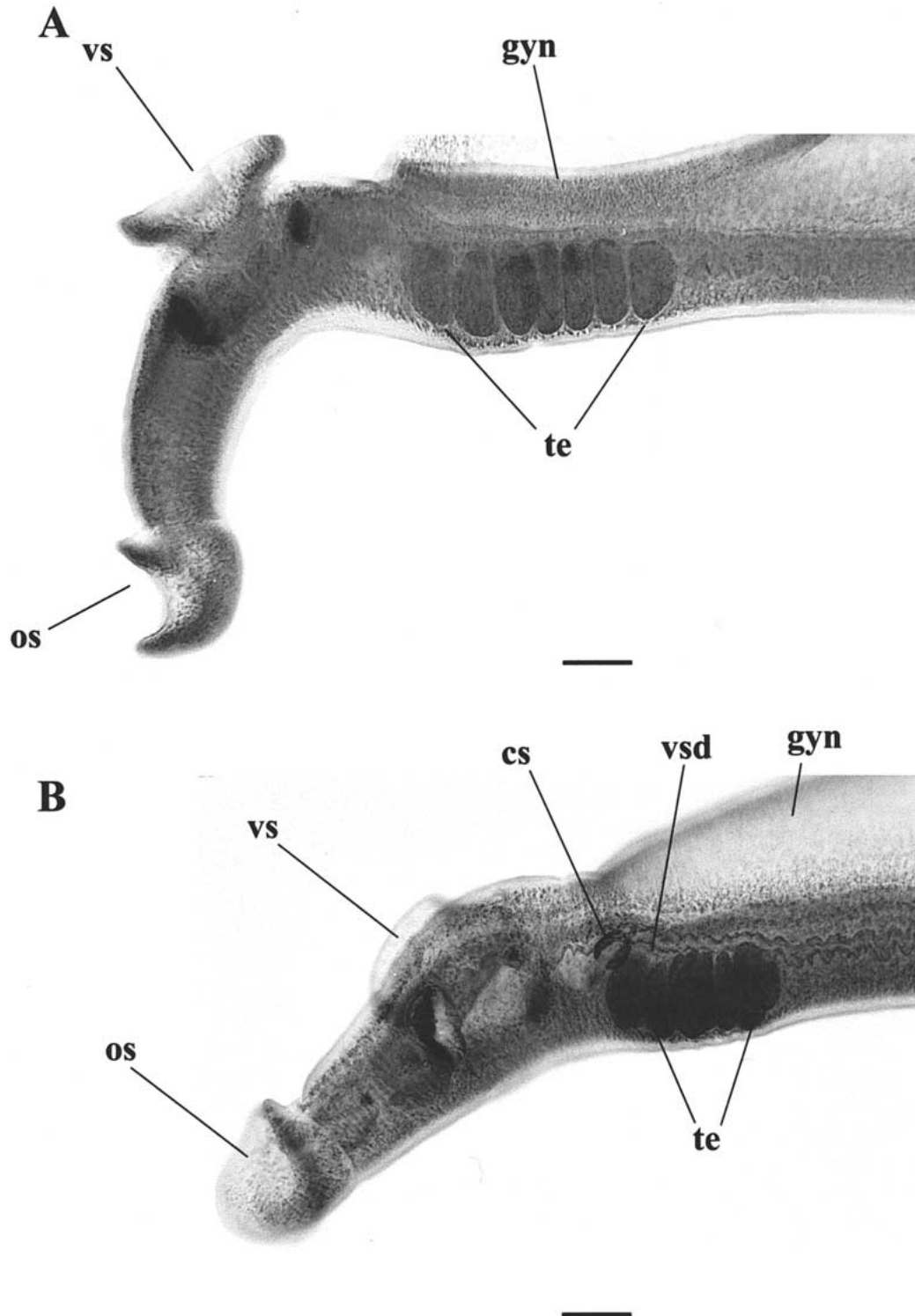


Figure 5. Male *Schistosoma ovuncatum* n. sp. A. Anterior end showing details of testes chain. B. Anterior end showing testes and cirrus-sac (cs) or seminal vesicle. Abbreviations: As for Figure 3; vsd, vas deferens. Scale-bars: 100  $\mu$ m.

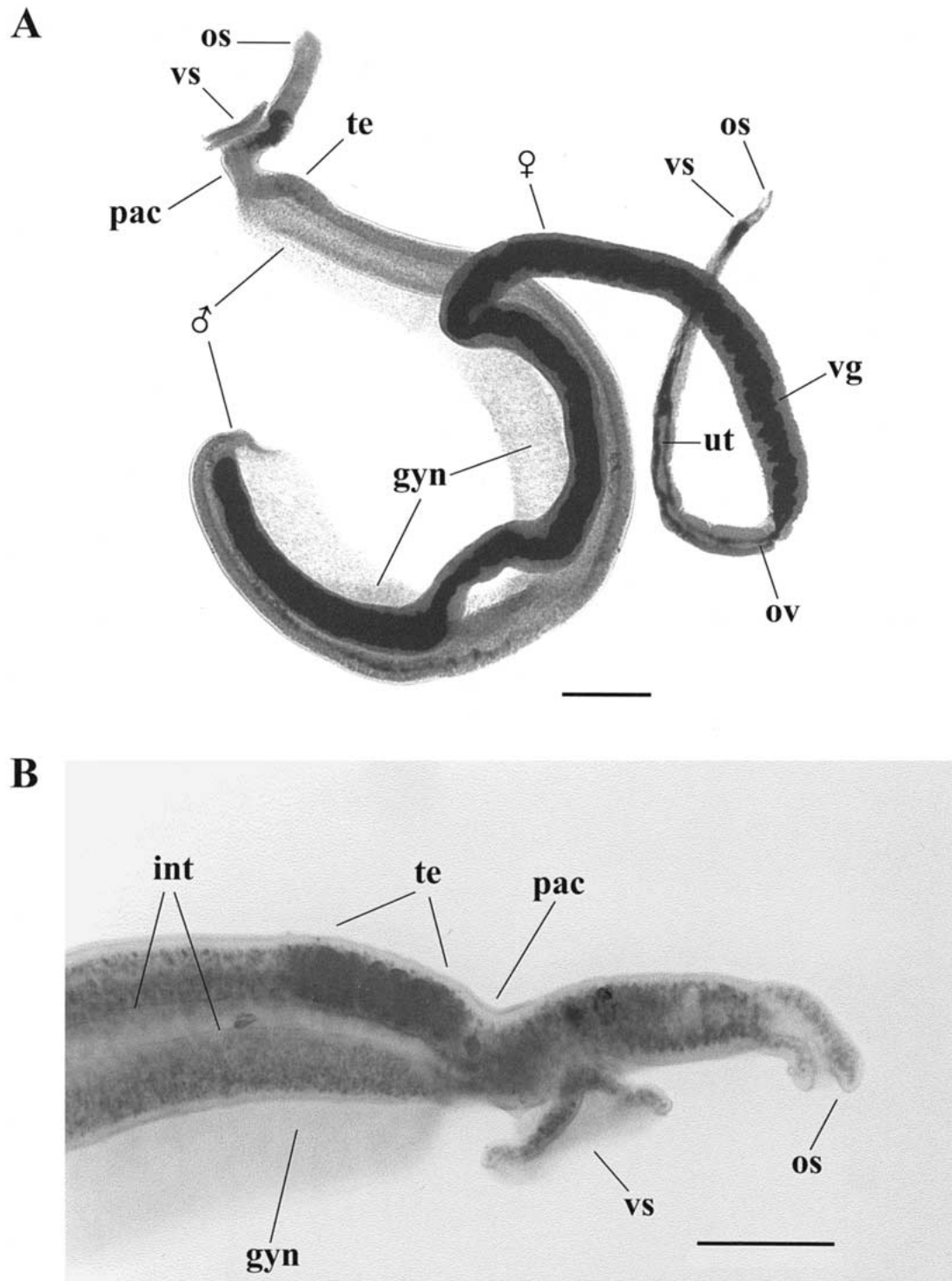


Figure 6. *Schistosoma sinensium*. A. Male and female in copula. B. Anterior of male showing the constriction just posterior to the ventral sucker (vsc) and gut caeca (int). For other abbreviations see Figure 3. Scale-bars: A, 200  $\mu\text{m}$ ; B, 100  $\mu\text{m}$ .

worm in which *S. sinensium* was the relatively broader worm on average (although there was some overlap in the ranges for the two taxa). The constriction just posterior to the ventral sucker (Table 1, row 8) was again more pronounced in female *S. sinensium*, but there was some overlap with the range for *S. ovuncatum*. In both male and female *S. sinensium* the posterior extremity was broader, not scuteiform as in *S. ovuncatum*. The diameters of the oral and ventral suckers in female *S. sinensium* were found to be significantly less than those of *S. ovuncatum*, again in keeping with the latter being the larger worm. Similarly, the forebody length of 85  $\mu\text{m}$  in female *S. sinensium* was less than that of *S. ovuncatum*. The ventral sucker in *S. sinensium* was found to originate in a more posterior fraction of the worm than in *S. ovuncatum*. The morphology of the gut caeca differed little between the two species. The vitellarium of *S. sinensium* occupied a greater percentage of the posterior fraction of the worm (65%) than that of *S. ovuncatum* (40%); this leads to the conclusion that the relative extent of the vitellarium is much greater in *S. sinensium*. Bao (1958) also recorded a value of 60% for the vitellarium of *S. sinensium*. The length and morphology of the ovary of *S. ovuncatum* did not differ significantly from that seen in *S. sinensium* (Figure 7), either here or in the study by Bao (1958). However, the ovary in *S. ovuncatum* was significantly wider than that seen in *S. sinensium* and also exceeded the width reported by Bao (1958) by some 75%. In this study and that by Bao (1958) only one egg was observed in utero in *S. sinensium*, whereas in *S. ovuncatum* it was not uncommon to find 10 or more eggs in utero at one time.

The egg of *S. sinensium* (Table 2, Figure 4C) in the present study closely resembled that described by Bao (1958). The egg is elongate-oval. The anterior part is somewhat narrowed anteriorly and curves (slightly) laterally away from the side bearing the spine. The posterior end of the egg is blunt and rounded with a conspicuous subterminal spine. The spine is long, straight and at an angle of about 30° from the central axis of the egg. Consequently, *S. ovuncatum* differs from *S. sinensium* in that the egg of the latter is not elongated anteriorly, and the spine is small and hook-shaped rather than long and straight. In addition, the egg of *S. sinensium* measures 105  $\mu\text{m}$  in length, which is the same as that reported by Bao, but significantly greater than the length of the *S. ovuncatum* egg which measures only 70  $\mu\text{m}$ . In contrast, the mean width of the *S. ovuncatum* egg in the present study is greater than that of *S. sinensium*. The spine of *S. sinensium*

was found to be 15  $\mu\text{m}$  long on average, similar to the 20  $\mu\text{m}$  recorded by Bao, and this is significantly greater than the 5  $\mu\text{m}$  observed for *S. ovuncatum*.

#### *Cercaria* (N = 5) (Table 2)

The cercaria of *S. sinensium* is similar in appearance to that of *S. ovuncatum*; however, there are morphometric differences. The average body length of *S. sinensium* is 160  $\mu\text{m}$ , which is significantly greater than that for *S. ovuncatum* (125  $\mu\text{m}$ ); the value reported by Bao for *S. sinensium* was also 160  $\mu\text{m}$ . In contrast, the mean body width for *S. sinensium* cercariae of 65  $\mu\text{m}$  was found to be significantly different from that recorded for *S. ovuncatum* (75  $\mu\text{m}$ ). Consequently, the body of the *S. ovuncatum* cercaria is somewhat shorter and broader than that of *S. sinensium* and also bears a narrower tail (Table 2).

#### Remarks

The schistosome recently collected from Mianzhu County is considered to be *S. sinensium* Bao, 1958 on the basis of similarity in worm and egg morphology. Good agreement is found in terms of body width, number of testes, relative length of the vitellarium, size, shape and position of ovary, and number of eggs observed *in utero*. Marked differences from the account of Bao (1958) were observed for worm length and width (males only), diameter of suckers and forebody length (males). However, the measurement techniques used by Bao are unclear and differences in fixation and mounting procedures also have an effect on such absolute measurements. The worms used by Bao were also some 2 weeks older than those used in the present study, and worms such as *S. japonicum* are known to continue growing after achieving sexual maturity (Cort, 1921). *S. ovuncatum* is considered to be a valid sibling species of *S. sinensium* on the following grounds. The male *S. ovuncatum* is larger in terms of both length and width but is a more slender worm (relative to its body length). The female *S. ovuncatum* is also larger but is a relatively less slender worm. The constriction just posterior to the ventral sucker is less pronounced in both sexes and, in keeping with *S. ovuncatum* being an overall larger worm, the diameter of the oral and ventral sucker is greater. The testes are larger and number 6-7 (rather than 8-9 in *S. sinensium*). The vitellarium occupies a lesser proportion of the posterior fraction of the female worm in *S. ovuncatum*; that is, it is proportionally shorter in terms of body length. The ovary is larger and the

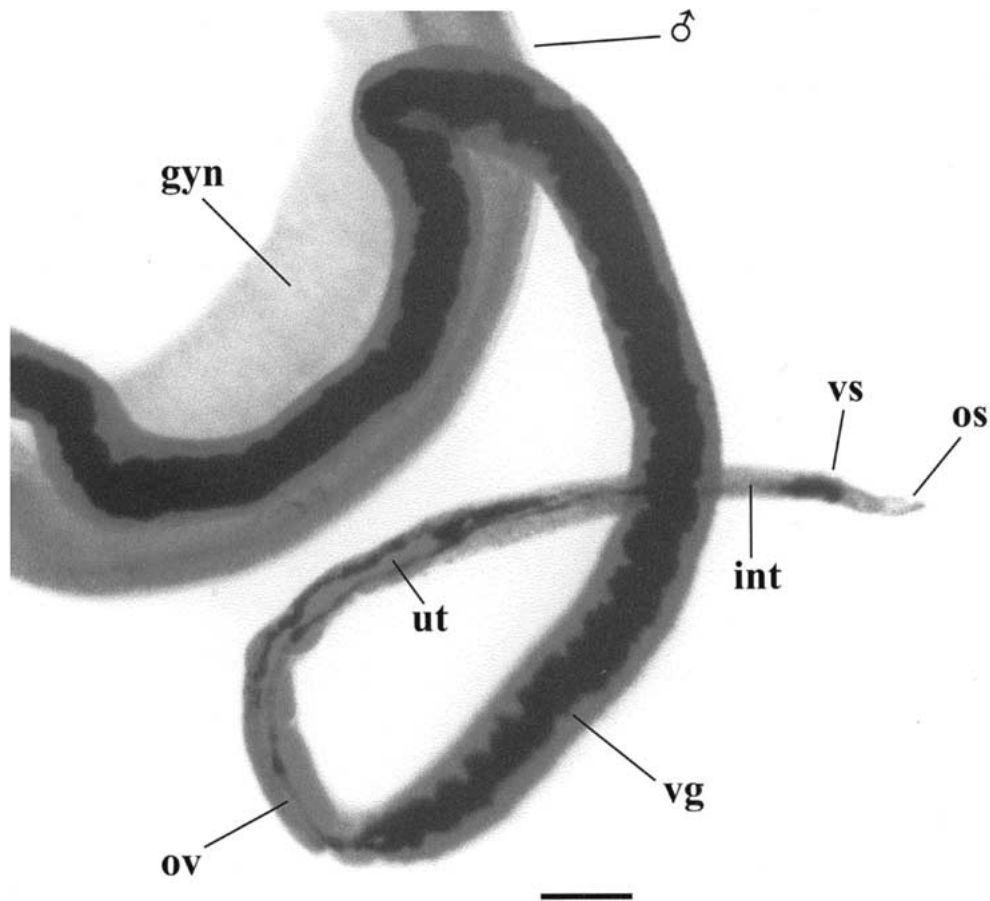


Figure 7. Female *Schistosoma sinensium* showing details of reproductive system. For abbreviations see Figures 3 and 6. Scale-bar: 100  $\mu$ m.

number of eggs found in utero much greater. Kruatrachue et al. (1983) also collected cercariae from *T. bollingi* at Wat Tam-Tab-Tao and obtained worms from laboratory infections based on these cercariae; these authors did not describe the morphometrics or anatomy of these worms but did undertake a scanning-electron-microscope (SEM) study of the tegument of the worms. As the schistosome studied by Kruatrachue et al. may well have been *S. ovuncatum*, the findings of these authors should be considered. In keeping with the present findings, SEM microscopy showed the worms to be atuberculate; however, the subequatorial dorsal tegument of the male was found to bear large sharp spines, particularly at the posterior. The tegument was considered to resemble that of *S. mekongi* or *S. japonicum* under SEM (Kruatrachue et al., 1983). Spines were not notable in either the present study or that of Bao (1958); however, it is unlikely that the spines observed by Kruatrachue et al., which were around 1.5  $\mu$ m long, would have been a major feature

under the light microscope. The egg of *S. ovuncatum* is broader, shorter and more rounded. The spine on the egg is much shorter and is hook shaped. The body of the cercaria is broader, shorter and the tail is less stout. Finally, there are differences in the intermediate host species between the two taxa.

## Discussion

### *Comparison of the new species*

*Schistosoma ovuncatum* n. sp. (Figure 8) differs from the worm described by Greer et al. (1989), also from Wat Tam-Tab-Tao, Chiang Mai, Thailand, in a number of respects. The female *S. ovuncatum* is wider, the ventral sucker is larger in both sexes, the fore-body is longer, the reunion of the gut caeca is in a more anterior position, the testes and ovary are shaped differently, and the number of eggs found *in utero* is much greater. In addition, the egg of *S. ovuncatum*

is smaller, more rounded and has a hook-shaped and shorter spine. It is possible that two species of *Schistosoma* are transmitted by *Tricula bollingi* at one site and that each research group found only one of these species; perhaps *S. sinensium* is no longer present in this snail population and may even have been displaced by *S. ovuncatum*. Alternatively, as the worms and eggs described by Greer et al. were recovered from field-trapped rodents, it is possible that these worms were not transmitted by *T. bollingi*. Baidikul et al. (1984) also provide photographs of eggs from naturally infected rodents; the egg photographed from a laboratory mouse by these authors does resemble *S. sinensium* (*sensu stricto*); however, this egg was from a laboratory infection based on Chinese material. A common schistosome infecting rodents in Thailand, and which also produces an egg bearing a subterminal spine, is *Schistosoma incognitum* Chandler, 1926, and possible intermediate hosts (*Lymnaea* and *Radix* spp.) were found at Wat Tam-Tab-Tao. The egg of *S. incognitum* is laid singly and measures  $95 \times 40 \mu\text{m}$ , on average, thus being similar in size to those described by Greer et al. In addition, the number of testes in *S. incognitum* is 2-7, which encompasses the 5-6 reported by Greer et al. (1989), and the ovary is similarly situated in the two taxa (Kumar & Burbure, 1986). However, the adult *S. incognitum* is tuberculate and slightly larger than the worms described by Greer et al. Certain *Orientobilharzia* spp. possess an egg most similar (in both size, shape and spine) to that described by Greer et al., and the genus also includes atuberculate species; the intermediate hosts (again *Lymnaea* or *Radix* spp.) were present at Wat Tam-Tab-Tao. Unfortunately, *Orientobilharzia harinasutai* Kruatrachue, 1965 (also referred to as *Thailandobilharzia harinasutai* in Baugh, 1977), the species commonly found in Thailand, is tuberculate, has in excess of 50 testes, the oral sucker is subterminal and the ovary smaller and pre equatorial. More importantly, *Orientobilharzia* spp. generally inhabit Artiodactyla, only being found in rodents from experimental infections. It is unlikely that earlier authors such as Greer et al. (1989) would have missed these differences and further surveys for *S. sinensium* around Wat Tam-Tab-Tao are still required.

*Schistosoma mekongi* is found in the Mekong River of Cambodia and southern Laos. As the Mekong River flows only 140 km from Wat Tam-Tab-Tao, it is important to distinguish *S. ovuncatum* from *S. mekongi*. In *S. mekongi* the male is completely spined and up to four times the length of *S. ovuncatum*, in the fe-

male the ovary is longer and differently positioned, the uterus can contain 10 or more eggs at one time, the gut caeca unite near the posterior extremity of the worm, the cercaria bears a relatively longer tail, and the egg is smaller, rounder and bears a very short spine (sometimes described as a "knob") (Voge et al., 1978). *S. mekongi* and *S. japonicum* are morphologically similar, although *S. japonicum* appears to be slightly smaller and the eggs somewhat rounder with a smaller knob. *S. malayensis* is also similar to *S. mekongi* and cannot be confused with *S. ovuncatum*. Neither *S. mekongi* nor *N. aperta* is known from the Ping River valley, in which the stream at Wat Tam-Tab-Tao runs; nor is *S. japonicum*, which is not found in Thailand. Consequently, we conclude that *S. ovuncatum* is indeed a species distinct from other members of the *S. japonicum*-group. Similarly, on the basis of morphology and intermediate host, the new taxon cannot be confused with *S. incognitum* or any other known taxon of the *S. indicum*-group; these schistosomes are transmitted by pulmonate snails rather than caenogastropods such as the Triculinae.

#### *Historical biogeography*

The consensus view on the evolution and colonisation history of *Schistosoma* in Southeast Asia has largely been based on the ideas presented by Davis (1979, 1980, 1982, 1992). These hypotheses exploit the reciprocal illumination gained by an examination of phylogenies for both the schistosomes and their snail intermediate hosts and are based on an extensive set of molluscan morphological characters (e.g. see Davis et al., 1994). The Pomatiopsidae have a Paen-gaeian distribution; however, that of the Pomatiopsinae is Gondwanan with extant taxa showing a "southern continental" (vicariant) distribution (Davis, 1979). A Gondwanan origin was thus proposed for Asian Pomatiopsidae and *Schistosoma*, with the ancestors of *S. mansoni* Sambon, 1907 and *S. haematobium* (Bilharz, 1852), being isolated on the African Plate; these taxa would then be separated as Gondwana broke up in the late Mesozoic (Davis, 1979, 1980). Colonisation of mainland Southeast Asia would have begun after the collision with the Indian Plate (about 40 mya) and the onset of the Tibetan uplift which initiated the main rivers of Asia (Hutchinson, 1989). Davis (1979) suggested that the main route of colonisation was via the Northwest Burma-Brahmaputra corridor which opened approximately 18 mya. Palaeogeographical models adopted by Davis (1979) indi-

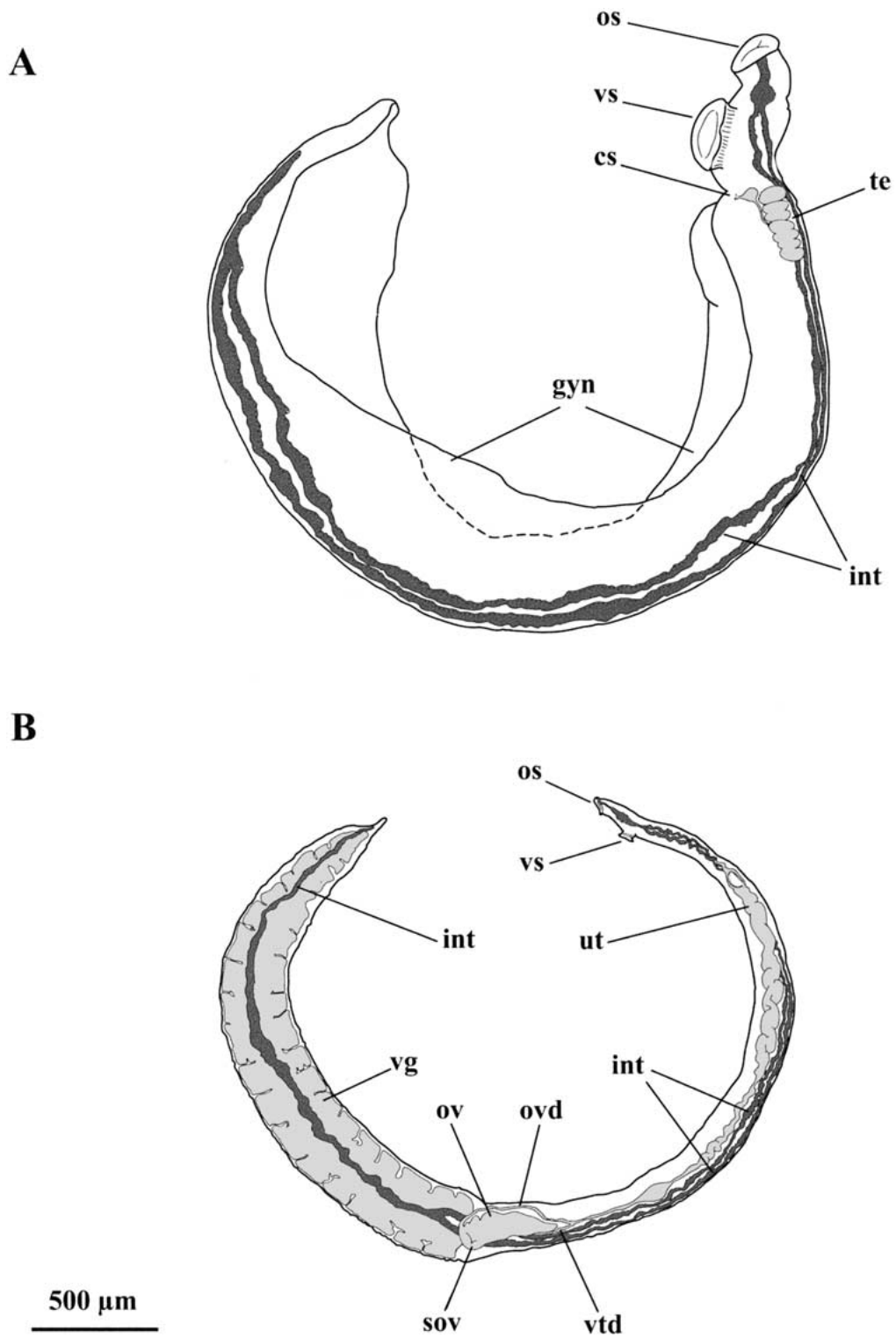


Figure 8. Drawing summarising the morphology of *Schistosoma ovuncatum* n. sp. as observed in the present study. A. Male. B. Female. Abbreviations: As for Figure 3; cs, cirrus-sac; int, gut caeca.

cated that separation of the Yangtze River from the Irrawaddy/Ping/Mekong River systems occurred in the late Miocene. According to this model Chinese *Schistosoma* (e.g. *S. japonicum* today) and Lao or Thai *Schistosoma* (including *S. malayensis*, *S. mekongi* and *S. ovuncatum* n. sp.) have been geographically separated for around 12 million years. However, this traditional view of a series of “linear” radiations down the major rivers of Southeast Asia, from a common origin in southern China (Yunnan), is now questionable in the light of more recent palaeogeographical models. It now appears most likely that connexions between the upper Mekong and Yangtze Rivers were broken in the late Miocene (7–5 mya).

Current models suggest that the mountains of Tibet were of moderate stature until the Pliocene, similar to those of northern Burma and Thailand in which *T. bollingi* is found today (Mitchell, 1981). Consequently, at that time Tibet would have been suitable for colonisation by the ancestors of *Oncomelania* and extant Triculinae introduced from India along with the schistosomes they transmitted. Following the initial orogeny, to an average elevation of 2,500 m, the Tibetan Plateau is thought to have experienced a second Pliocene uplift to achieve the present average of 5,000 m (Xu, 1981). The second uplift probably initiated the separation of the upper Yangtze from the other major rivers of Southeast Asia, such as the Irrawaddy and Mekong, as the faster waters cut deeper channels into the Plateau and through across Yunnan. As the climatic changes associated with the second uplift would have been intolerable, any pomatiopsid snails present must have already dispersed separately into the middle reaches of their respective rivers in Yunnan and Burma. Consequently, the progenitors of *S. japonicum* and *S. sinensium* (sensu stricto) (Yangtze River taxa) and those of *S. malayensis*, *S. mekongi* and *S. ovuncatum* (Mekong River taxa) were separated no later than 5 mya. Similarly, *S. sinensium* of Sichuan is likely to have been separated from *S. ovuncatum* of Thailand for over 5 million years, with little chance of genetic exchange over the extensive mountain range and 850 km running between the two populations. This again supports the case that the two taxa are indeed separate species.

It is now known that Southeast Asia experienced marked tectonic activity throughout the Cenozoic, with marked changes in drainage configuration (see Tapponier et al., 1982, 1986; Hall, 1998; Lacassin et al., 1998). These changes afford the possibility of the direct colonisation of Thailand, by antecedent

Triculinae and *Schistosoma* from Burma (originally India), rather than via China and the upper Mekong River as proposed by Davis et al. (1994). One scenario is that the ancestors of both “Yangtze” and “Mekong” *Schistosoma* (see above), and Triculinae, dispersed throughout northern Burma and Tibet until 5 mya. Isolation of the Yangtze and Mekong taxa would then have occurred during the Pliocene orogeny. For example, taxa may have dispersed separately into the upper and lower Irrawaddy River (Yangtze taxa to the upper river), which then existed as two separate drainages (Hutchinson, 1989) (Figure 9A). Precursors of *S. ovuncatum* and *S. mekongi* could then disperse into Thailand via the lower Irrawaddy and the extended Mekong-Salween River which flowed together during the Pleistocene (c. 1.5 mya, Figure 10). Subsequent introductions from Burma into northern Yunnan (Yangtze River) were probably prevented by the ongoing elevation of the West Burma Block (see Hutchinson, 1989). A second possible scenario is one in which pre-*S. japonicum* and/or pre-*S. sinensium* entered southern China much earlier (in the Miocene) off one of the Tibetan (Cimmerian) blocks which collided with Asia before the Indian Plate. This would permit the direct colonisation of the upper Yangtze in Tibet by early *Schistosoma* and Pomatiopsidae, which then dispersed southwards into the Sichuan Basin (Figure 9B). The Yangtze platform basin has remained quite stable over the last 600 million years, and the course of the Yangtze has changed relatively little throughout its history (Hutchinson, 1989). In addition, the mountains of southern Yunnan, northern Laos and northern Vietnam are likely to have provided a barrier to dispersal over the last 200 million years. The absence of *Oncomelania* and *S. japonicum* from mainland Southeast Asia is evidence for this barrier. This hypothesis of an independent origin for Yangtze taxa, off a Cimmerian block, and Mekong taxa, in northern India and Burma, implies that the ancestors of *S. sinensium* and *S. ovuncatum* were separated some 200 mya, rather than the 5 mya suggested by scenario one. Clearly, the relationships between these two taxa are important in distinguishing between historical biogeographical models for Southeast Asian *Schistosoma*.

*The evolutionary position of Schistosoma ovuncatum n. sp.*

During the Pliocene the Mekong River probably flowed due south from Chiang Rai (Figure 10), down what is today the Ping River valley, to enter the Gulf

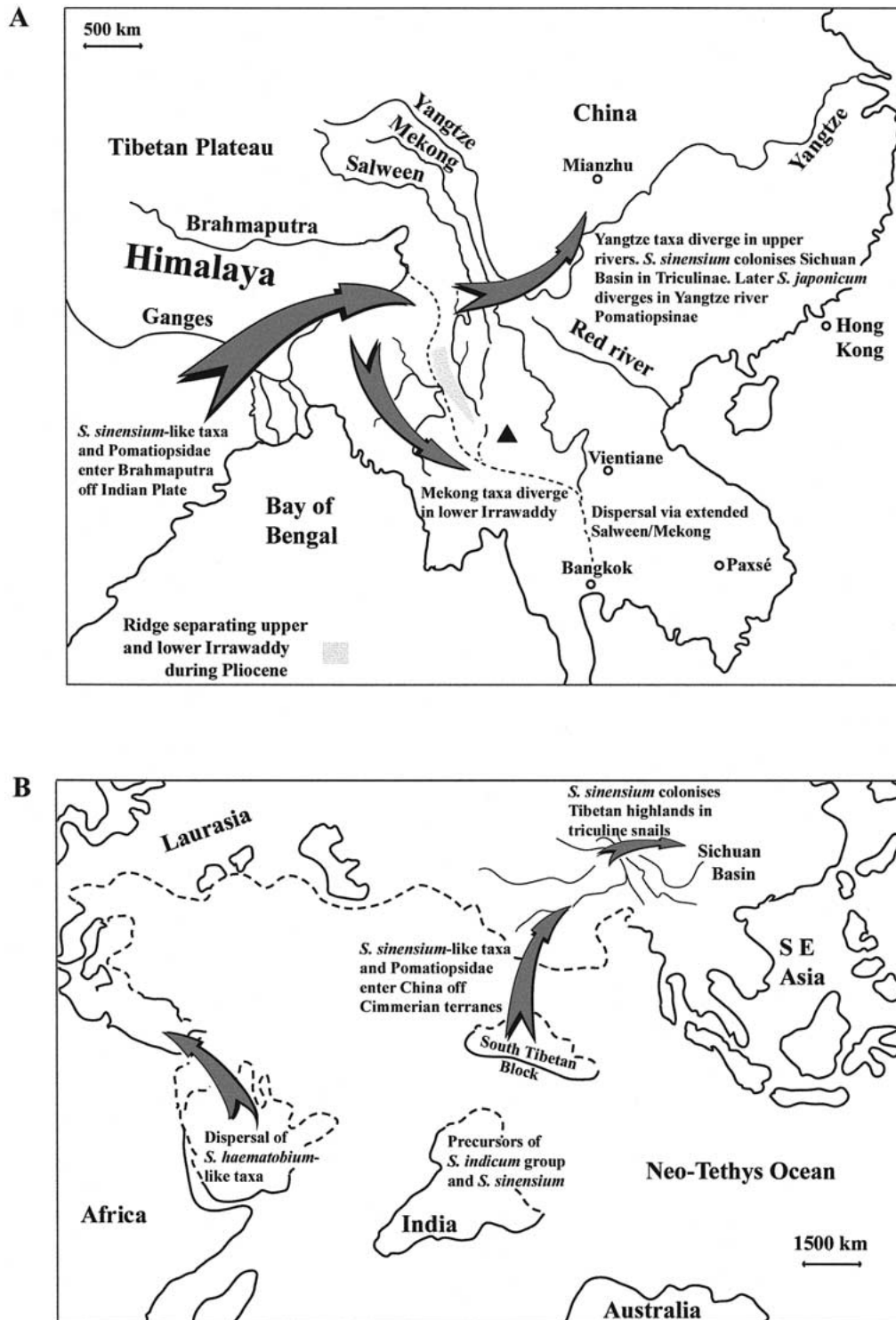


Figure 9. Semi-schematic illustration showing two possible scenarios for the radiation of *Schistosoma* across China and Southeast Asia. A. Isochronous colonisation by ancestors of Yangtze taxa (*S. sinensium*/*S. japonicum* and the Pomatiopsidae) and Mekong taxa (*S. ovuncatum* n. sp., *S. mekongimayensis* and the Triculinae) during the Pliocene. The type-locality of *S. ovuncatum* is shown by a black triangle. Prehistoric river courses are denoted by broken lines. B. Heterochronous colonisation during the Cretaceous and late Tertiary. The ancestor of *S. ovuncatum* (and other Mekong taxa) enters Southeast Asia, from Burma, after the Indian Plate collides with Asia, having arisen in India from *S. indicum*-like taxa able to utilise triculine snails. The Mekong taxa thus colonise Southeast Asia during the Pliocene much later than the ancestors of the Yangtze taxa. The Yangtze taxa are shown to have colonised southern China after rafting northwards on one of the Cimmerian terranes accreted during the Cretaceous. Scale and palaeogeographic features approximate.



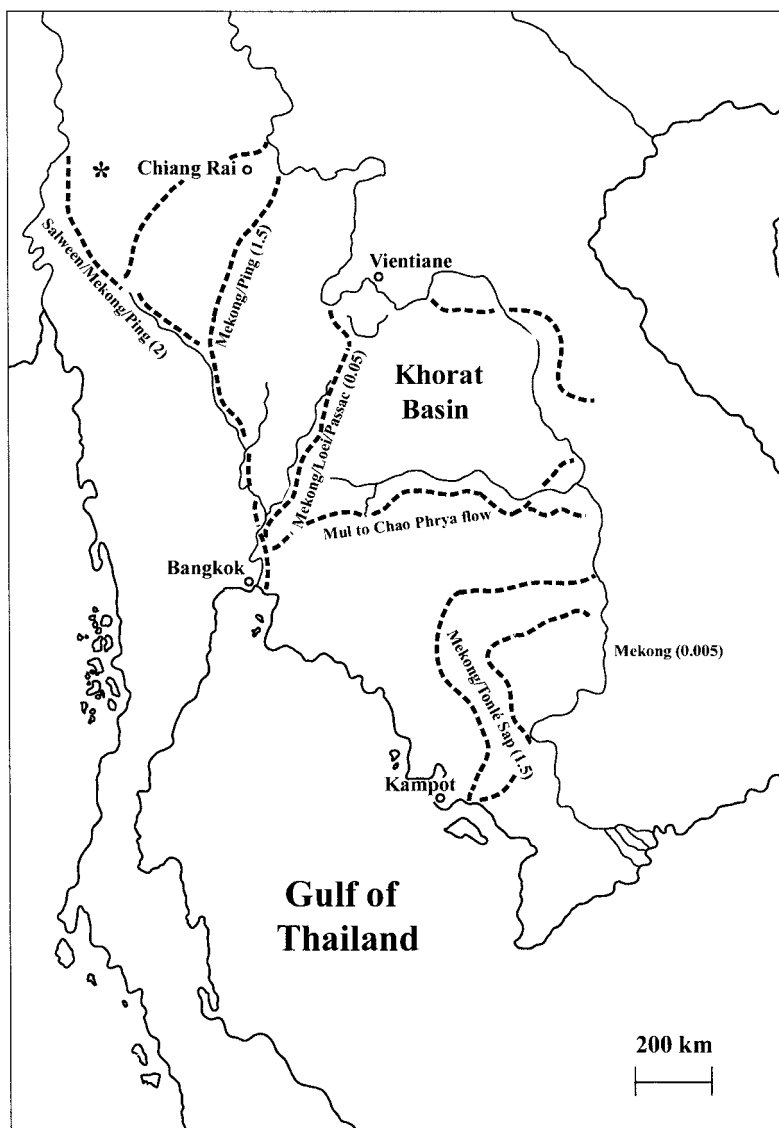


Figure 10. Major course changes along the Salween, Mekong and Ping Rivers of Southeast Asia during the late Cenozoic. Prehistoric river courses are shown as broken lines; the names of the captured rivers are given along with approximate times of capture ( $\times 10^{-6}$  years) in parentheses. The type-locality of *Schistosoma ovuncatum* n. sp. is also shown (\*). Condensed from the palaeogeographic maps of Hutchinson (1989).

of Thailand via the present Chao Phraya River delta (Hutchinson, 1989; Lacassin et al., 1998). This may explain the finding of *S. ovuncatum* and *Tricula* in the Ping River system in northwest Thailand. Late Cenozoic faulting probably diverted the Mekong eastwards along its present course towards Vientiane (Figure 1). The time of divergence between *S. mekongi* and *S. ovuncatum* is thus estimated to be approximately 1.5 mya. Yuan et al. (1984) reported that, although *Neotricula aperta* could not be infected with *S. japon-*

*icum*, this snail was susceptible in the laboratory to *S. 'sinensium'* from Wat Tam-Tab-Tao (infection rate 4.7%). These authors also reported that *T. bollingi* was susceptible to *S. mekongi* (infection rate 16.7%). The laboratory infection rate for *S. 'sinensium'* in *T. bollingi* was 57.1%. The findings of Yuan et al. thus support the view that *S. ovuncatum* and *S. mekongi* are closely related taxa. However, it has not been possible to replicate these compatibility experiments and compatibility was assumed where the snails shed cercariae;

the work did not examine the success of the resulting cercariae (the identity of adults was not confirmed) and the *T. bollingi* used were field-collected and could have harboured earlier (natural) infections.

The ancestral form of *Schistosoma* that existed in northern Burma, Tibet and Yunnan during the Pliocene may well have resembled *S. sinensium* of Sichuan today. This ancestral schistosome could have entered China, off the Indian Plate, at the same time as ancestors of the Southeast Asian taxa or perhaps have dispersed much earlier off one of the Gondwanan fragments accreted earlier (scenario two). The status of the *S. sinensium*-like taxa has become more important following the report of Snyder & Loker (2000) suggesting that *Schistosoma* originated in Asia. This is in contrast to the view of authors, such as Davis (1979), who considered *Schistosoma* as having an African origin. Bao (1958) noted that *S. sinensium* was of particular interest as it possessed characters seemingly intermediate between those of members of the *S. indicum*-group (e.g. a subterminal spined egg and single egg *in utero*) and the *S. japonicum*-group (e.g. a triline intermediate host and an atuberculate tegument). Members of the *S. indicum*-group are widespread throughout India and those found in Southeast Asia (*S. incognitum* and *S. spindale* Montgomery 1906) are thought to have dispersed from India during the Holocene, probably as a result of human seafaring. As current hypotheses suggest an Indian origin for the *S. japonicum*-group, with dispersal via the Brahmaputra and northern Burma, taxa with characters of both groups, such as *S. ovuncatum* and *S. sinensium*, may best represent the ancestral form of *Schistosoma* that colonised southern China and Southeast Asia during the late Tertiary. The present host of *S. sinensium* is a triline, and this is the case for all but one member of the *S. japonicum*-group. Indeed, *Schistosoma* may have colonised southern China and Southeast Asia in a snail of the Triculinae. The later colonisation of the Yangtze Basin by Pomatiopsidae may have facilitated the divergence of *S. japonicum* from *S. sinensium*-like stock, with subsequent colonisation of the mid-Yangtze plain by the former in oncomelanid snails. The extant Triculinae appear well adapted to mountain streams and minor rivers and, as such, represent a better vehicle than the Pomatiopsinae for the colonisation of northern Burma, Laos and Thailand. The lack of involvement of pulmonate snails in the transmission of *S. japonicum*-group parasites (and their utilisation in India and elsewhere) may be similarly explained. Once southern China had been

colonised the ecological situation would have been reversed and the amphibious *Oncomelania* (Pomatiopsinae) was clearly better adapted to the less fluviatile and more ephemeral habitats of the wetlands and canals of the middle Yangtze River system. Transmission of *S. sinensium* in Sichuan occurs in small streams draining the eastern margin of the Tibetan mountains and involves a species of *Tricula*; this would be expected given an origin for this clade in northern India and Burma. The definitive hosts of *S. sinensium* are bandicoots and other rodents. Humans would have become available to Asian *Schistosoma* once the lower rivers had been colonised. Human settlements are sparse in the highlands of Tibet and Sichuan but common around the habitats of *Oncomelania* and *N. aperta* in the south of China and Laos, respectively. Similarly, the invasion of human hosts by pre-*S. mekongi* may have led to its divergence from the highland taxon *S. ovuncatum* in Burma. *S. ovuncatum* is likely to be found throughout northern Burma, and its apparent restriction to one site on the Burmese border in Thailand probably reflects the undisturbed nature of these border areas. Unfortunately, it has not as yet been possible to undertake surveys in Burma where such new species of *Schistosoma* and *Tricula* may be found.

In view of the above, we would expect *S. sinensium* to be basal to a clade containing both *S. mekongi* and *S. japonicum*. The time of separation between *S. ovuncatum* and *S. mekongi* is estimated to be 1.5 mya; this is supported by palaeogeographical data and the biogeography and phylogenetics of the intermediate hosts. The time of separation between *S. ovuncatum* and *S. sinensium* is less clear and is between 200 and 5 mya. The relationships between these *S. sinensium*-like taxa and members of the *S. indicum*- and *S. japonicum*-groups are now central to studies of the historical biogeography of Southeast Asian *Schistosoma*. Consequently, work in this laboratory is now underway to collect DNA sequence data for these taxa. It is hoped that such data will enable the detection of character polarity for these schistosomes and reveal the direction and timing of colonisation and speciation events early in the evolution of human schistosomiasis.

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