CHAPTER VI

KARYOTYPES OF OPERCULATE LAND SNAILS OF THE GENUS *CYCLOPHORUS* (PROSOBRANCHIA: CYCLOPHORIDAE) IN THAILAND

6.1 Introduction

With high species diversity and a wide-ranging distribution, *Cyclophorus* is an attractive subject for systematic and biogeographical study. However, while the intraspecific shell morphology of *Cyclophorus* is variable, general shell form in *Cyclophorus* is conservative and morphology of the reproductive organs has not been reported as being of value in species discrimination. Thus, no meaningful investigations have been carried out into relationships between *Cyclophorus* species based on anatomical characters. There is enormous potential for genome sequencing-based studies on terrestrial snails, but to date these have been conducted almost exclusively on Stylommatophoran pulmonates (Wade *et al.*, 2000) and involve relatively expensive consumables. In contrast, karyotyping is a low cost methodology that provides useful tools for alpha systematics, can be used in evolutionary interpretations, and is ultimately complementary to sequencing. Although *Cyclophorus* is a diverse genus and clearly presents an interesting focal group for diversification studies, less than 5% of the total species in the genus have been karyotyped to date (Kasinathan and Natarajan, 1975; Choudhury and Pandit, 1997).

Here we present karyotypes of ten Thai species of *Cyclophorus*, which brings the total examined to 16 (Table 1), and for the first time allows some discussion of systematic implications.

6.2 Results of Karyotype analysis

Locality, sex (determined on the basis of the presence of a male penis located at lower right tentacle) and number of snails karyotyped are given in Fig. 1, 2 and Table 2.

In all the samples we examined, sex chromosome heteromorphism or secondary constrictions were evident. The karyotype of all species consists of 2n = 28 chromosomes with the fundamental number (FN) 56 and only metacentric and submetacentric chromosomes were identified (Fig. 3, 4). There is a size discontinuity between chromosome pairs 9 and 10, and two size groups can be easily recognized within each genome in two species, *C. cantori* and *C. fulguratus,* collected from Kokplasiew, and between pairs 11 and 12 in *C. speciosus*. Other species exhibit size continuity. Interestingly, exclusively metacentric elements (14m) are observed in *C. volvulus*, whereas each of the remaining nine species exhibits gradually decreasing numbers from 13m to 5m (Table 2) and the karyotype arrangements are different from each other. The karyotype of *C. courbeti* is almost identical to that of *C. fulguratus*, but differs from the latter in having two fewer metacentric pairs. The two larger species *C. aurantiacus* and *C. malayanus* possess similar karyotypes (7m + 7sm). Examples of three smaller species *C. cantori, C. speciosus* and *C. subfloridus* exhibit similar karyotype characteristics to those of *C. aurantiacus* and *C. malayanaus*. However, these smaller species exhibit size discontinuity between pairs 9 and 10, 11 and 12, and 10 and 11, respectively.

The three southern species, *C. cantori, C. diplochilus* and *C. malayanus,* exhibit lower metacentric chromosome numbers than the four northern species, *C. aurantiacus, C. fulguratus, C. subfloridus* and *C. volvulus*. The Eastern populations of *C. saturnus* and *C. speciosus*, which have distributions in Central Thailand, have intermediate metacentric numbers 9m and 8m, and the western species *C. aurantiacus* exhibits a low to intermediate metacentric number 7m as in *C. malayanus*. Only *C. volvulus* located in an isolated area of the Central Region has all metacentric karyotypes of 14m. Intraspecific variation was found between populations of *C. fulguratus* located in central and in northeastern regions. Specimens in the central range possess karyotypes of 12m + 2sm whereas those from the northeast are 13m + 1sm (Table 2). Male karyotypes of northeastern populations from Kokplasiew (13) show size differences from Phuwiang (15).

Females were karyotyped for three of our study species, *C. fulguratus* from Phuwiang, *C. malayanus* from Sramorakot (21) and *C. volvulus* from Wang Kanlueang (7), and all showed distinct size heteromorphism of metacentric pairs (ZW) (Fig. 4). All males of all 10 species have homologous pairs. This suggests the presence of a ZZ-ZW type of sex chromosome (ZZ in male, ZW in female).

6.3 Discussion

The number of chromosomes among *Cyclophorus* is highly conservative. Several characteristics are common to all karyotyped species, including the invariable diploid number (28), the presence of a size continuum and the numerical balance of metacentric chromosomes in the larger and smaller size groups (Kasinathan and Natarajan, 1975; Choundhury and Pandit, 1997; the present study).

This conservation of haploid number contrasts with the subclass Streptoneura (Caenogastropoda), for which haploid chromosome numbers have been reported from 7 to 60 (Burch, 1967). As with the Euthyneura, the

Streptoneura exhibits a conservative chromosome number in each taxon, this seldom varies by more than ± 2 bivalents (Burch, 1967). An investigation of ten tiny cyclophoroideans belonging to *Diplommatina* also showed a similar conservative chromosome number (2n = 26) and chromosome morphology (metacentric and submetacentric) (Ieyama and Tada, 1991; Ieyama, Henmi and Tada, 1993; Ogaito and Ieyama, 1997; Ieyama and Ogaito, 1998, 2000).

The chromosomal variation in our specimens identified as *C. fulguratus* indicates that this is a cluster of species, as chromosomal variation may represent a primary species-isolating mechanism as discussed by King (1993). As currently defined *C. fulguratus* ranges from Thailand (type locality) to Indochina and is notable for exhibiting significant variation in shell morphology (Habe, 1964). Our data suggest that we sampled two species or subspecies within Thailand. On the basis of evidence presented here it seems likely that *C. fulguratus*, as currently understood, consists of a number of biological species. Further chromosomal surveys, combined with comparative analyses of geographic variation in morphological characters and DNA sequence of the potential *C. fulguratus* species complex, are needed to establish species limits.

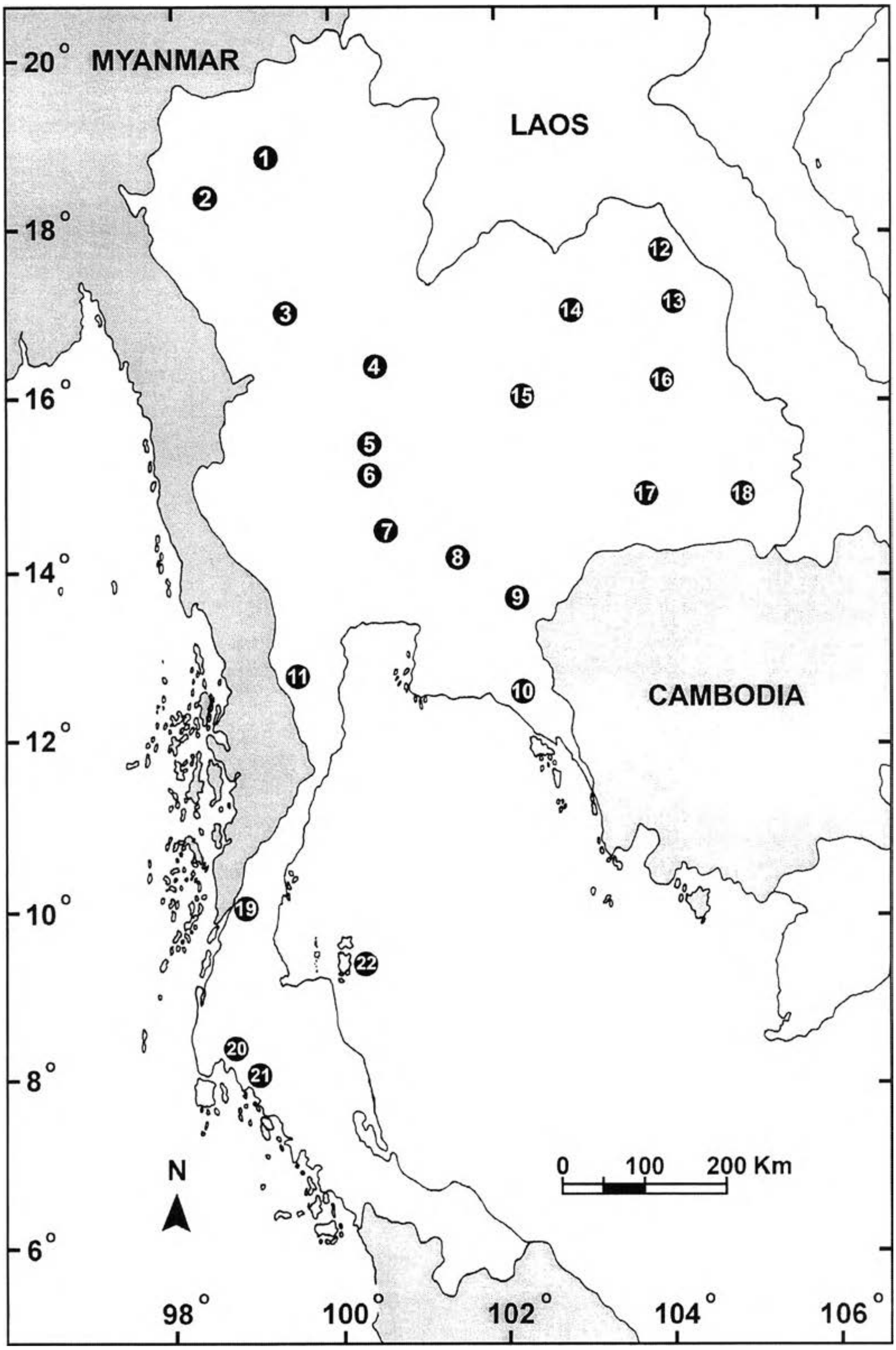
Karyotypes of the two larger species, *C. aurantiacus* and *C. malayanus* , are similar in having 7m + 7sm, but differ in arm length ratios. Moreover, *C. aurantiacus* exhibits small satellites on the short arms of pair 8 and a secondary constriction on the long arm of pair 9 (Fig. 4). These characters are highly variable between a wide range of organisms (Swanson et al., 1967), are associated with a high level of conservation between species and therefore associated with species status (Clark and Wall, 1996; Rickart *et al.*, 1999).

The present study is the first to demonstrate the presence of ZZ-ZW type sex chromosomes in *Cyclophorus*. Baršienė and Ribi (1998-1999) reported that the family Neritidae, order Archaeogastropoda, has XX-XO type (XX in female, XO in male), and the Mesogastropoda has the ZW type. In the Caenogastropoda ZZ-ZW sex-chromosome determining has also been observed in some *Viviparus* spp. (Baršienė *et at.*, 2000).

The karyotype is generally a species-specific character useful in species discrimination (King, 1993; Clark and Wall, 1996). Karyological data has been used for species-level discrimination in several molluscan groups such as *Goniobasis* (Dillon, 1991); *Bellamya* (Zhou *et al.*, 1988); *Atlanta* (Thiriot-Quièvreux and Seapy, 1997) and *Viviparus*. Baršienė et al. (2000) used karyotyping to improve on existing schemes of classification and to explain the evolutionary role of chromosomes. Cytogenetic studies are particularly valuable for clarifying phylogenetic relationships and investigating mechanisms of chromosomal evolution in closely related species.

It is worth emphasising that chromosome numbers in the Cyclophoridae are highly conservative. Sixteen species have now been reliably investigated and all of these have 14 pairs of chromosomes. The absence of any examples of aneuploidy in *Cyclophorus* or in other members of the Cyclphoridae demonstrates that, on current evidence, aneuploidy has not played a role in species isolation and evolution of the group.

Figure 6.1 A map of Thailand showing the collecting localities of *Cyclophorus* for karyotype analysis. 1 = Kunkorn, Chiang Rai; 2 = Brijinda, Chiang Mai; 3 = Lansang, Tak; 4 = Nernmaprang, Phitsanulok; 5 = Teppitak, Nakhonsawan; 6 = Sangkat Rattanakeri, Uthaithani; 7 = Wang Kanleuang, Lopburi; 8 = Wang Takrai, Nakhonnayok; 9 = Khao Chakan, Sra Kaeo; 10 = Phlieu, Chanthaburi; 11 = Kang Krajan, Phetchaburi; 12 = Nawa, Nakhon Phanom; 13 = Kokplasiew, Sakon Nakhon; 14 = Chaiwan, Udonthani; 15 = Phu Wiang, Khon Kaen; 16 = Nongkungsri, Kalasin; 17 = Srikhoraphum, Surin; 18 = Khueang Nai, Ubon Ratchathani; 19 = Phakayang, Ranong; 20 = Namphud, Phangnga; 21 = Sramorakot, Krabi; 22 = Samui, Suratthani.


MYANMAR
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106°
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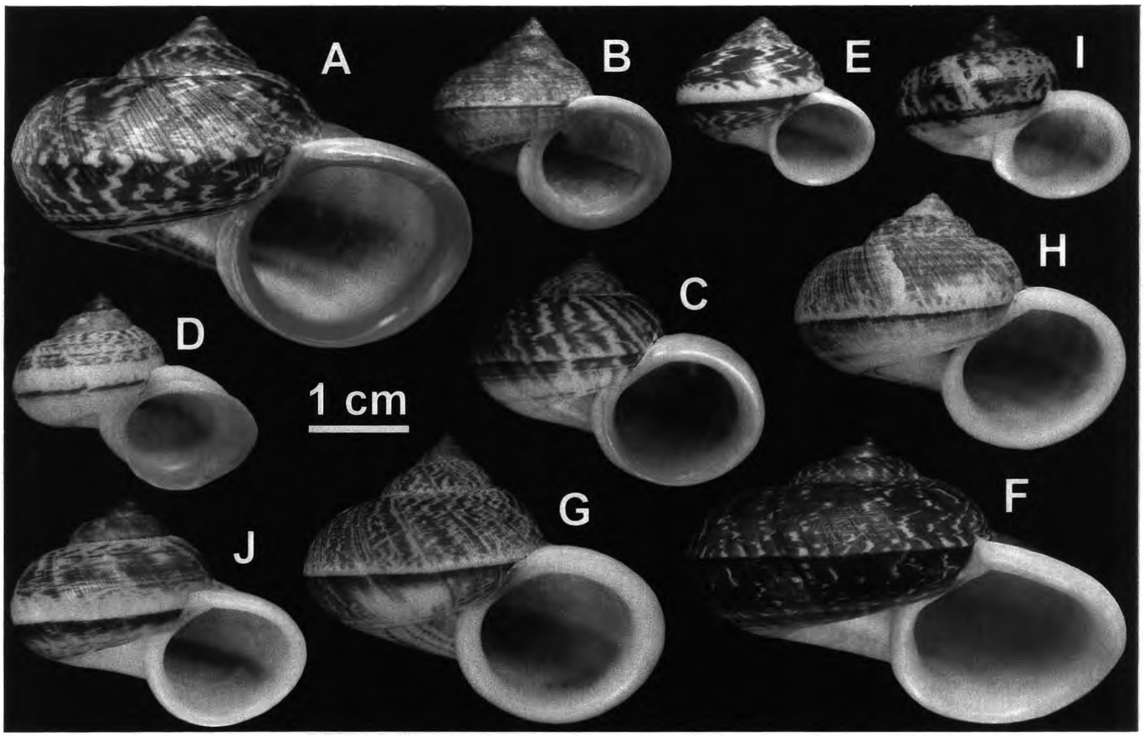

Figure 6.2 Shell specimens of, **(A)** *Cyclophorus aurantiacus*, **(B)** *C. cantori*, **(C)** *C. courbeti*, **(D)** *C. diplochilus*, **(E)** *C. fulguratus*, **(F)** *C. malayanus*, **(G)** *C. saturnus*, **(H)** *C. speciosus*, **(I)** *C. subfloridus*, **(J)** *C. volvulus*.

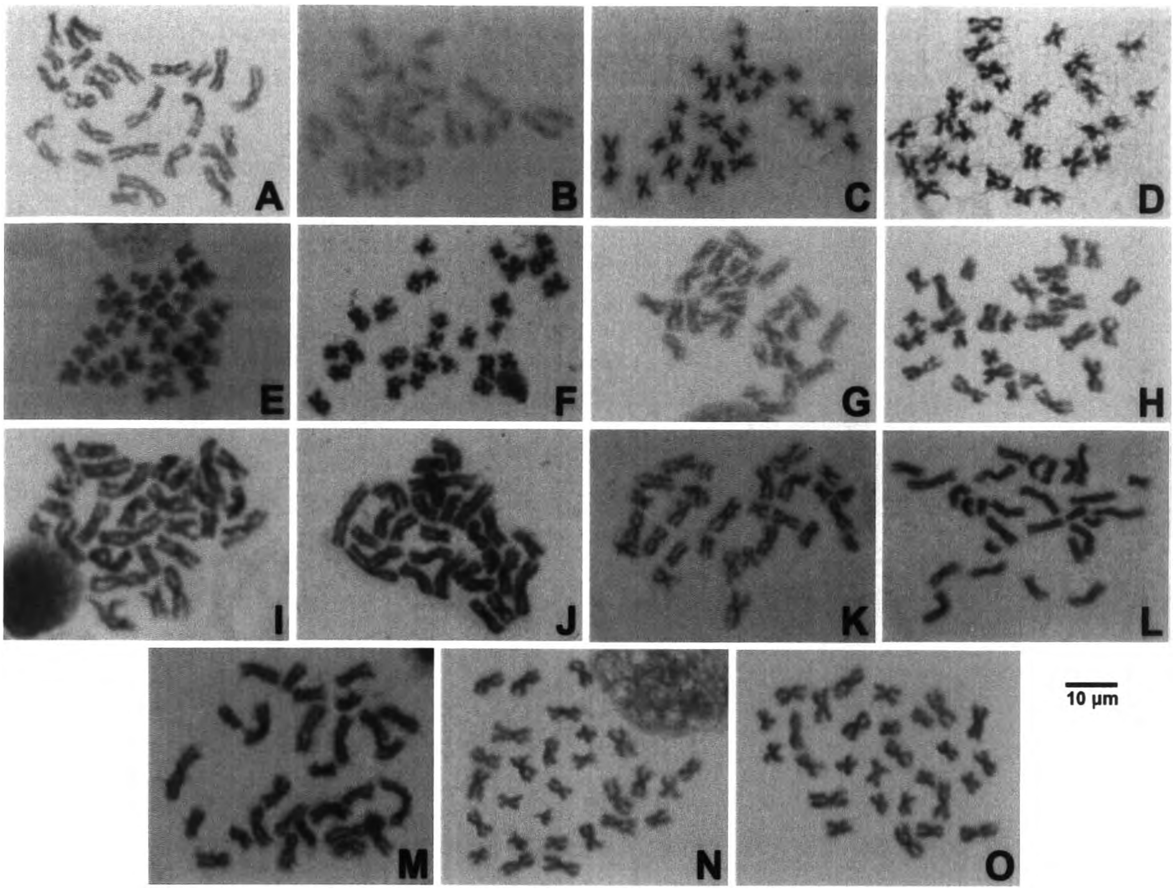

Figure 6.3 Mitotic chromosomes of, **A**, *Cyclophorus aurantiacus* (male, CUMZ 800); **B**, *C. cantori* (male, CUMZ 804); **C**, *C. courbeti* (male, CUMZ 805); **D**, *C. diplochilus* (male, CUMZ 826); **E**, *C. fulguratus* (male, CUMZ 809); **F**, *C. fulguratus* (male, CUMZ 813); **G**, *C. fulguratus* (male, CUMZ 815); **H**, *C. fulguratus* (female, CUMZ 815); **I**, *C. malayanus* (male, CUMZ 819); **J**, *C. malayanus* (female, CUMZ 819); **K**, *C. saturnus* (male, CUMZ 822); **L**, *C. speciosus* (male, CUMZ 823); **M**, *C. subfloridus* (male, CUMZ 824); **N**, *C. volvulus* (male, CUMZ 825); **O**, *C. volvulus* (female, CUMZ 825).

Figure 6.4 Karyotypes of ten *Cyclophorus* species as indicated in Figure 3, showing submetacentric (sm) and metacentric (not labelled). The chromosome markers are indicated by satellite chromosome (arrow head) and secondary constriction (arrow). ZZ and ZW represent sex chromosomes of male and female, respectively. Karyotypic variation within *C. fulguratus* found between two populations of central region (C) and northeastern region (NE).

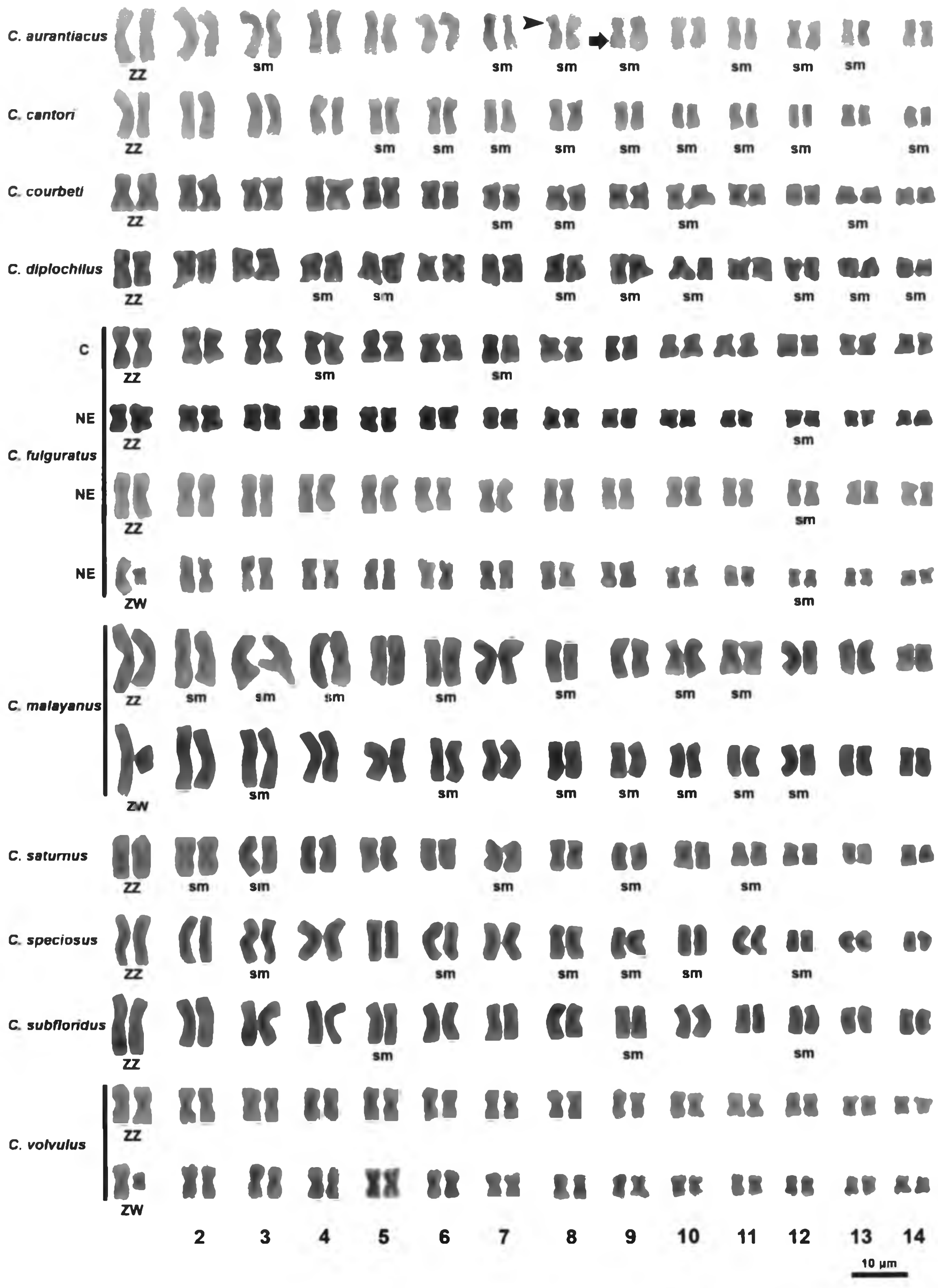
C. aurantiacus
C. cantori
C. courbeti
C. diplochilus
C
NE
C. fulguratus
NE
NE
C. malayanus
C. saturnus
C. speciosus
C. subfloridus
C. volvulus
ZZ
ZW
sm
2 3 4 5 6 7 8 9 10 11 12 13 14
10 µm

Table 6.1 The diploid (2n) and fundamental number (FN) in the known karyotypes of species of cyclophorid snails.

Species	Origin	2n	FN	References
Cyclophorus jerdoni (Benson, 1851)	India	28	-	Kasinathan and Natarajan (1975)
Cyclophorus polynema (Pfeiffer, 1854)	India	28	-	Choundhury and Pandit (1997)
Pterocyclus bilabiatus (Sowerby, 1853)	India	28	-	Kasinathan and Natarajan (1975)
Theobaldius ravidus (Benson, 1851)	India	28	-	Kasinathan and Natarajan (1975)
Theobaldius shiplayi (Pfeiffer, 1856)	India	28	-	Kasinathan and Natarajan (1975)
Micraulax scabra Theobald, 1876	India	28	-	Kasinathan and Natarajan (1975)
Cyclophorus aurantiacus (Schumacher, 1817)	Thailand	28	56	Present study
Cyclophorus cantori (Benson, 1851)	Thailand	28	56	Present study
Cyclophorus courbeti Ancey, 1888	Thailand	28	56	Present study
Cyclophorus diplochilus Möllendorff, 1894	Thailand	28	56	Present study
Cyclophorus fulguratus (Pfeiffer, 1852)	Thailand	28	56	Present study
Cyclophorus malayanus (Benson, 1852)	Thailand	28	56	Present study
Cyclophorus saturnus Pfeiffer, 1862	Thailand	28	56	Present study
Cyclophorus speciosus (Philippi, 1847)	Thailand	28	56	Present study
Cyclophorus subfloridus Ancey, 1888	Thailand	28	56	Present study
Cyclophorus volvulus (Müller, 1774)	Thailand	28	56	Present study

Table 6.2 Species, localities, karyotype formula, sex and number of species used in the present study (N).

Species	Localities (voucher specimens)	Sex	N	Karyotype formula
C. aurantiacus	Kang Krajan, Phetchaburi Province (CUMZ 800)	Male	10	7m + 7sm
C. cantori	Namphud, Phangnga Province (CUMZ 802), Phakayang, Ranong Province (CUMZ 804), Sramorakot, Krabi Province (CUMZ 801)	Male	15	5m + 9sm
C. courbeti	Brijinda, Chiang Mai Province (CUMZ 805), Kunkorn, Chiang Rai Province (CUMZ 806), Lansang, Tak Province (CUMZ 807)	Male	15	10m + 4sm
C. diplochilus	Samui, Suratthani Province (CUMZ 826)	Male	10	6m + 8sm
C. fulguratus	Teppitak, Nakhonsawan Province (CUMZ 809), Sangkat Rattanakeri, Uthaithani Province (CUMZ 810)	Male	15	12m + 2sm
	Chaiwan, Udonthani Province (CUMZ 814), Khao Chakan, Sra Kaeo Province (CUMZ 811), Khueang Nai, Ubon Ratchathani Province (CUMZ 818), Kokplasiew, Sakon Nakhon Province (CUMZ 813), Nawa, Nakhon Phanom Province (CUMZ 812), Nongkungsri, Kalasin Province (CUMZ 816), Phuwiang, Khon Kaen Province (CUMZ 815), Srikhoraphum, Surin Province (CUMZ 817)	Male, Female	28, 15	13m + 1sm
C. malayanus	Samui, Suratthani Province (CUMZ 820), Sramorakot, Krabi Province (CUMZ 819)	Male, Female	11, 7	7m + 7sm
C. saturnus	Phlieu, Chanthaburi Province (CUMZ 822)	Male	15	9m + 5sm
C. speciosus	Wang Takrai, Nakhonnayok Province (CUMZ 823)	Male	10	8m + 6sm
C. subfloridus	Nernmaprang, Phitsanulok Province (CUMZ 824)	Male	12	11m + 3sm
C. volvulus	Wang Kanleuang, Lopburi Province (CUMZ 825)	Male, Female	10, 7	14m

Abbreviations: m, metacentric; sm, submetacentric chromosome.