SOCIAL BEHAVIOUR AND ITS EVOLUTION IN SCYTODID SPIDERS (ARANEAE: SCYTODIDAE)

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SUMMARY

Sociality is a major innovation in the history of life. Understanding how social behaviour evolved and what maintained it has thus been a focus of evolutionary, behavioural, ecology, genetic and molecular biological research since Darwin. Despite being a minority, social and subsocial spiders are widely distributed in 10 distinctly unrelated families, including the spitting spider family Scytodidae. Scytodid spiders are known for their unusual habit of spitting sticky gum through the slit-like opening of their cheliceral fangs. With spitting being used in prey capture and in defense against predators, it is easy to think that spiders that spit are unlikely to be social. Solitary life may, in fact, be characteristic of most scytodids. Yet various forms of sociality have been documented in Scytodidae, hence making it a suitable and useful system for studying the evolution of sociality. Nevertheless, this group of spiders is poorly-studied and detailed studies of scytodid social behavior and the evolution of scytodid social behaviour are scarce. However, an important first step is to determine precisely what the social characteristics are of individual species, and so it is necessary to have a complete knowledge of the intraspecific behaviour of each species. Here I provide details concerning the social behaviour of a number of scytodid species from Africa and Asia. The main aim of the thesis is to investigate the social behaviour and its evolution of the scytodids by using field and laboratory observations and experiments as well as molecular phylogenetic analyses. In chapters 2, 3 and 4, the social behaviour of 19 scytodid species living in three different habitat types – caves, ground and vegetation - were investigated. Nine criteria for sociality were chosen to determine the social status of the scytodids and to examine whether there would exist any correlation between the ecological factors and the evolution of sociality. A total of four species of *Scytodes* (two from ground substratum and two from aerial vegetation habitats) are found to be subsocial exhibiting traits such as extended maternal care, cooperative prey capture, communal feeding, delayed natal dispersal and a degree of tolerance among brood-mates. The other species were solitary. My results partially support Kullmann's hypothesis that maternal care is ancestral to derived cases of permanent-sociality. I suggest the possibilities that extended maternal care through food provisioning improves the spiderlings' survivability and group living enhances prey capture abilities. I also propose that ecological factors such as low prey availability and high predation pressures may expalin why cave scytodids are solitary, whereas abundant food supply and the presence of numerous large-sized prey may promote sociality in the ground and aerial leaf zones.

In the absence of a robust phylogenetic tree for scytodid spiders, it is impossible to reconstruct social trajectories. In chapter 5, I reconstructed a phylogenetic tree using molecular data for rigorously testing hypotheses about the evolution of scytodid sociality. A total 87, 701 base pairs of molecular data from 49 species were used to reconstruct the relationships within the Scytodidae and with that of the sister families, Sicariidae and Drymusidae. The family Scytodidae as a whole is recovered as monophyletic, as well as *Dictis* within Scytodidae thus re-validating the current standing of this genus. I propose that some species (e.g. '*Scytodes*' *magna*, Guangxi '*Scyloxes*' sp. 1 and Guangxi '*Stedocys*' sp. 1) be placed in a new genus or subfamily within the Scytodidae. Although support for many nodes is weak (and a few are unresolved), the consensus tree indicates that at least six generic level clades exist within Scytodidae. Until the present study, a phylogenetic hypothesis of this family has never been presented and the relationships within Scytodidae have never been investigated using either morphological or DNA sequencing data. Resolution of the phylogenetic relationship of the Scytodidae is an important prerequisite to interpreting the evolution of sociality

By mapping social behavioural characters onto the molecular phylogeny of the scytodids, I tested the hypotheses about scytodid social evolution (Chapter 6). I show that sociality may have evolved at least four times in Scytodidae. In the phylogeny, subsocial species appear towards the tip clades and they are not monophyletic, suggesting that the subsocial scytodids are derived. The maternal care route hypothesis is thus supported. The dominant trend in the evolution of scytodid sociality is the presence of extended maternal care and tolerance among siblings. Interestingly, a solitary way of life occurred with ancestral habitats, the caves and leaf litter. This could be due to prey scarcity or an anti-predator strategy. The ability to evolve into (sub)social spiders may be due to changing environments. This study should be considered inconclusive because more scytodid species need to be included in the analysis. However, it implies a significant finding for exploring the causes and consequences of social evolution in this group of spiders.

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CHAPTER 1

GENERAL INTRODUCTION

Social behaviour refers to the cooperative interactions among individuals of the same species (Michener 1969). Most animals exhibit some form of cooperative social behaviour at some point during their life cycles. Cooperation can be defined as an act of working together simultaneously for a mutual benefit (Downes 1995). This behaviour may fall anywhere from complex cooperative nest building as seen in highly social animals such as termites where group members interact throughout their lives, to solitary individuals whose only social exchanges involve reproduction often over a limited mating period.

Sociality means group-living (Alexander 1974) and according to Kullman (1972), three traits must be exhibited by the animal in order for it to be social. These are tolerance, inter-attraction and cooperation. For years, one of the most perplexing and enduring questions in animal behaviour is how sociality evolved and what maintains it (e.g. Darwin 1859; Fisher 1930; Haldane 1953; Hamilton 1964; Wilson 1971; West Eberhard 1975; Costa and Fitzgerald 1996; Whitehouse and Lubin 2005). The basic principle must be that in general groups form and persist because all the individuals involved somehow attain benefits, in spite of detriments such as increased intensity of competition for resources, increased likelihood of disease and parasite transmission, and increased conspicuousness rendering a species more vulnerable as prey (Alexander 1974). This begs the question: what are the benefits of group living that offset these detriments? These include:

1) Lowered susceptibility to predation due to aggressive group defense, as with the ‘early warning’ effect in colonial web-building spider *Metepeira incrassata* (Uetz et al. 2001), or group cover as with schooling fish (Alexander 1974; Alcock 2009);
2) Access to scattered large supplies of food that individuals locate too infrequently on their own, as is often the case for social spiders (e.g. Nentwig 1985; Riechert 1985; Uetz 1989, 1992; Krafft and Pasquet 1991; Pasquet and Krafft 1992; Kim et al. 2005; Guevera and Avilés 2007);

3) Localization of resources such as safe nesting/sleeping sites or suitable breeding sites for animals such as birds (Ashmole 1963) and mammals (Bartholomew 1952); and building of communal webs which are capable of capturing larger prey and where cooperative brood care can take place.

Social behaviour in spiders

In invertebrates, sociality is mostly studied and modeled after social insects, while few models based on spider sociality have been studied (Avilés 1997; Crespi and Choe 1997; Whitehouse and Lubin 2005). Many of the terms used in the study of social behavior originated from the context of halcitine bees (Michener 1969) and then extrapolated onto the hymenoptera (Wilson 1971) and so on. As a result, sociality in spiders is often discussed using the same levels of classification as insects, which may not be appropriate due to the vast evolutionary divergence of the two groups (Uetz and Hieber 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007).

Spiders are generally envisaged as being solitary predators, aggressive when they encounter conspecific individuals and sometimes cannibalistic (e.g. Uetz 1983; Foelix 1996; Aviles 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). Nonetheless, sociality is a rare, but significant, phenomenon in spiders (Avilés 1997; Whitehouse and Lubin 2005; Agnarsson et al. 2006; Avilés et al. 2007; Johannesen et al. 2007; Lubin and Bilde 2007), hence presenting a unique opportunity to study these “exceptions to the rule’. Sociality is found in about 40 out of approximately 40,000 known (Platnick 2010) spider species, and how sociality is expressed in spiders has not been studied as extensively as in insects and vertebrates (Wilson 1971; Hermann 1982; D’Andrea 1987; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). Yet examples of sociality in spiders are of exceptional interest in the context of controversy over the role of *kin selection and *haplodiploidy in explaining the evolutionary origin of sociality in insects (Hamilton 1964; Wilson and Wilson 2007; Wilson 2008).

Why is it important to distinguish between the insect and spider models of sociality? Firstly, spider societies are more defined by their foraging function of the group rather than a reproductive one (Whitehouse and Lubin 2005). This is because in spiders, every individual is a reproductive unit, while in insects like ants, sterile workers serve one reproductive queen. The structure of spider societies differ vastly from insects because they depend on the web for prey capture, while an analogous nest structure in insects serves a protective purpose (D’Andrea 1987; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007).

Evolution of sociality in spiders

The evolution of sociality in spiders is thought to follow two alternative pathways (Shear 1970). One route is from the subsocial precursor, where group living evolved from extended maternal care. The other route is from a parasocial precursor, where group living evolved from aggregations around a resource (Evans 1958; Michener 1958; Wilson 1971). These two pathways exert different phylogenetic constraints on the evolution of group living. The same environmental factors (e.g. large prey items) promoting group living can give rise to different expressions of sociality. One such phylogenetic constraint is the degree of maternal care. Social (read: true social) spiders have evolved via the subsocial route and have a phylogenetic history of feeding communally with closely related siblings in the maternal nest (Whitehouse and Lubin 2005; Lubin and Bilde 2007).

Colonial (i.e. territorial permanent or periodic social) spiders originate via the parasocial route and individuals are territorial on their own webs. The groups are formed in part by aggregation, and therefore the individuals are not necessarily closely related. The lack of extended maternal care in these spiders and the defense of individual web-territories lead to a different group character to that of the cooperative spiders (Whitehouse and Lubin 2005; Lubin and Bilde 2007)

The subsocial pathway arose from extended parental care, with the juveniles tending to remain in their mother’s web until they develop into later instars and subsequent delayed natal dispersal (Buskirk 1981; Avilés 1997; Crespi and Choe 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). Natal dispersal here is defined as a movement of an animal from its birth site (Johnson and Gaines 1990). While sharing a nest with siblings, spiders should adopt tolerance and cooperation (Buskirk 1981). This highlights the evolutionary importance of maternal care in spiders. Baylis and Haplin (1982) proposed that parental care is the most common antecedent behaviour from which sociality has evolved. The behavioural antecedent to parental care is the parent-offspring association.

Behavioural and life history traits used in defining spider sociality

A variety of traits must be present before a spider can be considered truly social (but see examples of *Mallos gregalis* (Opell 1997), *Tapinillus* sp. (Avilés 1994) and *Delena cancerides* (Rowell and Avilés 1995)). They include: maternal care, extended maternal care, tolerance, cooperation in web building and prey capture, delayed natal dispersal, communication, female-biased sex ratio, inbreeding and generational overlap (Agnarsson 2006). It has been found in *Anelosimus* that more complex sociality generally occurs by 'terminal addition' (see Agnarsson 2006). For example, egg-sac guarding precedes post-hatching maternal care, which precedes cooperation in web building and prey capture, and so on. 'Maternal care', 'subsociality' and 'sociality' albeit useful as summaries for discussion, are categories that contain a mosaic of primitive and derived characters and overlap considerably (Agnarsson 2006). It is therefore important to define discrete criteria for defining sociality in a particular study system because such detailed categorizations can not only facilitate the comparison of species, but also suggest testable hypotheses about the evolution of sociality.

For the purpose of this study, I defined a species as being solitary if the progeny receives maternal care but disperses soon after eclosing from the egg-sac. In some species, newly eclosed progeny passively receive maternal care before they disperse, for example they remain in the maternal web which may offer some form of protection from the harsh environments. I still classify such species a solitary. Here, maternal care is defined as egg-sac attendance where the female protects the eggs from predation and parasites, thermoregulates the eggs and assist in the emergence of the spiderlings from the sac. Species are subsocial if the progeny, typically of one female, remain together long enough, due to the occurrence of extended maternal care, to contribute to cooperative activities such as group prey capture, but disperse prior to mating. In other word, in the subsocial spp. the social phase is terminated at the point of pre-mating dispersal. How is **extended** maternal care different from maternal care? Extended maternal care means that the duration of maternal care is extended and involves maternal food provisioning as well as defense of the brood such that juvenile aggregations maintained beyond the time when they are physiologically and morphologically capable of dispersing due to receipt of protection and nourishment from the adult female.

Species are social if colony members remain in the natal nest past reproductive maturity, so that the nests typically contain multiple adult females and their progeny. Communal brood care in the social species is performed by multiple females in the nest. Inbreeding and female-biased sex ratios also distinguish social from subsocial spp. In most social species, all stages overlap. These non-territorial permanently social spiders are sometimes termed quasisocial species.

The following behavioural traits were studied in each of the 19 species of scytodids:

Maternal care

Trivers (1972) defined parental investment as “any investment by the parent in an individual offspring that increases the offspring’s chance of surviving at the cost of the parent’s ability to invest in other offspring.” Parental investment will increase during evolution whenever a parent improves its overall reproduction (e.g. choosing a fitter mate or selecting a suitable site to breed in order to ensure reproductive success), resulting in a subsequent decrease in brood, litter size and rate of offspring production (Alexander 1974). Parental care is characterized by trade-offs in investment, for example between egg size and number of young or providing resources at the egg stage versus the post-hatching stage (Salomon et al. 2005).

Any form of parental behaviour that appears likely to increase the fitness of a parent’s offspring (Clutton-Brock 1991) is defined as parental care. Parental care may be essential to offspring survivorship in one or more of the following ways:

1) Protection against predators and parasites;
2) Regulation of nests/burrows or the egg itself which may perish if invaded with fungi;
3) Provisioning young after hatching; removal of mother might result in mortality or reduction in growth rates;
4) Protection from competition with older conspecifics

Maternal care is the dominant form of uniparental care that occurs in invertebrates and reptiles (Clutton-Brock 1991). This behaviour by which the female parent improves the fitness of her offspring has been of particular interest in the literature on spiders and ranges from spiders enclosing their eggs in silken sacs, and protecting the sac contents to the provision of juveniles with prey, shelter and anti-predator protection (Kullman 1972; Avilés 1997; Schneider and Lubin 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). As a step toward deriving a framework that can be used for explaining why maternal care occurs in certain groups and not others and why the details of maternal care vary, we need detailed comparative studies on maternal behaviour from a wide range of species.

Baylis and Haplin (1982) proposed that parental care is the most common antecedent behaviour from which sociality has evolved. Since the behavioural antecedent to parental care is the parent-offspring association, there is potential parent-offspring association in scytodids because females practice close egg attendance. It is therefore essential to understand the forms of maternal care provided by scytodid females because maternal behaviour may be the basis of sociality, if present, in scytodid spiders.

Extended maternal care

It has been argued that an extension of the initial period of tolerance (i.e. period when spiderlings are confined within the common egg-sac) can be facilitated through extended maternal care as suggested by Kullmann (1972). Extended maternal care is defined as juvenile aggregations maintained beyond the time when the young are physiologically and morphologically capable of dispersing from the natal site due to receipt of protection and nourishment from the adult female (Miller 1989). Based on the phylogeny by Coddington and Levi (1991), Avilés (1997) showed that the majority of social spiders with the exception of *Mallos gregalis* (Bond and Opell 1997) appear in clades where maternal care has been reported. Buskirk (1981) suggests that in spiders, more advanced form of sociality could evolve from the extension of parental care and delayed dispersal of the young. Extended maternal care and delayed dispersal of the young are characteristics of the subsocial species *S. intricata* (Eberhard 1986), Los Baños *Scytodes* sp. (Li et al. 1999) and the Singapore *Scytodes* sp. (Yap and Li 2009). As detailed intraspecific behaviours are needed, I divided extended maternal care into specific behaviours. The more elaborate maternal-care behaviours that may pre-adapt spiders to sociality include:

(1) ***Food provisioning***. Strong maternal effects, mediated by provisioning the young with food via prey capture and transport to natal nest (Foelix 1996), trophic eggs (Evans et al. 1995), regurgitation feeding (Kullmann and Zimmerman 1975) or matriphagy (Seibt and Wickler 1987), influence the size of the young at dispersal, and more importantly the tolerance levels among brood-mates in turn delaying the dispersal of juveniles. By reducing the chances of cannibalism and increasing the individual tolerance, food provisioning should be considered an important step in the evolution of permanent sociality.

(2) ***Protection***. One of the main arguments for the evolution of parental care is that it enhances offspring survival and reduces the risks to juveniles of dispersal away from the maternal nest

(Lambin et al. 2001). Protection can come in two forms: passive protection of the web or nest, as well as active protection from the maternal female against predators. Active defense of young and even cooperative defense is found in a number of the social arachnids (Buskirk 1981; Polis and Lourenco 1986; Mori and Saito 2005). When frightened, young amblygids, *Damon diadema*, moved significantly closer to their mother or siblings and mothers threatened or actively attacked potential aggressors (Rayor and Taylor 2006);

(3) ***Absence of maternal aggression***. Bessékon (1997) showed that the reproductive state of a female exerts a major influence on its aptitude to tolerate the young and ensure maternal care, and that only pre-emergence females were found to be aggressive towards young homospecifics. Tolerance of conspecifics by a highly predaceous spider requires that the predator be able to distinguish conspecifics from prey (Kullmann 1972). For example, *Coelotes terrestris* females can discriminate conspecific young from prey (i.e. cricket larvae), more of which they kill and ingest, and this discrimination is at least partially based on cuticular chemical stimuli during body contact (Bessékon 1997). Young whip scorpions, *Phrynus marginemaculatus* and *Damon diadema,* remain closely associated and highly interactive with their mother and siblings for approximately one year. Interactions include both active aggregation and frequent amicable tactile interactions through whip stroking (Rayor and Taylor 2006). *Scytodes fusca* (Bowden 1991), *S. longipes* (Nentwig 1985) and *S. intricata* (Eberhard 1986) adult females tolerate early-instar juveniles on the web.

(4) ***Close contact with maternal female upon eclosion***. It has been found that contact with the maternal female and siblings affect hunting behaviour, learning and central nervous system (CNS) development in spiderlings of a lycosid, *Hogna carolinensis* (Punzo and Ludwig 2002). Spiderlings emerged from their egg sacs and climbed onto the dorsal surface of their mother's abdomen where they remained until their yolk supply was depleted. Maintaining early contact with the maternal parent has profound effects on the CNS development of these spiderlings, and influences their ability to capture prey. Gundermann et al. (1988) showed that the maternal provisioning activity (i.e. food provisioning either through prey supply or regurgitation) is essentially regulated by exogenous factors, that is, stimulations emanating from the developing progeny. When funnel-web spider *Coelotes terrestris* females were separated from their brood, the “prey handling time” of these females shifted from a few minutes to more than two hours (Gundermann et al. 1988). Thus, it may be possible that by aggregating around the mother, either by mounting her body or in the maternal nest, spiderlings stimulate a ‘food supply activity’ in the maternal female.

Cooperation

Cooperative behaviour is often envisaged as a particularly important characteristic of social animals. For social spiders, behaviour that suggests cooperation includes web building, group prey-capture and food sharing, web cleaning and brood care (Buskirk 1981; Avilés 1997; Lubin and Bilde 2007), with benefits being in terms of improved foraging success, anti-predatory protection or both (e.g. Kullman 1972; Krafft 1979; Riechert 1985; Pasquet and Krafft 1992; Rypstra 1993; Avilés 1997; Gundermann et al. 1997; Avilés and Tufiño 1998; Uetz et al. 2002). Siblings are often the closest relatives within a population, and more often than not they are one another's most direct competitors. Therefore, the extremes of cooperation and competition may both be represented in their interactions (Alexander 1974). For example, subsocial *Amaurobius ferox* practice collective prey-capture but individual spiderlings have been observed to pull in different directions (Kim et al. 2005). Cooperative hunting, which is one of the most widely occurring forms of cooperation in animals, has been hypothesized to be an important evolutionary trait of sociality in certain animal groups (Packer and Ruttan 1988; Dugatkin 1997). Indeed, cooperation in prey capturing is an essential characteristic in permanently social spiders (Kim et al. 2005; Whitehouse and Lubin 2005; Lubin and Bilde 2007).

Tolerance

Tolerance (i.e. suppression of intraspecific aggression and cannibalism) is another important characteristic that has often been considered to be important in the evolution of spider sociality (Kullman 1972; Buskirk 1981; D'Andrea 1987; Avilés 1997; Whitehouse and Jackson 1998; Whitehouse and Lubin 2005; Lubin and Bilde 2007). Dispersal from the natal nest in the subsocial spiders has been linked to loss of mutual tolerance (Kullmann 1968; Buskirk 1981; Wickler and Seibt 1993). Despite living in groups, some subsocial spiders show little tolerance towards conspecifics and some even practice cannibalism after an early social phase (Bowden 1991; Wickler and Seibt 1993; Avilés 1997; Whitehouse and Jackson 1998; Whitehouse and Lubin 2005; Lubin and Bilde 2007). This suggests that highly pronounced tolerance is not a condition that must be present in order for sociality to evolve in spiders.

Nonetheless, I chose to investigate whether tolerance exists in the more social scytodids because I adhere to the notion that mutual tolerance may be the first requirement for the transition from solitary to social living to occur (Kullmann 1972; Krafft 1982; D'Andrea 1987).

Avoidance of cannibalism within family groups would lead to increased tolerance, which could facilitate prolonged association and in turn cooperative behaviour such as joint prey capture and brood care.

Delayed natal dispersal

Insufficient food and intraspecific competition, including aggressive interactions and cannibalism may all be responsible for the early dispersal observed in broods containing small spiderlings (Salomon et al. 2005), whereas delayed juvenile dispersal is often associated with extended parental care that benefits the offspring (Clutton-Brock 1991). The juveniles benefit in that they obtain a greater and steadier intake of resources. The transition from subsocial to quasisociality involves suppression of the dispersal phase. Dispersal behaviour is a factor constraining socialization (Kim 2000) because the spiderlings, should they decide to remain in the maternal nest, can bring about a permanent social organization. There is evidence that the time of dispersal is influenced by prey availability (Morse 1980; Krafft et al. 1986; Ruttan 1990). Understanding why and how the dispersal phase is suppressed is core to understanding the evolution of advanced social systems in spiders (Avilés and Gelsey 1998).

The subsocial spiders provide an excellent opportunity in addressing these questions. While the majority of spiders are solitary with dispersal occurring a few days after eclosing from the egg sac, the social spiders remain in the maternal web for their entire lives resulting in multigenerational system. Subsocial spiders, however, are the intermediate, dispersing much later than their solitary counterparts and show plasticity in the time of dispersal (Kim 2000). The transition from periodic to permanent sociality, therefore, must have involved suppression of this dispersal phase. Here, I study and describe the natal dispersal patterns (e.g. asynchronous dispersal? Which spiderlings disperse early?) of the scytodids and discuss aspects of this pattern that may be of relevance to understanding the transition to sociality in the spitting spiders. Initial colony composition surveys performed in the field would give me a good idea on the dispersal patterns. For example, a colony that comprises of mother-late instars association may imply that the spiderlings are delaying the dispersal time due to advantages such as protection, gaining more nutrition via capture of large prey and conserving silk.

Fecundity

Investment in young via post-hatching maternal care is relatively uncommon among invertebrates (Clutton-Brock 1991), but it is evident in several spider families. Young that obtain a large amount of resources from the mother are more likely to grow to large adults and thus have a higher fitness (Salomon et al. 2005). In social spiders, females are less fecund (i.e. lay fewer eggs) than their solitary relatives (Riechert 1985; Vollrath 1986; Wickler and Seibt 1993) because of the heavier resource investment into her brood. A large proportion of the resources an offspring needs for its development are obtained in the juvenile stage (Bernardo 1996), and it is for this reason that mothers who practice extended care include food provisioning as a major part of the repertoire of extended maternal care behaviours. For example, *Stegodyphus lineatus* females produce a single small clutch with small eggs, feed their young by regurgitation and are finally consumed entirely by their young (Schneider 1996), thus, the female invests all her resources in a single reproductive event.

I chose to study the reproductive traits of scytodids because females that produce fewer eggs and have longer intervals between successive clutches will be an indication of the more social spiders. That is, females of species that have low fecundity may be more social as an increase in investment per individual young is often associated with a decrease in the number of offspring produced (Schneider 1996).

Role of ecological factors in the evolution of sociality in spiders

Factors such as predator protection (e.g. Henschel 1998), access to prey too large for individual spiders to capture (e.g. Pasquet and Krafft 1992), reduced per capita investment in silk (Riechert 1985) and thermal control (Seibt and Wickler 1990) have been proposed to promote group living in the more derived social species (reviewed in Avilés 1997). I suggest the following ecological factors shape the evolution of scytodid sociality, and include them in my discussion.

Three-dimensional web

Within the araneomorph spiders, cooperative or communal behaviour occurs more frequently within genera which depend on webs (irregular or orbicular) (Shear 1970; Krafft 1979; Buskirk

1981; D’ Andrea 1987; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). There are two reasons why a web may be important for sociality to arise. First, webs provide a physical link among nest-mates thus providing a means of communication (Shear 1970) and cohesiveness to the group (Krafft 1979; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). Second, webs are an expensive structure thus providing an incentive for nest-sharing and communal web-building (Riechert 1985; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007).

The ability to capture large prey insects is a major driving force for sociality in spiders (Nentwig 1985). Social web-builders such as *Anelosimus* (Brach 1975; Christenson 1984), *Stegodyphus* (Kullmann 1972) and *Agelena consociata* (Krafft 1969, 1970) construct extremely large 3-D webs, possibly to effectively capture larger insects such as grasshoppers and roaches which cannot be caught by the web of one individual spider. As individual spiders fuse their webs to form a communal sheet, nest-sharing and group-living occurs.

The Madagascan spitting spider, *Scytodes socialis*, lives in groups of up to 16 individuals, including juveniles, adult males and females. The baseball-sized webs that are constructed with debris, leaves, branches and strands of silk, not only provide shelter for the spiders but also help to ensnare passing prey. Mature males and females and even the juveniles will participate in the prey capture, and captors share prey with the non-captors (Miller 2006). By working as a team, these spiders are able to subdue large prey such as roaches, moths and flies which a single individual is incapable of doing (Miller 2006). Most species of spitting spiders do not build webs to capture prey. *S. socialis*, however, follows a trend of almost all other social spiders by building communal webs. The vibrations of such webs serve the function of alerting multiple colony members to the presence of prey.

Prey abundance

Many researchers have stressed the importance of food supply in determining the length of the tolerant phase, maintaining that periodic-social (i.e. subsocial) spiderlings can be made to remain together in the natal nests for significantly longer durations if subjected to high-prey treatments (e.g. Krafft et al. 1986; Ruttan 1990; Gundermann et al. 1993; Kim 2000). Year-round prey availability and large prey size can explain in part the mostly tropical distribution of social spiders (reviewed in Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007).

Plentiful food supply may facilitate a delay in the timing of dispersal, and with large prey cooperation becomes more profitable (Ward 1986; Rypstra and Tirey 1990). Prey availability is thus an important factor for the size of the territory of a spider (Riechert 1978) and for spider sociality (Nentwig 1985).

Prey abundance has been implicated in evolution of sociality in spiders in many cases. Aggregations of the orb-weaver, *Nephila clavipes* form around areas of high prey abundance in Peru (Rypstra 1985). Some species that build big web conglomerates do so to take advantage of high insect densities (Uetz et al. 1982). These examples, including *S. fusca*, indicate that high prey abundance is important in the evolution of sociality.

Predations risk

Benefits of living in social groups often include a reduction in predation risk (Alexander 1974; Rayor and Uetz 1990, 1993). Seibt and Wickler (1988) found that predation on *Stegodyphus* spiders is more significant in promoting sharing silk and sociality. Comparing solitary and socially living *S. dumicola*, Henschel (1993) found the former particularly vulnerable to *Crematogaster* ants, whereas spiders in groups could effectively ward off ant troops by blocking their way with cribellum silk. Offspring of *Stegodyphus* benefit from a remarkable degree of safety in a large nest (Wickler and Seibt 1993), showing that group-living may stem from predator pressures.

Intense predation on eggs by generalist predators such as ants may be an important selective pressure promoting parental care in the Neotropical harvestman, *Bourguyia albiornata*, which oviposit almost exclusively inside the tube formed by curled leaves of a bromeliad (Machado and Oliveira 2002). Fink (1987) proposed that ants are the major selective factor favouring egg-sac guarding in the green lynx spider, *Peucetia viridians*. She suggested that some of the features of its maternal care, such as silk cutting and egg-sac suspension, may be specialized anti-ant adaptations. In the presence of predators, groups may form to provide protection. In such a protective society, the function of the group is the sum of individual behaviours in the group that enhance the survival of group members (Whitehouse and Lubin 2005). However, if the average fitness benefit to individuals living in a group is less than the fitness of those living solitarily then individuals will start to disperse from the group (Whitehouse and Lubin 2005).

Phylogeny and its implication in the evolution of sociality

Comparative biology typically relies on a phylogeny to interpret the evolution of character systems (e.g. Brooks and McLennan 2002). Mapping of these traits onto a phylogenetic tree enables the examination of evolutionary patterns in diverse data sets such as biochemistry, karyology, morphology, ecology, and behaviour (Lim and Dunlop 2008). The transition from solitary to social living is one of the key events in evolution. The rarity of this transition has provided a classic problem for evolutionary biologists and the unparalleled success of social animals is of central interest to all ecologists. To investigate the factors underlying this transition, it must be studied in a phylogenetic context and require clades of extant species that have several independent origins and different degrees of sociality. Such clades are exceptionally rare.

Fewer than 25 species are known to be truly social (Avilés 1997; Whitehouse and Lubin 2005; Agnarsson et al. 2006; Lubin and Bilde 2007). Despite the small number, social species are widely distributed across nine genera in six families (Lubin and Bilde 2007). There is now evidence to support at least 18 independent origins of sociality (Agnarsson et al. 2006). Within the family Theridiidae, which holds the majority of social species, there are 11-12 social species distributed across 3 genera representing 9 independent origins of sociality (Avilés 1997; Agnarsson 2006; Avilés et al. 2006;). There are three independent origins of sociality in the genus *Stegodyphus* of the family Eresidae (Johannesen et al. 2007). Therefore, with variations in the degree of sociality, social spiders may provide a unique opportunity for analyzing the evolution of sociality.

Study system

Within the family Scytodidae Blackwall 1864, there are five genera, namely *Dictis* Koch 1872, *Scyloxes* Dunin 1992, *Scytodes* Latreille 1804, *Soeuria* Saaristo 1997, and *Stedocys* Ono 1995. The spiders in the genus, *Scytodes*, are found all over the world, with a total of 225 described species (Platnick 2010). These spiders are known for their unusual habit of spitting sticky gum through the slit-like opening of their cheliceral fangs (Eberhard 1986; Li et al. 1999; Miller 2006). With spitting being used in prey capture and in defense against predators (e.g. Bristowe 1958; McAlister 1960; Gilbert and Rayor 1985; Li et al. 1999), it is easy to think that spiders that spit would be unlikely to be social. Solitary life may, in fact, be characteristic of most scytodids. Yet various forms of sociality are also known from research on the species in this genus (see Table 1-1). For example, *Scytodes fusca* Walckenaer from Queensland lives in colonies (Bowden and Jackson 1988; Bowden 1991), whereas *S. intricata* Banks from Panama (Eberhard 1986) and *Scytodes* sp. from the Philippines (Li et al. 1999) are subsocial, exhibiting extended maternal care. For these two species, there is also evidence of cooperation in prey-capture and delayed offspring dispersal. Animals with these traits are often called subsocial. Yet another type of organization is known for a scytodid, this coming from a study of *S. socialis* from Madagascar: non-territorial multiple-adult (Miller 2006). Other known *Scytodes* species are solitary (Valerio 1981; Eberhard 1986). *Scytodes*, being a genus comprising of species that ranges in social organization from solitary to social, appears to be especially suitable for research concerned with the evolution of spider sociality.

However, an important first step is to determine precisely what the social characteristics are of individual species, and so it is necessary to have a complete knowledge of the intraspecific behaviour of each species. Here I provide details concerning the social behaviour of a number of scytodid species from Africa and Asia. The main aim of the thesis is to investigate the social behaviour and its evolution of the scytodids by using field and laboratory observations and experiments as well as molecular phylogenetic analyses. Prior work indicates the presence of solitary, subsocial and social behaviour in the focal family, Scytodidae. This study began with a survey to verify the presence of each of these forms of sociality in wild populations, as well as to check for the possibility of further, as yet unrecorded forms.

The rapid diversification of scytodids together with their ecology and high sociality potential makes this genus a promising candidate for future studies of the evolution of sociality. The phylogeny of these spiders, coupled with detailed behavioural studies provides an ideal background for comparative studies of the relative influence of various ecological factors on the evolution of sociality.

Table 1-1. Social behaviour of *Scytodes* species that have been studied so far

Species	Origin	Social status	Social behaviour characteristics	Reference
Scytodes fusca	Queensland, Australia	Social: Communal-territorial	• Cohabiting male/female pairs • Females tolerant of juveniles • *'Communal-territorial' with up to 50 individuals occupying a web complex.	Bowden and Jackson 1988
S. intricata	Costa Rica; Panama	Subsocial	Existence of mother-offspring associations, but lack of joint prey capture and prey sharing.	Eberhard 1986
S. longipes	Neotropics	Solitary	Carries egg-sac but without other social tendencies	Dabelowe 1958
Los Baños Scytodes	Los Baños, the Philippines	Subsocial	• Existence of mother-offspring associations • Female provides food for young	Li et al. 1999
S. thoracica	Luzon, the Philippines	Solitary		Dabelow 1958
S. socialis	Madagascar	Social: Communal-non-territorial	• A single web occupied by males, females and juveniles. • Cooperative prey capture. • Participants and non-participants feed together	Miller 2006
Scytodes sp.	Singapore	Subsocial	• Existence of mother-offspring associations • Female provides food for young	Yap and Li 2009

* The term 'communal-territorial' refers to adults of one generation using a composite nest but each territorial of its own web

Objectives

The aim of this study has three folds. First, to determine the social status of scytodids (i.e. solitary, subsocial or social) and find out whether there exists any correlation between the ecological factors and the evolution of sociality, I categorized the scytodids into their habitat types: cave-dwelling, ground-dwelling and aerial leaf-dwelling. Three of the thesis chapters (Chapters 2, 3 and 4) are based on the sociality of the scytodids. To do so, detailed and complete knowledge of the intraspecific scytodid behaviour is required. To determine the sociality of each species, eleven criteria for sociality were chosen, including (1) the presence/absence of parental care (e.g. egg-carrying or not); (2) the presence/absence of extended parental care; (3) the presence/absence of group foraging behaviour; (4) natal dispersal patterns; (5) adult-juvenile and juvenile-juvenile interactions (i.e. social signals used between conspecifics such as aggressive interactions between siblings); (6) total number of egg-sacs; (7) number of spiderlings produced by a female in her life span; (8) the interval between batches (egg-sacs); (9) the interval between hatching and subsequent egg-sac production; (10) egg hatching time; and (11) number of spiderlings per clutch. Maternal effects can slow or accelerate the rate of evolution of a character compared to the case when no maternal effect is involved. Because of the notion that maternal care appears to be ancestral to derived cases of permanent-sociality (i.e. sociality is generally thought to evolve via the ‘‘maternal care route’’) (Kullmann 1972; Kraus and Kraus 1988; Johannesen et al 1998, 2007), in other words, maternal care precedes subsociality, which in turn precedes quasisociality (permanent sociality) in the evolutionary history of social lineages (e.g. Burgess 1978; Buskirk 1981; Schneider 2002; Agnarsson 2002, 2004), many of the behavioural traits defined in this study focus on maternal behaviours, that is maternal-offspring interactions.

Many benefits have been proposed for group-living in spiders. The main ecological factors invoked as responsible for the formation of groups are foraging advantages, predator defense and the establishment and maintenance of nest-sites (Alexander 1974; Emlen 1984; Crespi and Choe 1997). Overall, average fitness should be higher for individuals living in groups compared with solitary individuals (Bilde et al 2007). In this study, I tested whether groups of spiderlings captured prey more efficiently than singletons, as well as investigated the effects of large-sized prey on joint prey capture. Due to the advantages of remaining in the natal nest, I also studied their natal dispersal patterns to see whether late dispersers gained more body mass than their siblings that left the nest early. I suggest the possibilities that group living enhances prey capture abilities and extended maternal care through food provisioning improves the spiderlings' survivability.

My second objective is to reconstruct a robust phylogenetic tree for Scytodidae using various nuclear and mitochondrial genes. The family Scytodidae is in need of revisionary taxonomic work because some of the earlier studies have been made on misidentified species. Morphological data traditionally have been used to delimit species and continue to be widely used today, but many recent studies have used DNA sequence data to test traditional, morphology-based taxonomies (Wiens and Penkrot 2002). With the help of a molecular phylogeny, augmented by morphological data, I re-validate a current standing genus (e.g. *Dictis*) and corroborate Lehtinen's (1986, 2010) proposal of erecting new genera within this family.

Regardless of the level of sociality, all scytodids maternal female demonstrate care for their eggs. I hypothesize that sociality in scytodids arises through temporal extension of juvenile web sharing during maternal care – the 'maternal care hypothesis' (Burgess 1978; Vollrath 1982; Uetz 1983; Smith 1986, 1987; Avilés 1986, 1997, 1999, 2000; Avilés and Gelsey 1998; Agnarsson 2002). In order to test this hypothesis, a phylogeny is essential. Using molecular data from both mitochondrial and nuclear genes, this study is the first to test this hypothesis phylogenetically. Until the present study, a phylogenetic hypothesis of this family has never been presented and the relationships within Scytodidae have never been investigated using either morphological or DNA sequencing data. Resolution of the phylogenetic relationship of the Scytodidae would be an important prerequisite to interpreting the evolution of sociality. The phylogenetic pattern of sociality can also help answer the questions about the number of origins of sociality, or if the social scytodids form a monophyletic group and what the molecular phylogeny suggest about trends in the evolution of social behaviours such as extended maternal care, cooperative foraging and delayed dispersal patterns.

My third specific objective is to test the hypotheses about the origin and evolution of scyodid social behaviour by mapping the social behavioural traits onto the molecular phylogenetic tree. Nineteen behavioural characters, of which 13 were mapped as discontinuous data, were defined and mapped onto the preferred phylogeny in order to study the evolutionary trends of specific social behavioural traits in the scytodids. In addition, one ecological character i.e. habitat type is also included to see whether ecological correlates of cooperation and sociality in scytodids exist. Specifically, I wanted to investigate whether habitat type, which indirectly affects prey abundance and prey types, can influence the degree of sociality in scytodids. I also compared the sociality of scytodids to the different social systems found in other spider families, and using the phylogenetic data, explore the causes and consequences of social evolution within Scytodidae.

The sequence of the chapters in the dissertation reflects how the project evolved:

The environment may play a role by either providing incentives for sociality or by placing insurmountable obstacles. Caves are among the most fascinating environments on Earth, where a specialized underground biota provides evidence of evolutionary adaptations for life under severe conditions. Spiders are commonly found in caves, but little attention has been paid to their behaviour. **Chapter 2** deals with behaviour and life history, including maternal care, cooperative prey-capture, tolerance among siblings and reproduction, of five cave spitting spiders. *Scytodes magna* and Guangxi *Scyloxes* sp. 1 occur exclusively in the aphotic zone, whereas *Scytodes fusca*, *S. cavernarum* and the Philippines *Scytodes* sp. 2 can be found relatively close to the cave entrance, known as the light zone. Like other typical spitting spiders, *Scytodes fusca*, *S. cavernarum* and the Philippines *Scytodes* females carry their egg-sacs with their chelicerae. However, *S. magna* and Guangxi *Scyloxes* sp. 1 females do not carry their egg-sacs in their chelicerae, and instead hang their egg-sacs on their webs. *S. fusca*, a widespread species that inhabits a wide range of habitats, is studied for the first time from caves. My results demonstrate that all five species of cave scytodids are asocial despite being found in different zones of the caves. However, I found a few traits that deviate from the typical solitary characteristics. This includes late natal dispersal in Guangxi *Scyloxes* sp. 1 spiderlings and low fecundity in *S. cavernarum*.

In **Chapter 3**, I report two subsocial spitting spiders with extended maternal care, prolonged mutual tolerance (in relation to low cannibalism rates) and cooperative prey-capture; *Scytodes gooldi* and *S. rubra*, two species of spitting spiders discovered in South Africa from field and laboratory observations. After hatching there is an extended mother-offspring and sibling association, with juveniles remaining in the maternal web up till the seventh instars in the case of *S. rubra*. This extended tolerant phase and highly cooperative behaviour led me to classify *S. rubra* as a “transition into social” species. In contrast, the third species Yunnan *Scytodes* sp. 3 from China, exhibits maternal care but the tolerant phase for this species is much shorter than *S. gooldi* and *S. rubra*. It terminates when second-instar spiderlings dismount from the body of females to disperse. The social systems in *S. gooldi*, *S. rubra* and Yunnan *Scytodes* sp. 3 were compared to those of other ground-dwelling spiders and arachnids. The advantages of prolonged tolerance in living under rocks and in dead logs, as well as the possible reasons for a solitary life strategy in leaf litter are also discussed.

Previous studies have shown that scytodid species exhibiting social behaviour all live in the aerial vegetation microhabitats. In **Chapter 4**, I examined the social status of 11 scytodids species from two genera (*Scytodes* and *Dictis*) that inhabit the leaves of shrubs and herbs by examining colony structure, eleven behavioural and reproductive traits. Two *Scytodes* species (*Scytodes* sp. 1 and *S. pallida*) are found to exhibit extended maternal care with food provisioning, joint prey-capture and communal feeding among siblings, low cannibalism rate, and delayed natal dispersal, confirming their status as subsocial species. Other three species of *Scytodes* and all six *Dictis* species were not seen to exhibit extended maternal care, cooperate in prey-capture, or share food among siblings, in addition to starting cannibalism and dispersal from nests early, suggesting they are solitary. I also compare the behaviours of two different populations of *S. pallida* (Singapore and Malaysia) and *Dictis* sp. 2 (Singapore and China). The two populations of *S. pallida* display the same social organization, but there are population variations in the reproduction and cooperative prey-capture efficiency.

The spitting spiders (Scytodidae) are an assemblage of five poorly defined genera – *Scytodes*, *Scyloxes*, *Soeuria*, *Stedocys* and *Dictis* - among which hypothesized relationships have yet to be studied thoroughly. Understanding these relationships is important because they will serve as a basis for testing the hypotheses concerning scytodid social evolution. In **Chapter 5**, I constructed the first molecular phylogeny of the spider family Scytodidae using the nuclear 18SrDNA, 28SrDNA and Histone 3 genes and the mitochondrial COI gene from 44 ingroup taxa from a wide geographic range. Although the monophyly of the most speciose genus *Scytodes* has not been resolved, my results strongly support the monophyly of Scytodidae. The parsimony analysis (49 taxa) resulted in five equally most parsimonious trees, and six well-defined clades – the tentative ‘*Magnodes*’; *longipes*; *fusca*; *thoracica*; *pallida*; and *Dictis* groups. At least three other clades (containing *Stedocys*, putative *Scyloxes* and *Stedocys*) remain poorly resolved. Based on their placement on the phylogeny and unique morphologies, I found that scytodid taxonomy is in need of a revision and propose that more genera should be erected. This phylogeny may provide a framework for analyzing patterns of evolution in social behaviour of spitting spiders.

By mapping social behavioural characters onto the molecular phylogeny of the family Scytodidae, I tested the hypotheses about scytodid social evolution in **Chapter 6**. I show that sociality may have evolved independently at least four times in Scytodidae. In the phylogeny, subsocial species appear towards the tip clades and they are not monophyletic, suggesting that the subsocial scytodids are derived. The maternal care route hypothesis is thus supported. The dominant trend in the evolution of scytodid sociality is the presence of extended maternal care and tolerance among siblings. Interesting, a solitary way of life occurred with ancestral habitats, the caves and leaf litter. This could be due to prey scarcity or an anti-predator strategy. The ability to evolve into (sub)social spiders may be due to changing environments. This study should be considered inconclusive because more scytodid species need to be included in the analysis. However, it implies a significant finding for exploring the causes and consequences of social evolution in this group of spiders.

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CHAPTER 2

Comparative Biology of Cave-Dwelling Spitting Spiders: Parental Care, Cooperative Prey-Capture, Cannibalism, Natal Dispersal, and Reproductive Behaviour

ABSTRACT

The environment may play a role by either providing incentives for sociality or by placing insurmountable obstacles. Caves are among the most fascinating environments on Earth, where a specialized underground biota provides evidence of evolutionary adaptations for life under severe conditions. Spiders are commonly found in caves, but little attention has been paid to their behaviour. Here the life history, including maternal care, cooperative prey-capture, tolerance among siblings and reproduction, of five cave spitting spiders (Scytodidae) was examined. *Scytodes magna* and Guangxi *Scyloxes* sp. 1 occur exclusively in the aphotic zone, whereas *Scytodes fusca*, *S. cavernarum* and the Philippines *Scytodes* sp. 2 can be found relatively close to the cave entrance, known as the light zone. Like other typical spitting spiders, *Scytodes fusca*, *S. cavernarum* and the Philippines *Scytodes* females carry their egg-sacs with their chelicerae. However, *S. magna* and Guangxi *Scyloxes* sp. 1 females do not carry their egg-sacs in their chelicerae, instead they hang their egg-sacs on their webs. This behaviour is previously undocumented in scytodids. *S. fusca*, a widespread species that inhabits a wide range of habitats, is studied for the first time from caves. All five species of cave scytodids are asocial despite being found in different zones of the caves. However I found a few traits that deviate from the typical solitary characteristics. This includes late natal dispersal in Guangxi *Scyloxes* sp. 1 spiderlings and low fecundity in *S. cavernarum*. Possible explanations for the solitary strategy adopted by the cave scytodids were also given.

INTRODUCTION

The specialized underground biota found in caves provide evidence of evolutionary adaptations for life under severe conditions that include reduction or absence of light, constant (usually very high) humidity, extremely stable temperature, high carbon dioxide and low oxygen levels, and severely limited energy (food) input (Culver, 1982; Howarth, 1983, 1993; Parzefall, 1985; Culver & Sket, 2000; Culver et al., 2003). The energy input to caves is also low and usually allochthonous as there are no primary producers. In spite of these challenges, many animals live in cave environments, with invertebrates being especially common. Cavernicolous environments select for loss of characters that are unnecessary and costly to produce or maintain (e.g. eyes, pigmentation, thickened cuticle), and accentuation of characters that assist in resource location or compensate for the unnecessary characters lost (e.g. attenuated appendages, reduced metabolic rate) (Prendini et al., 2010). Cavernicolous environments also create natural barriers, limiting dispersal, restricting gene flow, and resulting in the evolution of localized endemics (Crouau-Roy, 1989; Lamoreaux, 2004).

Depending on the extent of physiological, behavioural and morphological specialization, various cave ecomorphotypes can be recognized (Howarth 1983, 1993). Obligate cavernicoles or troglobites are restricted to deep cave environments and are highly stenotopic. They exhibit pronounced troglomorphies, ecomorphological adaptations to hypogean habitats, including the loss or reduction of eyes and pigmentation. Troglobites include various species of blind Crustacea, Collembola and Psedoscorpiones (Levy 2007). There are a few species of troglobitic arachnids, including the eyeless scorpion, *Akrav israchanani*, from the Ayyalon caves in Israel (Levy 2007), the Mexican cave-dwelling harvestman, *Hoplobunus boneti* (Mitchell 1971), and *Islandiana* sp. (Linyphiidae) of Florida caves (Peck 1970). Facultative cave-dwellers or troglophiles are found in all cave habitats, from the entrance to the deep zone, and show intermediate degrees of adaptation. Trogloxenes are species that utilize caves for shelter during the day but feed outside at night and there are the cave accidentals as well (Barr 1967).

Sociality in cave arthropods is rare. Having analysed what was known about cave ants, Wilson (1962) hypothesized that social insects “never become truly troglobitic” because “they are unable to maintain sufficiently large cave demes”. It is implicit that the underlying reason is food scarcity. However, this view was challenged by the discovery of the first truly troglobitic ant, *Leptogenys khammouanensis*, from the caves of Laos. Voids of Laos, by providing large reservoirs of food, may have created an unusual opportunity for such evolution to occur (Roncin & Deharveng, 2003). Many arachnids live in caves. Previous work on cave arachnids includes studies of reproductive behaviour (e.g. Doran et al. 2001), analysis of adaptations to the hypogean environments (e.g. Hadley et al. 1981), examination of population genetic structures and speciation mechanisms (e.g. Hedin 1997), and research on systematics and evolution (e.g. Arnedo 2007), but little is known about the social behaviour of cave arachnids, including spiders. Parental care has been documented in the neotropical cavernicolous harvestman, *Goniosoma longipes* (Machado and Oliveira 1998; Machado et al. 2000). *G. longipes* females lay their eggs on the cave walls and guard their eggs for about two months until the first instar juveniles disperse. Defense against egg dehydration, fungal attack, cannibalism and interspecific predation have been proposed to be important selective pressure for the evolution of parental care in cave *G. longipes* (Machado and Oliveira 1998). Notes on the natural history of the newly described cave spider, *Goeldia zyngierae* (Titanoecidae), show that the interconnected webs contained two to thirty spiders of different life stages, living together. Almeida-Silva and colleagues (2009) suggested the possibility of subsocial behaviour in this species. However, this species is not exclusively cavernicolous since it occurs in the surrounding epigeic region as well. Also, spiders were not observed to feed together but when the web was disturbed a group of spiders would move quickly towards the disturbed area. *G. zyngierae* may be a colonial spider, formed via the parasocial route. What this means is that individuals of this species, whether related or not, aggregate (for colonies), with the presence of a common resource (e.g., food) being an important factor favouring aggregation (Lubin & Bilde, 2007).

Drymusid spiders (Drymusidae) that are considered close to the spitting spider family Scytodidae (Labarque and Ramírez 2007) are sometimes found in caves. Drymusid females usually rest on the underside of the web and also lay their eggs there (Labarque and Ramírez 2007). Females of *Drymusa dinora* Valerio and *Drymusa spectata* Alayón García usually build white, spherical, wrinkled egg-sacs, which are occasionally hung in the web, close to them. They also carry and transport the egg-sac (Valerio 1974; Alayón García 1981). However, they sometimes leave the egg-sacs to attack a prey or because of a web perturbation (Valerio 1974). However, maternal care of eggs and spiderlings has not been investigated in detail in drymusid spiders.

Scytodids have a prey-capture method that is unique for spiders. They spit on their prey, with the sticky substance expelled from their fangs gumming down the prey long enough for the scytodid to finish the attack by injecting venom (Foelix, 1996). Scytodids are also unique because of the social behaviour of some species, commonly found in the genus *Scytodes*. For example, Australian *Scytodes fusca* is known to exhibit complicated social structure in terms of web sharing and prey capture (Bowden and Jackson 1988). *S. intricata* from Panama (Eberhard 1986) and *Scytodes* sp. from the Philippines (Li et al. 1999) show extended maternal care of eggs and young. Other known *Scytodes* species are solitary (Valerio 1981; Eberhard 1986; Li et al. 1999). The variation of sociality in Scytodidae suggests that there are species within the family that may represent 'transitional' stages in the evolution of sociality, and this presents a rare opportunity to investigate the evolution of sociality in Scytodidae. Because the study of scytodid social behaviour has literally just begun, the biology and behaviour of many taxa are not yet known in sufficient detail to answer many simple and functionally important questions. In addition, the evolution of sociality in this family should be amenable to testing by comparative analysis of social behaviour. Comparative information about scytodids in other species of the genus *Scytodes* and in other genera is thus particularly important. However, only seven species from a single genus *Scytodes* have been subjected to detailed behavioural studies and our knowledge of the presence of sociality in the remaining species and the degree of maternal care in the closest species or sister species of social species in the family Scytodidae is lacking.

Some species of scytodids are known to live in caves, and more species will probably be added as systematic studies of the family advance. There are currently four scytodid species that are known to live in caves, including *Scytodes eleonorae* from Brazil (Rheims and Brescovit 2001), *S. cavernarum* and *S. magna* from Malaysia (Bristowe 1952; Roewer 1960), and *Stedocys uenorum* from Thailand (Ono 1995). The only previous work on cave *Scytodes* includes the description of parental care of *S. eleonorae* from Goiás, Brazil that is known to live in caves at distance of up to 800 m from the cave entrance (Rheims and Brescovit 2001). Like other non-cave *Scytodes*, females carry egg-sacs with their chelicerae, Detailed behavioural studies are lacking in *S. eleonorae* and other cave scytodid species. In this chapter, I aim to verify the presence of sociality of cave scytodids and to check for the possibility of further, as yet unrecorded forms of sociality by examining eleven behavioural traits of five cave species of scytodids, including maternal care of egg-sacs, extended maternal care (food provisioning), group prey-capture, delayed dispersal, tolerance, fecundity, number of clutches, interval between clutches, and interval between hatching and egg-sac production. These include *S. cavernarum, S. fusca* and *Scytodes magna* from Malaysia, *Scyloxes* sp. 1 from Guangxi, China, and *Scytodes* sp. 2 from the Philippines – representing possibly three different genera – *Scytodes* (*S. cavernarum*, *S. fusca,* and and Philippines *Scytodes* sp. 2), *Scyloxes* (Guangxi *Scyloxes* sp. 1), and a newly proposed genus '*Magnodes*' ('*Scytodes*' *magna*). If sociality exists in the cave scytodids, I ask the question whether it conforms to the *Goniosoma* model of gregariousness arising from defensive behaviour, or to that of *Goeldia* in which spiders aggregate around a common food resource, (i.e., sociality evolving from a parasocial precursor). If sociality arose from maternal care accompanied by an elaboration of the maternal care phase, this would be the first record of social cave spiders evolving via a subsocial precursor. Perhaps, as what Wilson (1962) hypothesized due to food scarcity it is difficult for troglobitic animals to evolve sociality and hence the scytodids may be leading a solitary lifestyle.

MATERIALS AND METHODS

Colony survey in the field

Five species of scytodid spiders (Fig. 2-1) were collected from various parts of caves (Table 2-1). *Scytodes magna* and Guangxi *Scyloxes* sp. 1 were found deep inside the caves where there was little or no light. Spiders were either hidden under large rocks or in holes carved deep into the cave walls. Many of Guangxi *Scyloxes* sp. 1 spiders constructed their webs on the ceilings of the caves. Just like the drymusids, Guangxi *Scyloxes* sp. 1 and *S. magna*, webs are attached to large supporting objects such as the crevices in the cave walls or under rocks. I found that *Scytodes magna* and Guangxi *Scyloxes* sp. 1 (as also in Yunnan *Scyloxes* sp. 2 and Guangxi *Stedocys* sp. 1) show an accentuation of the appendages such as legs and pedipalps, and thinning of the cuticle. Their long legs may be used in the assistance of resource and mate location. As these four species of scytodids were found in the deep dark zones of the caves and on the basis of their morphological characters, I classify them as ‘troglobites’ for the purpose of this study. *S. cavernarum* and the Philippines *Scytodes* sp. 2 were found mostly at the cave entrance and within 10-40 m of the entrance. A few individuals were also found at the edges of the dark zone (also known as the twilight zone). The difference was that the caves of *S. magna* and Guangxi *Scyloxes* sp. 1 were relatively undisturbed and in darkness whereas the caves of *S. cavernarum* and the Philippines *Scytodes* sp. 2 were opened to the public and hence had lightings installed inside the caves. *S. cavernarum* and the Philippines *Scytodes* sp. 2 were found in tight crevices along the cave walls, usually with a mass of silk and moss or debris covering the entrance of the crevice. *S. fusca* were found both at the cave entrance and inside the cave but within 20 m of the entrance. Throughout this study I found *S. fusca* to be a widespread species as I also found them in abandoned buildings, between wooden panels of kampong houses and inside water pipes. Bowden (1991) studied the intraspecific interactions of

S. fusca that constructed web complexes in trees, and found them to be communal territorial. In order to see whether *S. fusca* living in a different habitat type displayed similar intraspecific behaviours as those described by Bowden (1991), *S. fusca* used in this study were from the caves.

For simplicity, I referred all webs occupied by scytodids as ‘colonies’ (Li et al. 1999; Yap and Li 2009). I conducted a one-week survey in each of the respective locations to study the social structure of each cave spitting spider species. At 1-m intervals, all spiders and their webs were photographed and had their activity (if any) recorded (e.g. feeding on prey, copulating or foraging), after which they were hand-collected. The following data were recorded: size and composition of the colony (if any); the instar and gender of individuals; developmental stage of the colony (juveniles, females with egg-sacs). I also identified any prey items being attacked or consumed. Colonies were sampled once during the survey at each site. The life stages of juveniles were noted as second or later instars, with reference taken from the sizes of laboratory-cultured spiders of known instars. Data collected from the field were augmented with laboratory observations (see following sections) of captive individuals kept in plastic, cylindrical cages (diameter × height: 6 × 8 cm).

Table 2-1. Details of locality and the microhabitats of the five cave scytodids sampled from the field

Species	**Locality**	**Coordinates**	**Habitat type**
Scytodes fusca	Malaysia: Batu caves, Selangor	N03°13'60 E101°42'0 93 m asl	Crevices of cave walls, mostly at cave entrance
Scytodes cavernarum	Malaysia: Gua Angin, Gunung Senyum, Pahang	N03° 43' 0 102° 25' 60 57 m asl	Crevices of cave walls, ranging from cave entrance to few metres from entrance
Philippines *Scytodes* sp. 2	Philippines: Biak-na-bato National park, Luzon	N15° 5' 60 E121° 4' 0 196 m asl	Crevices of cave walls, inside cool windy areas of cave ranging from cave entrance to few metres from entrance
Scytodes magna	Malaysia: Batu caves, Selangor	N03°13'60 E101°42'0 93 m asl	Under loose rocks; deep crevices on cave walls, in the dark zone of cave, range from 100 m to 800 m from cave entrance
Guangxi *Scyloxes* sp. 1	China: Xi’an caves, Fengshan county, Guangxi	N24°33.941’ E107°02.465 574 m asl	Ceiling of caves, in the dark zone of caves, range from 50 m to 1 km from cave entrance

Fig. 2-1. Four species of cave scytodid spiders. (A) Female and (B) male *Scytodes magna*; (C) female and (D) male *S. fusca*; (E) the Philippines *Scytodes* sp. 2 female; and (F) female *S. cavernarum*. Scale bars = 2.5 mm

Parameters used in determining the social status of the species in the laboratory

Spider collection and maintenance

For laboratory observations, spiders were collected from study sites and maintained by adopting protocol similar to that used in earlier scytodid studies (Li et al. 1999; Li 2002; Yap and Li 2009), and only the essential details are given here. Spiders were collected from caves and brought back. All spiders were housed in a laboratory with controlled environmental conditions (temperature = 24 ± 1°C; relative humidity = 70-90%; light/dark cycle = 12 L : 12 D; lights on at 0800 h). They were fed with fruit flies, *Drosophila melanogaster* Meigen once a week. Stoppered holes permitted the insertion of prey without damaging webs, and water was continuously available from moist cotton inserted through other holes or from glass vials filled with water, stoppered with cotton, and placed in the cages. Life stages were referred to as, for example, egg, first instar (see Whitcomb 1978). 'Penultimate instar' were one moult from becoming adults. Unless qualified, 'male' and 'female' referred to adults. No individual spider was used in formal observations more than once per day. Decisions such as which particular spider was used in a given observation were made randomly. All experiments were carried out between 1100 h and 1800 h.

In order to determine the social organization of each species, eleven behavioural traits were measured: (1) the presence/absence of maternal care (e.g. egg-sac guarding); (2) the presence/absence of extended maternal care (i.e. juvenile aggregations maintained beyond the time when they are physiologically and morphologically capable of dispersing from the natal site due to receipt of protection and nourishment from the adult female (Miller 1989)); (3) the presence/absence of group foraging behaviour; 4) natal dispersal patterns (i.e. do the spiderlings disperse from their natal nest in their early instars or remain until maturity? Does the dispersal follow a progressive sigmoidal function similar to that of *Amaurobius ferox* spiderlings (Kim 2000)?); (5) adult-juvenile and juvenile-juvenile interactions (i.e. social signals used between conspecifics such as aggressive interactions between siblings); (6) mean total number of spiderlings/female; (7) mean number of spiderlings/clutch produced by a female; (8) the time interval between clutches (egg-sacs); (9) the time interval between the hatch date and next egg-sac production; (10) the time taken for spiderlings to hatch; and (11) mean number of clutches produced by a female. I also recorded behaviour of the hatchlings immediately upon emergence (i.e. whether they attached themselves to the broken egg-sac or mounted the mother's body? If they aggregated around the mother in the natal nest or scattered around the cage). I predicted that the spiderlings of the social scytodids would cluster as a group staying close to the mother for protection, upon eclosion.

I hypothesized that for the higher level of sociality, the following would be present: (1) maternal care by guarding their egg-sacs against predators and parasites; (2) extended maternal care in the form of food provisioning – capturing prey and transporting it back to the natal nest for the brood to feed; (3) cooperative prey capture and food-sharing among siblings; (4) delayed juvenile dispersal due to benefits such as receiving more nutrition and hence being able to attain a larger body size before leaving the natal web; (5) the maternal female would share her prey with her offspring; (6) spiderlings that capture prey would share it with the other non-captors; (7) little or no cannibalism amongst siblings. If cannibalism occurs it should be seen only at later instars (e.g., 3rd instar and beyond); (8) females would produce fewer offspring due to more time and effort invested in the young which should ensure greater survivability amongst them, thus increasing her fitness; and (9) the intervals between hatching and the next clutch would be longer due to the aforementioned reason.

Maternal care of eggs

A number of cages (220 ×120 × 60 mm) were set up for observing maternal care of eggs for each species. One day after females reached maturation (i.e. undergo their final moult), they were individually housed and maintained in the cages for 7 days, during which they built a web in their cage. Soon after the female built her web, a male was introduced into the cage and allowed to mate with the female. The male was then removed from the cage. The mated females were then monitored daily until they produced eggs and also until the eggs hatched. I recorded the date of egg production, the presence/absence of egg-sac guarding, the attendance duration if present, the number of hatchlings, and details of the female's egg-sac guarding behaviour.

Maternal care of offspring: mother-offspring interactions

To determine whether females provided food for their spiderlings, whether spiderlings shared prey with females, or whether females consumed the prey alone (i.e. not allowing the spiderlings to gain access to the prey), the females with their egg-sacs maintained in the laboratory were used. They were monitored daily until the spiderlings emerged from the egg-sacs. The date of emergence, number and instar of hatchlings, and behaviour of hatchlings immediately after eclosion were recorded. After emergence, general brood care of the spiderlings (e.g. food provisioning) by the female were observed for 30 min daily for the next 28 days or until all the spiderlings dispersed to build their own webs. Large prey such as house flies (*Musca domestica*) and cricket nymphs (*Gryllus dimaculatus*) were offered to the females and then mother-offspring interactions including female food provisioning and offspring sharing food with their mother were recorded. Food provisioning by the maternal females could take the form of passive prey provisioning (i.e. capture and wrap the prey and then leave at the site of capture for the offspring to feed or regurgitation). Data on mother-offspring interactions were collected from the same colonies as those that were used for natal dispersal trials (see below).

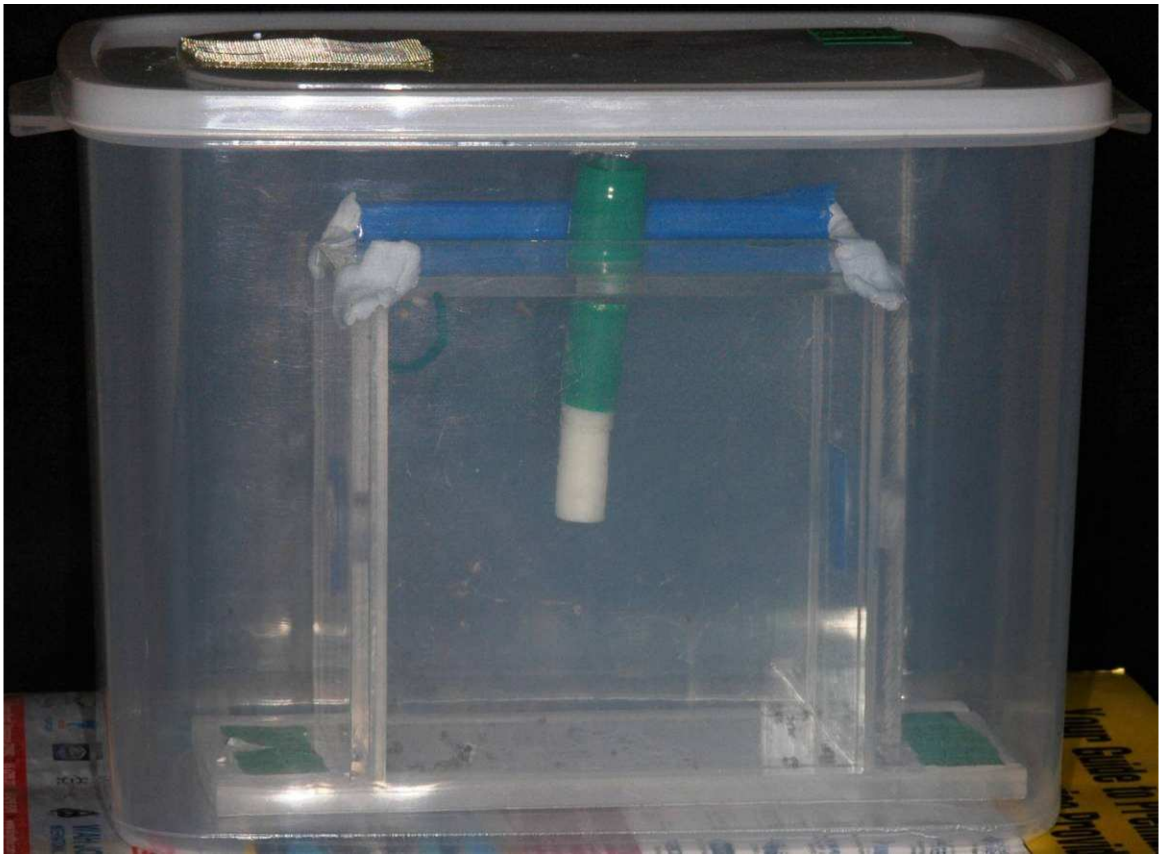

Fig. 2-2. The 'cage within a cage' set-up for studying the natal dispersal patterns of scytodid spiders. Modified from Ruttan (1990)

Natal dispersal pattern

To determine natal dispersal of spiderlings, I designed a ‘cage within a cage’ experiment (a vertical cage measuring 10 x 10 x 5 cm, with plastic frame and removable glass slides, inside a tupperware which had a hole cut out and covered with a piece of wire gauze (this allows for air movement) (Figure 2-2). This experimental design was adapted from Ruttan (1990). To ensure that the dispersal was intrinsic and not due to other causes such as maternal absence, I used colonies that contained both the female and her spiderlings. The spiders were provided fruit flies *ad lib*. once a week. Spiderlings were considered “dispersed” when they moved from the inner cage into the outer cage. The outer cage was thoroughly monitored daily for any dispersed spiderlings. Spiderlings found in the outer cage were removed, scored as having dispersed on that date, and weighed. Dead spiders were checked under the microscope for the cause of death i.e. by cannibalism or other natural causes (e.g. moulting). Spiders that had been cannibalized were easily identified by the presence of silk wrapping and a characteristic shrunken appearance (Yap and Li 2009). A few individuals always remained within the maternal web for up to several months after their siblings had dispersed. Hence based on these observations, I defined the end of dispersal as when 3 or fewer individuals had remained in the maternal web for at least one week. Species that showed both two criteria were not considered in this experiment: (1) ≥ 50% of the spiderlings dispersed before they were 3-weeks old. I assumed these species to have low tolerance among siblings and hence dispersed early; and (2) ≥50% of the spiderlings within the colony were cannibalised before the end of the third week of emergence.

Group foraging behaviour

To determine whether spiderlings participated in cooperative prey capture and fed as a group, they were put randomly into cages (10 x 10 x 5 cm) in groups of one, two, three, four, six and eight individuals 20 days after their emergence from egg-sacs (all in the late second instar). There were three replicates for each group. If an individual died during the experiment I replaced it with a new one. Before the start of the experiment the spiderling was weighed to ensure uniformity in size among the spiderlings (e.g. *S. fusca*: mean weight ± 1 SD = 0.00114 ± 0.00018 g; ranging from 0.00091 to 0.00139 g; 1.3 mm in body length, $n = 5$). The experiment started one day after the introduction of the spiderlings into their cages. The trials began immediately after a house fly was introduced into the cage and lasted 180 min, after which observations were made sporadically in the next 24 hours. We define ‘captors’ as individuals that attacked and fed subsequently on the prey and 'intruders' as those that did not participate in the capture but fed on the paralysed prey. If spiders fed together, we recorded how the prey was captured, time to paralysis (defined as the interval between the introduction of prey and the time when feeding began), the number of attackers (i.e., the number of spiderlings that spat at the prey), and whether or not the captors allowed the intruders to share in the prey. The presence of any aggressive behaviour (defined as forelegs raised and/or waving or a sudden forward jerk towards the counterpart) was also noted. All behaviours were observed by the observer and also video-recorded. Due to the fact that cannibalism and natal dispersal rates were high within the first five weeks of emergence and that group foraging was not observed during the early stages, the experiments for this group of (cave) spiders were not repeated when the spiderlings were 5-weeks of age.

Juvenile-juvenile interactions: sibling tolerance and cannibalism

The rate of cannibalism was used to measure the strength of sibling tolerance in five cave scytodid species. For determining whether cannibalism among sibling would occur, sibling groups, each comprised of 10 spiderlings (of approximately the same body size), were assembled in small vertical cages (10 × 10 × 5 cm) 7 days after their emergence (*n* = 3). All groups were provided with 20 fruit flies once a week. This feeding regime was to simulate low-prey conditions so as to investigate whether under such conditions siblings would withhold kin cannibalism (i.e. demonstrate high level of tolerance) or readily feed on each other to prevent starvation (i.e. low tolerance level). Each group was observed twice daily for a period of 14 days and thereafter once daily for 28 days. During each observation, the number of spiderlings that died from cannibalism or other causes was noted. Spiders that had been cannibalised were easily recognized by the presence of glue, silk wrapping and a characteristic shrunken appearance. Since scytodids prefer live prey (Li et al. 1999), it was presumed that the number of spiderlings that were eaten by their siblings after they died was small.

Reproductive behaviour

The mated females (same ones that were used for the 'observation of maternal care of eggs' experiment) were observed weekly and six variables were examined for each of five cave species: number of clutches (i.e., egg-sacs) per spider, number of spiderlings per clutch (i.e., egg-sac), total number of spiderlings produced by a female throughout her reproductive life-span, time for spiderlings to hatch (i.e. the interval between egg-laying and egg hatching), the interval between clutches, and the interval between hatching and subsequent egg-sac production.

Data analyses

Data were checked for normality by using Kolmogorov-Smirnov test and analysed with parametric or nonparametric procedures when appropriate. I performed linear regressions to examine the relationships between the timing of juvenile natal dispersal and juvenile body mass for each species. I also performed multivariate analysis of variance (MANOVA) to examine the differences in six reproductive traits (total number of spiderlings, time taken for spiderlings to hatch, number of clutches, number of spiderlings per clutch, the time interval between clutches, and the time interval between the emergence of spiderlings and the next egg-sac production) among five species. If a significant overall difference was detected, one-way ANOVA was then conducted for each reproductive trait, followed by Tukey's HSD. All tests were two tailed and the data are reported as mean ± S.E. of untransformed data. All statistical analyses were conducted using IBM SPSS Statistics 19 (SPSS Inc., USA).

RESULTS

Colony survey

The nest structures of the five cave species of scytodids are presented in Table 2-2. *Scytodes magna* and Guangxi *Scyloxes* sp. 1 that were observed during the survey were either a single adult male or female, or a female carrying an egg-sac. On a few occasions, *S. fusca*, and *S. cavernarum* females were found with newly emerged spiderlings (Table 2-2). The colony composition for Guangxi *Scyloxes* sp. 1 included the components penultimate male and female because they made up 18% of the total nest inhabitants (Table 2-2).

Table 2-2. Data on the inhabitants of the five cave scytodid species nests in the field collected over a 7-day period

Nest inhabitant(s)		***Scytodes fusca* (*N* = 53)**	***Scytodes cavernarum* (*N* = 48)**	**The Philippines *Scytodes* sp. 2 (*N*=12)**	**Guangxi *Scyloxes* sp. 1 (*N* = 34)**	***Scytodes magna* (*N* = 18)**
Mature female	Alone	15	22	5	7	5
	alone with egg-sac	9	5	0	0	0
	with spiderlings (1st instar only)*	5	2	0	0	0
	with spiderlings (mix of 1st instar and later)	0	0	0	0	0
	with spiderlings (later instar only)	0	0	0	0	0
	with mature male	0	0	0	0	0
	with mature male with egg-sac	0	0	0	0	0
Mature male	Alone	4	4	1	4	2
	with spiderlings (1st instar)	0	0	0	2	0
	with spiderlings (mix of 1st instar and later)	0	0	0	0	0
Groups without adult	1st instar spiderlings without adult	0	0	0	3	0
	1st instar spiderlings and later without adult	0	0	0	0	0
	later instar spiderlings without adult	0	0	2	0	0
Spiderlings	1st instar spiderling alone	0	0	0	0	0
	2nd instar and beyond spiderlings alone	20	15	4	10	11

*spiderlings emerge as 1st instar for these species

Maternal care

Scytodes cavernarum, *S. fusca* and the Philippines *Scytodes* sp. 2 produced eggs throughout the year in the laboratory, whereas *S. magna* and Guangxi *Scyloxes* sp. 1 females produced only 1-2 egg-sacs in the laboratory. *S. cavernarum*, *S. fusca* and the Philippines *Scytodes* sp. 2 females practiced maternal care by carrying their egg-sacs in their chelicerae (Fig. 2-3) whereas females of *S. magna* and Guangxi *Scyloxes* sp. 1 did not carry their egg-sacs in their chelicerae, instead they hang their egg sacs in the centre of the web, attached by several threads (Fig. 2-4). However, *S. magna* and Guangxi *Scyloxes* sp. 1 females always remained in close proximity to the egg-sac, with their bodies touching or almost in contact with the sac (Table 2-3). There was a difference in the type of egg casing. In *S. magna* and the Guangxi *Scyloxes* sp. 1, their eggs were surrounded by dense amount of thick silk, while in the other three species of *Scytodes*, their eggs were wrapped in a fine, more loosely woven silk envelope (Fig. 2-5)

Fig. 2-3. *Scytodes fusca* female carrying her egg-sac in her chelicerae. Scale bar = 1 mm

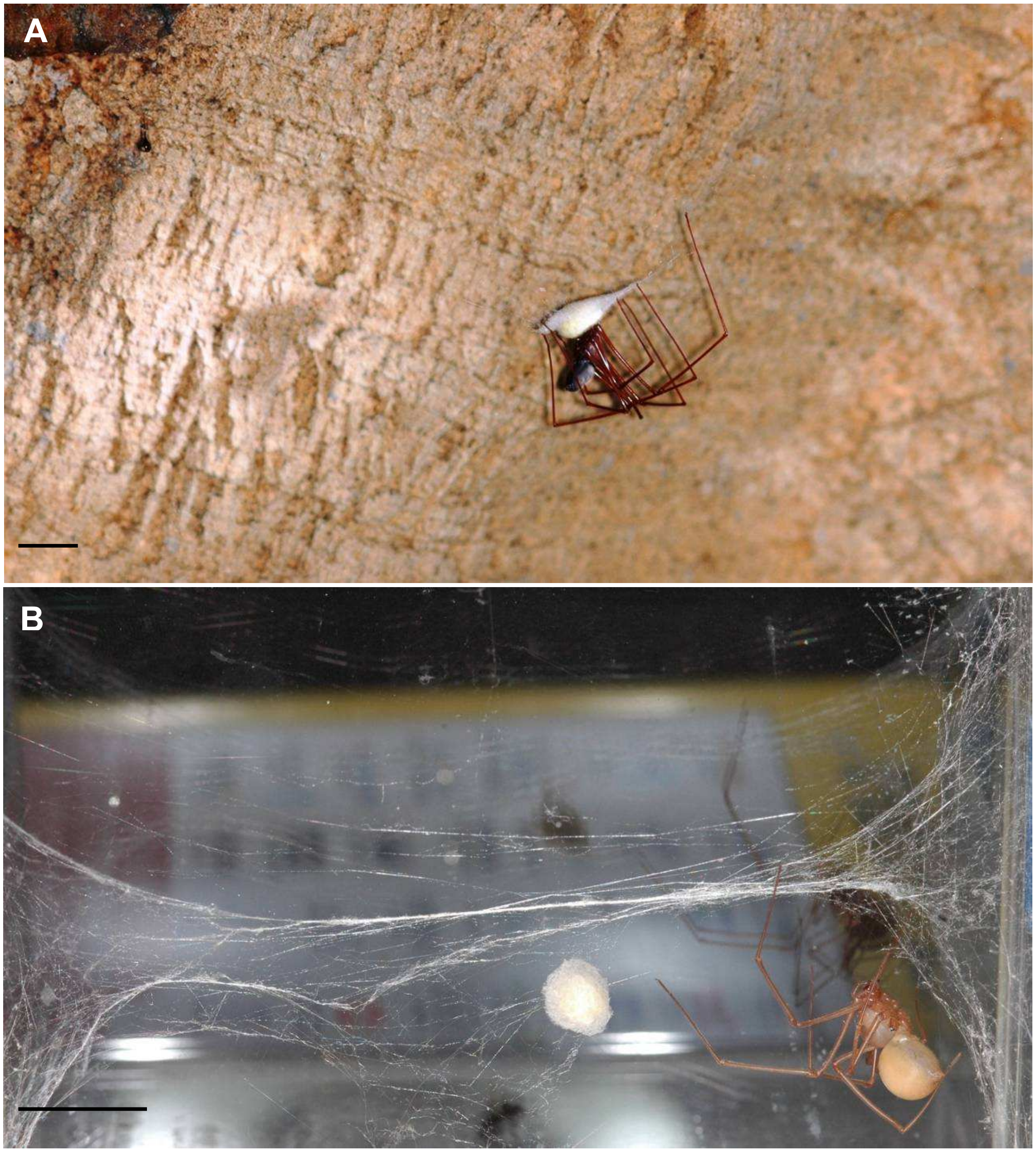

Fig. 2-4. (A) Guangxi *Scyloxes* sp. 1 female on the surface of the outer cave walls, staying close to her egg-sac. The egg-sac is suspended by two to three threads. (B) Web constructed by *S. magna* female. Her egg-sac is suspended by a few threads at the centre of the web. Scale bars = 10 mm

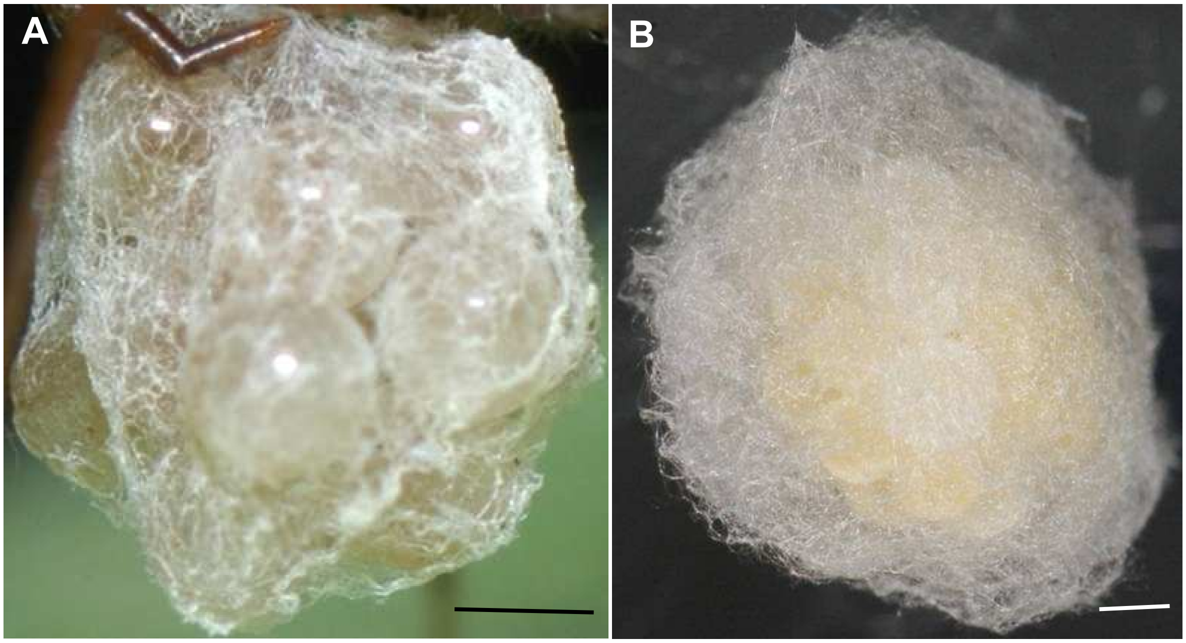

Fig. 2-5. Scytodid egg-sac. (A) Typical egg-sac of *Scytodes cavernarum*, *S. fusca* and the Philippines *Scytodes* sp. 2; and (B) *Scytodes magna* egg-sac. Note the denser silk surrounding the eggs of *S. magna*. Scale bars = 1 mm

When capturing prey, all *S. fusca* (*n* = 15) and the Philippines *Scytodes* sp. 2 females (*n* = 8), and 93% of *S. cavernarum* (*n* = 14) females were observed to drop their egg-sacs first. They usually left the egg-sacs in their maternal nest or suspended them by a thread and then spat at the prey (e.g., house flies) (Fig. 2-6). Upon subduing the prey the females usually transported it back to where they had left their egg-sacs and then started feeding on the prey. When the cage was tapped or moved, the females usually abandoned feeding and returned immediately to their egg-sacs and carried them again (Table 2-3). However, *S. magna* and Guangxi *Scyloxes* sp. 1 females rarely dropped their egg-sacs to catch prey. Instead, they usually ignored the prey (Table 2-3).

Fig. 2-6. *Scytodes fusca* female leaving her egg-sac aside to forage on the house fly

Table 2-3. The presence and absence of maternal care of egg-sacs and foraging during egg-sac attendance in the five cave scytodid species

	Scytodes magna	**Guangxi *Scyloxes* sp. 1**	***Scytodes cavernarum***	***Scytodes fusca***	**Philippines *Scytodes* sp. 2**
Carrying egg-sac in the chelicerae	No	No	Yes	Yes	Yes
Foraging during egg-sac attendance	No	No	Yes	Yes	Yes

Emergent phase and emergent behaviour

The spiderlings of all five cave species emerged as first instars and underwent their first moult into second instars between 3 and 6 days of emergence. Immediately upon emerging from the egg-sacs the spiderlings usually attached themselves to the broken sacs. Within 24 hours, all the spiderlings of *S. magna* and Guangxi *Scyloxes* sp. 1 spread themselves around the cage although still within close proximity of the mother. However, in *S. cavernarum*, *S. fusca* and the Philippines *Scytodes* sp. 2, about half the spiderlings still clustered in the maternal nest and the rest spread out in the cage.

Extended maternal care: food provisioning

Food provisioning (here I define it as the activity of supplying food items to the spiderlings by capturing of prey, injection of digestive enzymes and eliciting the spiderlings to feed on the captured prey) by females was absent in all five cave species. The females always captured the house fly and fed on it alone (Fig. 2-7). If the spiderlings approached the feeding female, the female usually raised the first pair of legs to chase them away. However, spiderlings of these five cave species were observed to scavenge on the prey remains. Should the spiderlings capture their own prey regardless of prey type (i.e. fruit flies or house fly), the females never snatched the prey away.

Natal dispersal

A spiderling's dispersal timing is positively correlated with its body mass in four out of five species except for *S. cavernarum* (Fig. 2-8). Light (small) juveniles dispersed earlier than heavier juveniles. The first spiderling to disperse was at third instar in *S. magna* (Fig. 2-8D), Guangxi *Scyloxes* sp. 1 (Fig. 2-8E) and *S. cavernarum* (Fig. 2-8B), at mid/late second instar in *S. fusca* (Fig. 2-8A), while for the Philippines *Scytodes* sp. 2 the first disperser was at early second instar (Fig. 2-8C). Although the first spiderling to disperse in the Philippines *Scytodes* sp. 2 was around 5 days after emergence from the egg-sac, the majority of the spiderlings dispersed after 47 days of emergence when they have undergone their third moult to become fourth instars. In *S. fusca*, after the first spiderling had dispersed many followed suit with a large number of the spiderlings dispersing on day 10 (Fig. 2-8A).

Fig. 2-7. Newly emerged Guangxi *Scyloxes* sp. 1 spiderlings spread out on the sparse silk nest and female feeding on house fly alone. Scale bar = 10mm

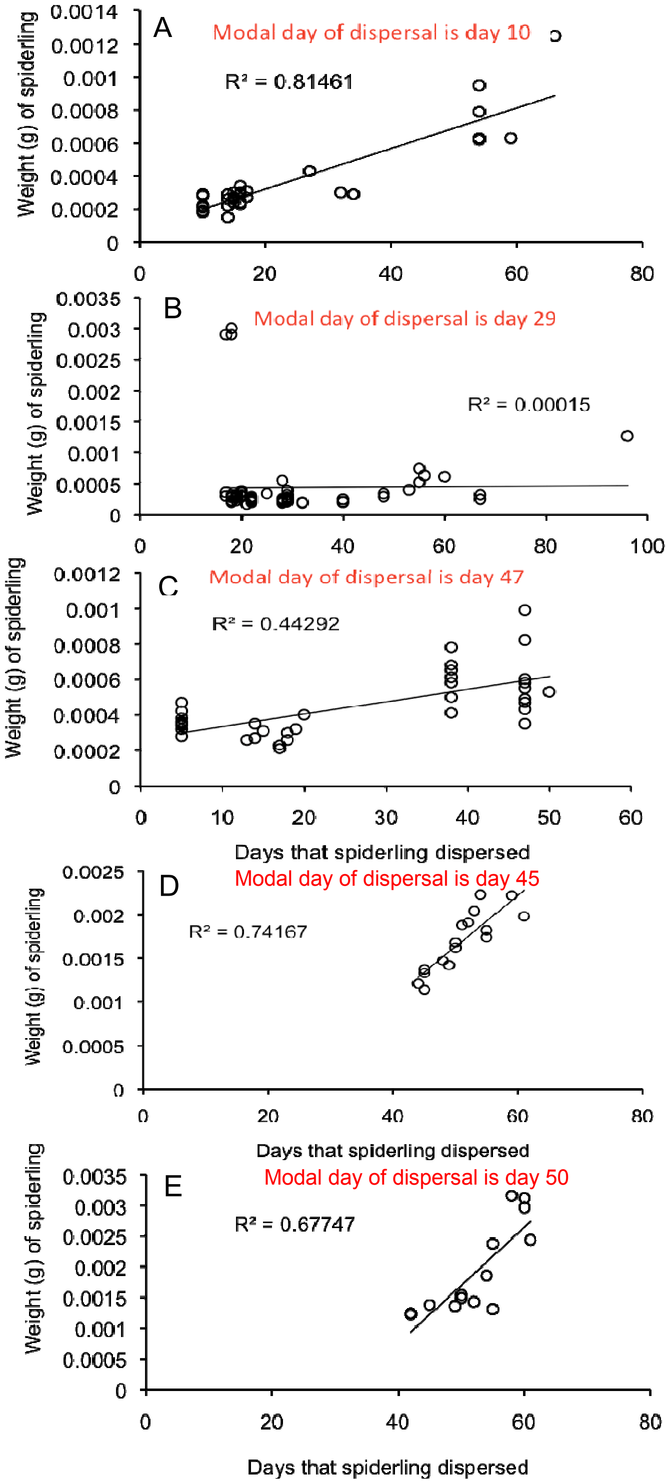

Fig. 2-8. Relationship between the days in which spiderlings dispersed and spiderling mass in five cave scytodid species. (A) *Scytodes fusca*; (B) *S. carvernarum*; (C) Philippines *Scytodes* sp. 2.; (D) *S. magna;* and (E) Guangxi *Scyloxes* sp. 1

Group foraging behaviour

Cooperative prey capture was not found in any of the five cave species. If the prey was too large for any one individual to subdue, it was eventually ignored. When the prey was captured by a spiderling, the captor never allowed the prey to be shared. The captor usually chased the intruders away by displaying sudden jerking movements (i.e. as if the captor is about to lunge onto the intruder) or leg raising and waving. In several instances, however, I observed one or two intruders feeding alongside the captor. This occurred when the prey was too large (e.g. house fly) to be monopolized by one spider and the intruders always “sneaked” in after the captor had settled down and was busy feeding on the prey.

Cannibalism among siblings

Cannibalism among siblings started 1-2 weeks after emergence from egg sacs (i.e. about the second instar) in all five cave species (Table 2-4). Most of the mortality was due to cannibalism. The causes of incidental mortality could be due to death during moulting or starvation due to the incapability of capturing fruit flies when they were small.

Table 2-4. Total cumulative mortality resulting from cannibalism in groups of spiderlings (three groups, 10 spiderlings per group except for *S. cavernarum* in which there were 8 spiderlings per group)

	Week 2 (7-13d)	**Week 3 (14-20d)**	**Week 4 (21-27d)**	**Week (28-34d)**	**Week 6 (35-41d)**	**Week 7 (42-48d)**	**Incidental mortality***
Guangxi *Scyloxes* sp. 1	0	2	5	9	17	22	4
S. magna	0	3	7	13	19	19	5
S. cavernarum	2	5	9	12	14	18	2
S. fusca	5	10	16	19	19	22	5
Philippines *Scytodes* sp. 2	3	7	10	15	19	21	7

*Incidental mortality refers to deaths not attributed to cannibalism (e.g. from moulting).

Reproductive traits

MAVONA revealed a significant overall difference in reproductive traits among five species of cave scytodids (Wilks' λ = 0.029, $F_{24.,60.}$ = 4.415, P < 0.001). There were significant differences in the egg hatching time, the interval between clutches, and the interval between hatching and next egg-sac production among the five species of cave scytodids. However, no significant difference in the total number of spiderlings produced (Fig. 2-9A) and the number of spiderlings per clutch (Fig. 2-9F) was found among five species (Table 2-5).

Table 2-5. Results from MAVONA comparing six reproductive traits among five cave scytodids

	F	***df***	***P***
Total number of spiderlings	2.308	4, 22	0.09
Egg hatching time (d)	4.138	4, 22	0.012
Interval (d) between successive clutches	19.965	4, 22	< 0.001
Interval (d) between hatching and next egg-sac production	20.175	4, 22	< 0.001
Number of clutches (or egg-sacs)	2.851	4, 22	0.048
Number of spiderlings per clutch	2.307	4, 22	0.09

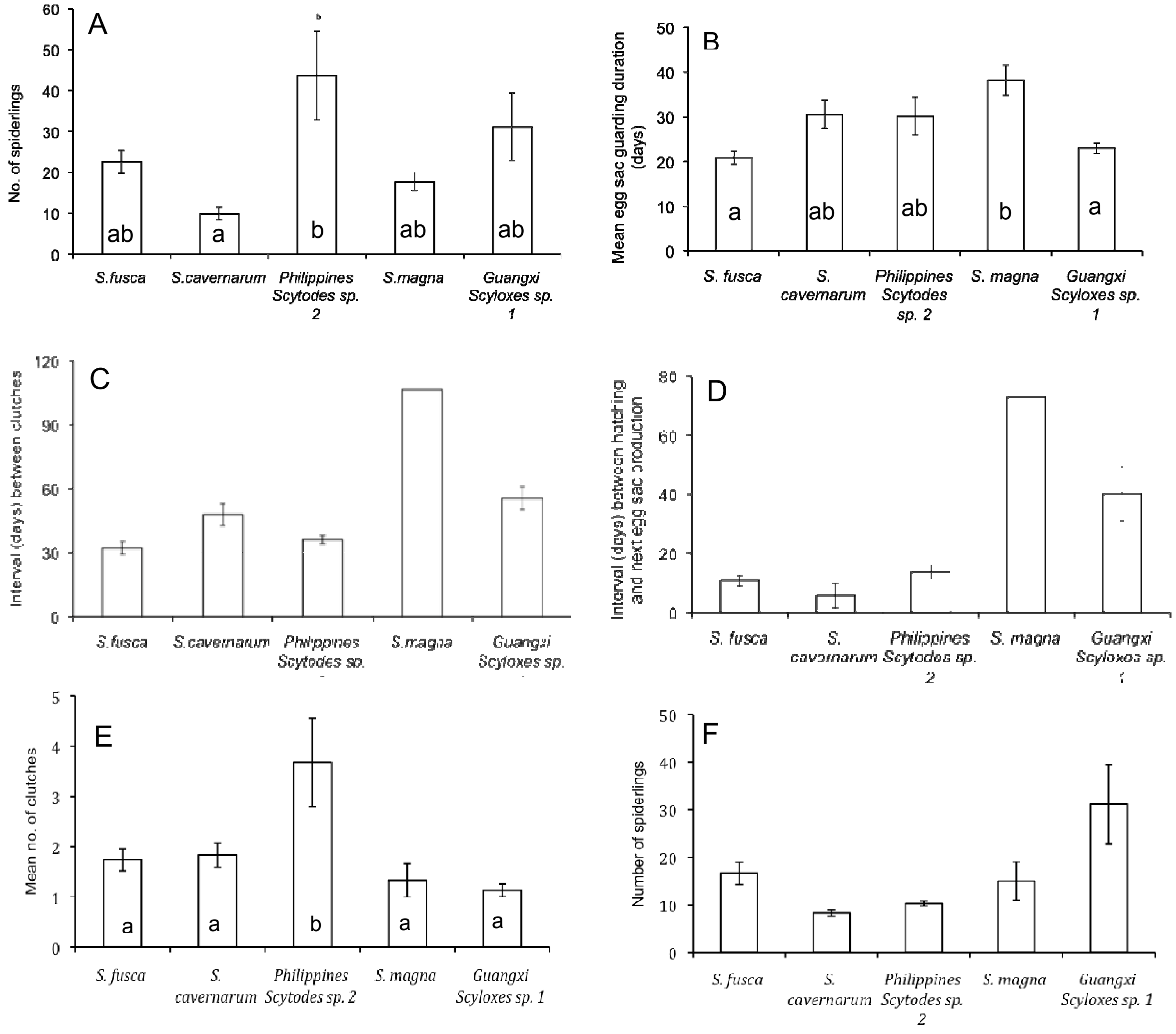

Fig. 2-9. Reproductive traits of five cave species of scytodids. (A) Mean (± S.E.) total number of spiderlings per female; (B) mean (± S.E.) egg hatching time (d); (C) mean (± S.E.) interval (d) between clutches; (D) mean (± S.E.) interval (d) between hatching and the next egg-sac production; (E) mean (± S.E.) number of clutches; and (F) mean (± S.E.) number of spiderlings per clutch. Different lower cases indicate significant differences.

DISCUSSION

My results show that all five species of scytodids living in caves are asocial (i.e. solitary). Nearly all the nests surveyed in the field contained only a matured male or female alone in all five species although some nests contained an egg-sac guarding female or a mother with first instar spiderlings. No nests with a female and spiderlings of later instars were observed. The nest may thus be founded by a solitary individual and offspring disperse early from the maternal nest. My laboratory observations and experiments also suggest that all five species of cave scytodid spiders show no extended maternal care, no food provisioning by mother, no cooperative prey-capture and food sharing among siblings, and lower level of tolerance among siblings. Therefore, cave scytodid spiders studied in this study seem to be asocial.

My study supports Wilson's (1962) view that perhaps prey scarcity in the caves makes it difficult for animals to attain sociality. In the entrance zones, there are numerous aerial prey items of a relatively small mass (Gillieson 1996), whereas in the dark zones, there are larger prey items but lower abundance (Gillieson 1996). Small prey items such as fruit flies and mosquitoes can be easily caught by singletons and would be insufficient for sharing. On the other hand, while the prey in the dark zones are of larger size, it also coincides with the fact that the spiders found in this part of the cave are large-sized scytodids (i.e. *S. magna* and Guangxi *Scyloxes* sp. 1) again making communal feeding unlikely. Furthermore, since prey are in low numbers there may be high competition for these prey items. Rector (2009) found that webs of the cave spider, *Meta ovalis*, in the dark zone were the widest spaced, whereas those in the entrance zones were nearer each other. A similar pattern was observed for *S. magna* and Guangxi *Scyloxes* sp. 1 implying that high predation of cave residents (Rector 2009) and prey scarcity probably lead to spiders opting for a solitary life strategy. The smaller species of scytodids in this study (i.e. *S. fusca*, *S. cavernarum* and Philippines *Scytodes* sp. 2) utilize cracks and crevices in the cave walls possibly as protection from predators and the changing environment. Perhaps insufficient space is another reason why sociality has not evolved in these species of *Scytodes*. Conditions of over-crowding and low prey supply can result in spiders readily cannibalizing each other (Avilés and Gesley 1998).

The subsocial pathway arises from extended parental care, with the juveniles tending to remain in their mother's nest until they develop into later instars (Li et al. 1999) and subsequent delayed natal dispersal (Buskirk 1981; Crespi and Choe 1997). There was no evidence of extended maternal care in the form of prey provisioning in any of the five species of cave scytodids. Maternal care ends at the point where the female aids in the emergence of the spiderlings by tearing open the silken egg case. Maternal females did not provide food and share the meals with their offspring (Fig. 2-7). If the spiderlings were capable of catching their own prey (e.g., fruit flies), the mother never snatched the prey item away, although among the siblings many of them tried to gain a free meal (i.e., intruders trying to share the prey item with the captors). While *S. cavernarum*, *S. fusca* and the Philippines *Scytodes* sp. 2 practice maternal care by carrying their egg-sacs in their chelicerae, *S. magna* and the Guangxi *Scyloxes* sp. 1 stay in close persistent contact with their egg-sacs without carrying them. This is the first report in spitting spiders. Females of *S. magna* and Guangxi *Scyloxes* sp. 1 do not carry their egg-sacs in their chelicerae probably because their fangs are not structured to transport. In a study of the character 'palpal claw' in the subfamily Heteropodinae, Jäger (2003) found that elongated palpal claw teeth and egg-sac carrying behaviour of heteropodines are a synapomorphic character. *Sinopoda*, a heteropodine genus, possesses shorter palpal claw teeth and show absence of egg-sac carrying behaviour. Whether the absence of egg-sac carrying is a plesiomorphic or derived (i.e. has been secondarily lost) in *S. magna* and Guangxi *Scyloxes* sp. 1 is unknown, but it is likely that their chelicerae are not adapted to carrying egg-sacs and perhaps any other items for that matter because in the laboratory, I observed that the females upon spitting and attacking the prey usually feed on the site of capture and no transportation has been observed.

Whether it is carrying egg-sac in the chelicerae or maintaining close persistent contact with the egg-sacs, maternal care may be of importance in these five species because potential egg predators such as cave crickets are common in the caves (Machado and Oliveira 1998). Constant exposure to heavy predation may be an important selective pressure favouring the evolution of parental care if unprotected eggs have a high chance of being preyed (Wilson 1971). Inside the caves, the scarcity of food and the strong predation pressure (cave fauna consists mainly of predators and detritivores) (Machado 2002) lead to a situation in which maternal care confers benefits to both mother and offspring. Although extended maternal care is absent in *S. magna* and Guangxi *Scyloxes* sp. 1, the first occurrence of natal dispersal in these two species was at the third instar, that is, around day 40 (Figs. 2-8D and E). This seems to imply that, although females do not provide food to their progeny due to reasons discussed above (i.e. prey scarcity and prey not large enough to be shared), they may tolerate their progeny remaining in their webs until they are more physiologically prepared for leaving the nests. In this way, the maternal web may offer protection to young spiderlings. *S. magna* and Guangxi *Scyloxes* sp. 1 females rarely leave their egg-sacs to forage, but foraging during egg-sac attendance was frequent in *S. fusca*, *S. cavernarum* and the Philippines *Scytodes* sp. 2. As the latter three species usually build their nests inside the crevices, the deep retreats (personal observations) where a female probably leaves her egg-sac to forage may serve as a natural barrier to egg-sac predators such as cave crickets and ants. *S. magna* and Guangxi *Scyloxes* sp. 1 females with their persistent close contact to their egg-sacs may indeed eat nothing during this time period by remaining inactive as spiders that remain inactive have low energy requirements. During times of starvation they can reduce their metabolic rate (Anderson 1974).

Tolerance has often been considered to be important in the evolution of spider sociality (Kullman 1972; Buskirk 1981; D'Andrea 1987; Whitehouse and Jackson 1998) although highly pronounced tolerance is not a condition that must be present in order for sociality to evolve in spiders. Cannibalism at early stage seems to be pronounced in all five species of cave spitting spiders, indicating that tolerance among siblings is almost non-existent. Thus, this low level of tolerance may prevent the evolution of sociality in cave spitting spiders. How could pronounced cannibalism occur in cave spitting spiders? There are two possible reasons (Polis 1988): a direct metabolic gain by eating the conspecific, and an indirect gain by reducing potential competitors thereby diminishing possible negative effects from interference competition (e.g. contest for nest sites). My results suggest that the direct metabolic gain from eating a conspecific could be particularly important in the early stages when individuals have not accumulated fat reserves necessary to withstand periods of food stress in caves where prey are scarce. In fact, I found that a large number of incidental mortality was due to spiderling starvation even in the laboratory with relative abundant prey.

Resource competition, mate competition and inbreeding avoidance have been proposed as three major ultimate causes of natal dispersal (reviewed in Johnson and Gaines 1990). The resource competition hypothesis states that siblings disperse in order to avoid competing with each other for resources in resource-limited habitats (Hamilton and May 1977) and predicts that there is no sex-biased dispersal when they have similar resource requirements (Gandon 1999). The mate competition hypothesis suggests that individuals disperse to avoid competing with siblings for mates and predicts that the sex suffering the higher cost from intrasexual mate competition will disperse further (Moore and Ali 1984). The inbreeding avoidance hypothesis suggests that individuals disperse to avoid mating with their siblings (Gandon 1999). The natal dispersal patterns varied among all five species. For example, the first *S. magna* spiderling to disperse was at third instar yet cannibalism commenced as early as in the second instars. A similar trend is seen in Guangxi *Scyloxes* sp. 1. In the Philippines *Scytodes* sp. 2, *S. fusca* and

S. cavernarum, the first disperser was at early second instar, late second instar and third instar, respectively. If we look at the time when more than 50% of spiderlings dispersed, the Philippines *Scytodes* sp. 2, *S. fusca* and *S. cavernarum* spiderlings dispersed at day 37, 18 and 29, respectively, which equate to late third instar, late second/early third instar and third instar in the same order. Thus, the general pattern observed is that spiderlings remain in the maternal nest and disperse at a later time despite the high rates of cannibalism among siblings. Why is this so? It could be that spiderlings that have not moulted into third instars are physiologically incapable of spitting and attacking their own prey because of possible reasons such as their glue glands are not yet fully developed or their legs are not strong enough hence the uncoordinated movements. As observed in the laboratory, second instar spiderlings were unable to capture fruit flies. The early dispersers of the batch may be fitter or they simply disperse a short distance away from the maternal nest, locate the first available site and remain there until the next moult when they are capable of capturing prey. Following or during dispersal, siblings may still compete for nest sites or resources. Such competition would be reflected in greater dispersal distances when the number of dispersing siblings is greater and over time as nearby sites become occupied. Spiders may also compete with individuals dispersing from neighbouring nests (Powers and Avilés 2003). Other spiderlings that remain in the maternal nest have to play a game of avoid being eaten or be the "eater". Spiderlings that successfully cannibalize on their siblings obtain more nutrition, hence attaining a larger body size. These fitter spiderlings have a higher chance of surviving and reproducing once they disperse from the maternal nest. I therefore suggest that natal dispersal in these five species of cave scytodids primarily reflect competition for resources within the natal nest because of the cave environments.

For Guangxi *Scyloxes* sp. 1 and *S. magna* spiderlings, dispersal started only at the mid third instar, but cannibalism among siblings was rampant, and a notable point is that there were no spiderlings that dispersed from the second instar in these two species. This could be attributed to the fact that *S. magna* and Guangxi *Scyloxes* sp. 1 live deep inside the caves where more aggressive predators such as huntsmen, crickets and centipedes are present. The individual spiders were found to be spaced some distance away from each other (approx. 1 m away) as compared to *S. cavernarum*, *S. fusca* and the Philippines *Scytodes* sp. 2 in which individuals were several centimeters from each other. This may be an indication that the *S. magna* Guangxi *Scyloxes* sp. 1 spiderlings would have to travel over a longer distance before settling down on a suitable site. Having to disperse over a long distance but with numerous predators lying ambush, there is an incentive for these spiderlings to remain a little longer in the maternal nest before dispersing. No doubt the incidence of cannibalism is high but perhaps the decision to remain in the maternal nest is the lesser of two evils. Furthermore, morphological immaturity in the first and second instars may preclude the spiderlings from leaving the maternal nest. *S. cavernarum* also displays a rather similar trend in that although cannibalism commences from the early second instar, the first dispersal occurred at the third instars. However, the first *S. cavernarum* spiderling(s) dispersed on day 18, which lies on the border between the second and third instars. For simplicity I recorded this/these spiderling(s) to be a (early) third instar because I found that ≥ 50% of the spiderlings moulted into third instars by day 16. Furthermore, although I observed cannibalism from the second instars, cannibalism in *S. cavernarum* only became rampant among the third instars. Due to asynchronous development as a consequence of lack of extended maternal care, some spiderlings will tend to grow faster and attain more body mass at a much faster rate than their siblings. As a result, these larger sized juveniles will continue to monopolize prey. Spiders are well-known for their cannibalistic nature in both sexual and non-sexual contexts (Wagner and Wise 1996), thus the tendency for the larger spiders to cannibalize their smaller-sized siblings is high. Hunger and high densities are important promoters of cannibalism (Bilde and Lubin 2001). Therefore, in order to escape being eaten, the small juveniles may be forced to make the decision of leaving the natal nest.

Solitary spiders are expected to produce large numbers of spiderlings compared with social species because an increase in investment per individual young is often associated with a decrease in the number of offspring produced (Schneider 1996). Like 99% of spiders, these five species of cave scytodid species are solitary, I have shown that these five species do not exhibit maternal care beyond the egg-sac phase; it ceases at the point of emergence of the spiderlings. Due to the lack of extended maternal care, spiderlings tend to have lower survivability rates, hence there may be a selection for more clutches and larger clutch size. It is evident that the Philippines *Scytodes* sp. 2 and Guangxi *Scyloxes* sp. 1 females produced many spiderlings throughout their reproductive stage, although there were no statistic differences in the mean total number of spiderlings produced by females among the five species (Table 2-5). My result for the Philippines *Scytodes* sp. 2 is consistent with the hypothesis that clutch size in spiders is correlated with sociality (Kullmann 1972; Smith 1982) and parental care (Buskirk 1981). With a mean of 3.33 clutches/female, this species has the highest clutch number (although not the highest clutch size) and in turn the highest fecundity rate among the five cave scytodid species. Being a solitary spider, the Philippines *Scytodes* sp. 2 produces more progeny due to the low investment put into each of her offspring. Based solely on their fecundity, however, it is difficult to conclude whether the other four species are solitary or not because the clutch size and clutch numbers are low, which is a character normally used to define the higher level of social spiders. Comparison of clutch size and numbers between social and solitary species of scytodids is needed.

A study carried out on the Tasmanian cave spider *Hickmania troglodytes* demonstrated that egg-sac production is sensitive to falls in humidity and other disturbances (Doran et al. 2000). Therefore, in the wild, *S. magna* could produce more egg-sacs. Many of the observed adult females were collected from their natural environment; hence the possibility that they had produced previous egg-sacs cannot be excluded. The age of the spiders collected can also affect the quantity of egg-sacs produced per spider. The results for *S. cavernarum*, however, contradict with our prediction because with each of the females producing a mean of only nine spiderlings in total, this species has one of the lowest fecundity and fertility. This could be attributed to a means of regulating the population density. I found *S. cavernarum* at two different sites: Gunung Senyum (where I collected the spiders and transported them back to the laboratory for experiments) and Batu Caves (where I was not permitted to collect). At Batu Caves I found *S. cavernarum* to be of very high abundance with the spiders located very close to one another thus requiring little effort in trying to locate them. Although I only found 48 colonies of *S. cavernarum* in Gunung Senyum, these spiders could have been more abundant in other parts of the caves. Population control is essential, without it competition for space, food and mates would be too stiff. Another possible reason is that of the five species, *S. cavernarum* females are the smallest in body size (mean body length = 5.32 mm). It is well known that fecundity tends to be correlated with body mass in invertebrates, including spiders (Higgins 1992; Schneider 1996). These differences in body size and mass may have resulted in a lower oviposition potential in *S. cavernarum*.

Social species are expected to delay egg production if females are performing maternal care, thus a longer interval between clutches. However, the interval between emergence and the next egg-sac production varies among the five solitary species although the interval was longer in *S. magna* and Guangxi *Scyloxes* sp. 1 compared to the other three *Scytodes* species. One would assume that this could be due to females of *S. magna* and Guangxi *Scyloxes* sp. 1 investing more time in their previous clutch of offspring. However, this was not the case because all five species show no extended maternal care. I postulate that the low metabolic rates of these two species of scytodids that live deep inside caves may be the cause of the females taking a longer time to produce another clutch. Carbon dioxide concentration is often significantly higher in caves than on the surface, especially in deep cave passages that have abundant decaying organic material (cited in Howarth 1983). The relatively higher carbon dioxide concentration and the lower oxygen concentration, as well as the greater variability of the concentration of these gases in underground environments, may play a role in the lowered metabolism (Howarth 1983). Troglobites often display a much reduced basal metabolic rate compared with their surface relatives, which has been interpreted as an adaptation for greater efficiency in their energy-poor environment (Poulson and White 1969).

With food scarcity in the caves, it is not surprising that the maternal females do not provide food to their young and cannibalism among siblings is rampant. Perhaps cave spiders are not predisposed to evolve into social species because the presence of colonies of spiders would be too conspicuous and become an advertisement for potential predators in caves. Many social spiders such as *Stegodyphus* and *Anelosimus* spp. are web-builders that have an incentive to form large colonies with extensive webs: to ensnare larger (usually flying) prey. Cave spiders with their rudimentary webs normally constructed in dark, hard-to-find spots such as in holes and crevices, have adapted to the harsh living conditions. An extensive web structure, which is a prerequisite for sociality in spiders as it provides a means of communication, are simply not suited for cave spiders.

It is interesting to note, however, that sociality can exist in cavernicolous arachnids, for example, in *Goniosoma* harvestmen. Machado (2002) suggested that the subsocial behaviour present in *Goniosoma* may confer a special advantage in this particular environment. *Goniosoma* females care for the eggs and first instar hatchlings before they dispersed. Maternal care is crucial for egg survival since predator may consume entire batches in a single night (Machado and Oliveira 1998). Up to 200 individuals can form diurnal aggregations, and this gregariousness confers defensive advantages to an individual harvestmen. Machado (2002) also proposed that the gregarious behaviour in *Goniosoma* may be induced primarily by microclimatic factors such as relative humidity and temperature, such that gregariousness could act as a behavioural mechanism for regulating and reducing evaporation among grouped individuals.

Absence of extended maternal care, predatory and cannibalistic behaviours pose barriers to social behaviour (Johannesen and Lubin 1999). Without prolongation of the maternal care phase, the requirement of a period of familiarization with members of the brood is not likely to be satisfied. The ability to recognize siblings and reduce kin cannibalism may facilitate a prolongation of the social period. Avoidance of kin cannibalism may be particularly beneficial en route to sociality under low food conditions as this is a situation often experienced by spiders (Avilés 1997), and this is also definitely the case in caves. Prey availability may be the most significant factor in determining mutual tolerance in spiders (e.g. Rypstra 1986). Two lines of thoughts emerge here: the first one puts a premium on the benefits that the members of the colony may gain through extension of maternal care and avoidance of kin cannibalism. This may lead to cooperative hunting which is hypothesized to be an evolutionary trait of sociality in certain animal groups (Packer and Ruttan 1988). By contrast the second line of thought puts a premium on the ecological constraints limiting the likelihood that an individual will stay home and form groups. The second line may be applied to these cave scytodids because as mentioned earlier cooperative hunting via an extensive web structure is not feasible for cave spiders. Moreover, Schneider (1996) has shown that single spiders always gained more mass than spiders in groups suggesting costs of competition. Witt et al (1978) also showed that social spiders digest prey more slowly than solitary ones probably due to spiders in groups injecting less digestive enzymes than when feeding alone. In a prey-scarce, predator-abundant environment such as caves, going solo might be the best survival plan.

In conclusion, all five cave scytodid species have been found to be solitary, although all females have been observed to practice maternal care in the form of close egg-sac attendance. I suggest the possibility that insufficient food supply and high predation risks can be attributed to the solitary strategy adopted by Guangxi *Scyloxes* sp. 1, *Scytodes cavernarum*, *S. fusca*, *S. magna* and the Philippines *Scytodes* sp. 2. More behavioural as well as morphological studies are required on a wider range of cave scytodids to confirm if they are the ancestors of all other scytodids distributed in other habitats including those found under rocks, in the leaf litter and live green leaves, and even those in houses and old buildings.

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CHAPTER 3

Social Behaviour of Spitting Spiders That Live in the Ground Zone

ABSTRACT

Spider sociality that involves web-sharing, group foraging, food sharing and brood care has arisen a few times in *Scytodes*. In this chapter I report two more cases. Extended maternal care, prolonged mutual tolerance (in relation to low cannibalism rates) and cooperative prey-capture are described for *Scytodes gooldi* and *S. rubra*, two species of spitting spiders discovered in South Africa from field and laboratory observations. After hatching there is an extended mother-offspring and sibling association, with juveniles remaining in the maternal web up till the seventh instar in the case of *S. rubra*. Laboratory observations also reveal that the time taken to subdue a prey decreases with increasing group size. This extended tolerant phase and highly cooperative behaviour led me to classify *S. rubra* as a “transition into social” species. In contrast, the third species Yunnan *Scytodes* sp. 3 from China, exhibits maternal care but the tolerant phase for this species is much shorter than *S. gooldi* and *S. rubra*. It terminates when second-instar spiderlings dismount from the body of females to disperse. The social systems in *S. gooldi*, *S. rubra* and Yunnan *Scytodes* sp. 3 were compared to those of other ground-dwelling spiders and arachnids. The advantages of prolonged tolerance in living under rocks and in dead logs, as well as the possible reasons for a solitary life strategy in leaf litter are also discussed.

INTRODUCTION

Insects and arachnids provide a wide range of examples and forms of evolution of cooperation in animals, hence they represent one of the most useful systems for the study of social evolution (Crespi and Choe 1997). The knowledge of social phenomena in insects is more developed than that of spiders, and for decades there have been numerous studies carried out on the social behaviour of ground insects, including, biparental care and social evolution in burying beetles (Eggert and Muller 1997), communication in ants (Jackson and Ratnieks 2006), evolution of eusociality in termites (Lacy 1980; Thorne 1997), and social behaviour and ecological characteristics of wood-feeding cockroaches (Park et al. 2002).

As the study of evolution of sociality becomes more revolutionized where data are available on a wide range of taxa, arachnid sociality, especially spider sociality, is making its mark in literature. As research progresses spiders, scorpions and mites show new levels of complexity and social sophistication. Cooperation and group-living have been well documented in web-builders of the *Anelosimus* (e.g. Marques et al. 1998; Agnarsson et al. 2007; Souza et al. 2007; Mailleux 2008; Agnarsson et al. 2010) and *Stegodyphus* genera (e.g. Salomon and Lubin 2007; Johannesen et al. 2009; Lubin et al. 2009; Ruch et al. 2009), but few studies have been conducted to investigate the social behaviour of spiders that live in the ground stratum. Perhaps the most well-documented sociality in ground spiders would be that of the wolf spider of the genera *Pardosa* (e.g. Nyffeler 2000; Bonte et al. 2007) and *Geolycosa* (Miller 1989), although there has been isolated cases of recordings of sociality in other spiders such as pholcids (Tomoji and Maryati 2001). This species, found in the southern part of Sabah, builds webs at the bases of large buttressed trees, and participate in web repair and defense.

The ground stratum comprises of species that nest in the soil, leaf litter, under rocks and all rotting wood on the ground, up to and including the largest rotting logs. Daily temperature fluctuations occur in these subsurface spaces. The dead wood habitat is for the most part in an exposed location where vicissitudes of temperature and humidity are great (Larkin and Elbourne 1964). During late morning, the light intensity is high; but temperature is still lower and relative humidity higher than at similar light intensities in the afternoon; spider activity in the herb stratum is strongly influenced by light intensity, temperature and relative humidity (Abraham 1983). The ground stratum provides an important component of biodiversity that is often overlooked (Hammond 1990). The leaf litter is usually moist and well-aerated. Despite being small in size and often overlooked, the ecological importance of spiders is undeniable as they are abundant predators of other forest litter arthropods (Platnick 1995). Forest litter provides a wide range of microhabitats for spiders through variations in moisture, cover material, and litter depth and structure. The spider fauna of leaf litter can be divided into various categories (Root 1973) based on methods of prey-capture and utilization of similar prey resources. One such category, the wandering spiders, may constitute up to 43% of ground-dwelling spider species in a forest (Drew 1967). These spiders do not use webs in prey-capture; they rely on a "sit-and-wait" foraging strategy, with frequent changes of site (Edgar 1969). Their prey consists mainly of insects of Collembola, Homoptera and small Diptera (Edgar 1969). The close similarity of cave adaptations among the diverse taxa in so many different cave areas indicates that cave adaptation is a general process and the result of similar selection pressures (cited in Howarth 1983). Similar adaptations are often found in boreal leaf litter species whose physical environment is similar to that in caves (Barr 1968).

Members of *Geolycosa turricola* are obligate burrowers with one-half to one-third of a given brood remaining in the maternal burrow past the time when dispersal could be successful (Miller 1989). These spiderlings display subsocial behaviour such as cooperative foraging and prey sharing, as well as a high degree of mutual tolerance towards members of the same group. The tolerant phase is important for *G. turricola* spiderlings because it allows for considerable growth and the larger size of the later dispersers affect their survival. Spiderlings that disperse at larger body sizes are capable of constructing deeper burrows which help them to survive the harsh winters (Miller and Miller 1984). Ground wandering wolf spiders (Lycosidae) are also known for their maternal care (Gertsch 1979, Wise 1993, Foelix 1996). After oviposition, the spherical egg-sac attached to the mother's spinnerets is carried around for several weeks. In the burrowing lycosids, the females guard their egg-sacs. The mother bites open the sac at the seam to assist the emergence of the spiderlings, after which the 40–100 pulli mount their mother's back, where they hold fast to her abdominal hairs (Nyffeler 2000).

The main questions motivating this chapter are: 1) whether sociality has arisen in ground-dwelling scytodids, and if so the advantages of being social and 2) the form of social behaviour exhibited by these spiders. In this chapter, I investigated social behaviour of three ground-dwelling scytodid species, *Scytodes* sp. 3 from Xishuangbanna, Yunnan, China that lives in leaf litter and may be a wandering spider since webs were not observed during the leaf litter sifting process (L-M. Yap Y.L., personal observations), *S. gooldi* that is found in dead logs and *S. rubra* occurring under rocks and stones of dry streams, the latter two being from South Africa.

Most of the communication mechanisms available to spiders (visual, vibratory, acoustic and chemical) have been implicated as important in social organization (Krafft 1982). Most consideration, however, has been given to the use of vibratory cues since complex social systems have arisen mostly in web spiders (Krafft 1982). In this situation one question arises: If Yunnan *Scytodes* sp. 3 is a wandering spider, does it preclude it from evolving into a social species or can it rely on other mechanisms of communication such as chemical communication to maintain a social structure? Although this study does not use any bioassay experiments to test questions concerning the nature of the mechanism of communication among the brood members of each species, this pilot study of the social behaviour of ground-dwelling scytodids may motivate further research in finding out the principal form of communication between group members of the subsocial/social species.

Dead logs are known to be teeming with invertebrates such as ants, termites, springtails, ticks, beetles, midges and mites (Irmler et al. 1996; Vanderwel et al. 2006). With such high prey availability and possibly an abundance of large-sized prey such as millipedes and beetles, would this create a favourable condition for *S. gooldi* to form colonies? Perhaps like the subsocial wolf spider *Geolycosa turricola* (Miller 1989), *S. gooldi* create burrows or tunnels in the soft interiors of the logs and use these nests as a means of communication among nest mates. Although not exactly a ground-dwelling spider, *Delena cancerides* (Sparassidae), a social bark-dwelling huntsman, live under the exfoliating bark of dead *Acacia*, *Callitris* and *Casuarina* thus its sociality cannot have its basis on an aerial web (Rowell and Avilés 1995). The hundreds of individuals that make up a colony feed communally and capture prey jointly. *S. rubra* constructs a rudimentary webbing on the flat under-surface of the rock or stone, thus any social behaviour that may be present in this species will also not be dependent on a web as a means of communication. In addition to both field and laboratory observations of Yunnan *Scytodes* sp. 3, *S. gooldi* and *S. rubra*, I also present parallel observations on some of their other social ground-dwelling spider counterparts.

MATERIAL AND METHODS

Colony survey

Three ground-dwelling species of scytodids were used in this study, *Scytodes* sp. 3 from China (Fig. 3-1 A), *Scytodes gooldi* (Fig. 3-1 B) and *S. rubra* (Fig. 3-1 C) from South Africa. The detailed information on localities, precise coordinates and habitats are presented in Table 3-1. Yunnan *Scytodes* sp. 3 were collected from the rainforest in the Xishuangbanna Tropical Botanic Garden (XTBG), Menglun, Yunnan Province. A matured forest with much disturbance in the past 20 years, Xishuangbanna is on the northernmost edge of tropical Asia, where tropical and subtropical flora inter-mingle. The result is an ecological mosaic of vegetation represented by tropical rain forest and subtropical evergreen broadleaf species (Zheng et al. 2006). The forest litter of Xishuangbanna Tropical Botanic Gardens (XTBG) is composed of topical broadleaf deciduous and broadleaf semi-deciduous (Zheng 2006). Yunnan *Scytodes* sp. 3 were collected from forest litter by using the sieving method in which the leaf litter and topsoil were gathered and thrown into the sieve at every 1-m interval. The sieve with a mesh wire at the bottom was then shaken vigorously and the small debris would fall through the tiny holes into a collection bag. The small debris, with all the invertebrates, was then laid out on a white cloth and I would wait for 5-15 min for spiders to emerge from under small pieces of leaves and small stones. There was a possibility that the number of females carrying egg-sacs might have been higher, but as a result of the shaking many of the egg-sacs could have been dislodged from the mother’s chelicerae.

Scytodes gooldi and *S. rubra* individuals and colonies were surveyed in Hanglip Picnic Spot, Limpopo, 6.5 km NW Makhado and Mkuze Game Reserve, Kwazulu Natal, respectively. When surveying *S. gooldi*, I tore out the outer pieces of bark to expose the soft wood of the dead logs (Fig. 3-2) and when surveying *S. rubra*, I found them by overturning rocks. At Hanglip Picnic Spot, all fallen rotting logs along the sides of a dirt track for a distance of 1 km were sampled. In the Mkuze Game Reserve, at every 1-m interval all rocks and stones within arm’s reach when squatting down were overturned. Unlike most other spiders, such as *Loxosceles* and salticids that live under stones, *S. rubra* have a body plan that is dorso-ventrally flattened. The stones where *S. rubra* were found were not deeply embedded and made up mostly the top layer; they were restricted to this area (i.e. dried-up stream) of the Mkuze game reserve. Spiders were photographed and had their activity (if any) recorded (e.g. feeding on prey, copulating, foraging or interactions between group mates). After surveys, spiders were collected and brought back to the laboratory.

Fig. 3-1. Three species of ground-dwelling scytodid spiders. (A) Female Yunnan *Scytodes* sp. 3, scale bar = 1 mm; (B) female *S. gooldi*, scale bar = 3 mm; and (C) a pair of penultimate *S. rubra* found under a rock, scale bar = 4 mm.

Table 3-1. Details of locality and the microhabitats of the three ground-dwelling scytodids sampled from the field

Species	**Locality**	**Coordinates**	**Habitat**
Yunnan *Scytodes* sp. 3	Xishuangbanna Tropical Botanic Gardens, Yunnan, China	N21º55', E101º15’ Elevation: 640m	Moist leaf litter in shady parts of forest
Scytodes gooldi	Hanglip Picnic Spot, Limpopo, South Africa	S23°0.0',E29°52.8' Elevation: 1154m	Soft wood inside fallen dead logs
Scytodes rubra	Mkuze Game Reserve, Kwazulu-Natal, South Africa	S27°36'20" E32°9'44" Elevation: 72m	Under top layer of loose rocks of a dried-up stream

Fig. 3-2. Fallen rotting log with soft wood exposed. *Scytodes gooldi* individuals and sometimes small colonies are found here.

Parameters used in determining the social status of the species in the laboratory

Spider collection and maintenance

For laboratory observations and experiments, spiders were collected from study sites and maintained by adopting protocol similar to that used in earlier scytodid studies (Li et al. 1999; Li 2002; Yap and Li 2009) and in Chapter 2, and only the essential details are given here. Spiders were collected in the field and brought back. All spiders were housed in a laboratory with controlled environmental conditions (temperature = 24 ± 1°C; relative humidity = 70-90%; light/dark cycle = 12 L : 12 D; lights on at 0800 h). They were fed with fruit flies, *D. melanogaster,* once a week. Life stages were referred to as, for example, egg, first instar (see Whitcomb 1978). 'Penultimate adults' were two moults from becoming adults. Unless qualified, 'male' and 'female' referred to adults. No individual spider was used in formal observations more than once per day. Decisions such as which particular spider was used in a given observation were made randomly. All experiments were carried out between 1100 h and 1800 h.

In order to determine the social step of each species, I measured eleven traits as used in Chapter 2. These include: (1) the presence/absence of maternal care of eggs; (2) the presence/absence of extended parental care (Miller 1989); (3) the presence/absence of group prey-capture; (4) natal dispersal pattern; (5) mother-offspring and juvenile-juvenile interactions; (6) total number of spiderlings/female; (7) number of spiderlings/clutch; (8) the time interval between clutches (egg-sacs); (9) the time interval between the hatching date and next egg-sac production; 10) the time taken for spiderlings to hatch; and (11) number of clutches produced by a female. The procedures for observing social behaviour of three ground-dwelling scytodid species were as described in Chapter 2.

Data analyses

As in Chapter 2, I first checked data for normality using Kolmogorov-Smirnov test and analysed data with parametric or nonparametric procedures when appropriate. Linear regressions were performed to examine the relationships between the timing of juvenile natal dispersal and juvenile body mass for each species. Multivariate analysis of variance (MANOVA) was run to test the overall difference in six reproductive traits (total number of spiderlings, time taken for spiderlings to hatch, number of clutches, number of spiderlings per clutch, the time interval between clutches, and the time interval between the emergence of spiderlings and the next egg-sac production) among three species. If a significant overall difference was detected, one-way ANOVA was then conducted for each reproductive trait, followed by Tukey's HSD. All tests were two tailed and the data are reported as mean ± S.E. of untransformed data. All statistical analyses were conducted using IBM SPSS Statistics 19 (SPSS Inc., USA).

Table 3- 2. Data on the inhabitants of the three ground-dwelling scytodid species nests in the field collected over a 7-day period

Nest inhabitant(s)		**Yunnan *Scytodes* sp. 3 (*N*=25)**	***S. gooldi* (*N* = 39)**	***S. rubra* (*N* = 35)**
Female	Alone	8	5	7
	alone with egg-sac	2	0	0
	with spiderlings (2nd instar only)*	0	1	0
	with spiderlings (mix of 2nd instar and later)	0	5	0
	with spiderlings (later instar only)	0	7	4
	with mature male	0	0	0
	with mature male with egg-sac	0	0	0
Male	Alone	4	3	2
	with spiderlings (2nd instar)	0	0	0
	with spiderlings (mix of 2nd instar and later)	0	0	0
Groups without adult	2nd instar spiderlings without adult	0	0	0
	2nd instar spiderlings and later without adult	0	2	0
	later instar spiderlings without adult	0	1	7
Penultimate	Female alone	0	0	3
	Male alone	0	o	2
Single spiderling	2nd instar spiderling alone	0	0	0
	later instar and beyond spiderlings alone	11	15	10

RESULTS

Colony survey

The social structures of the three species of ground-dwelling *Scytodes* in the wild are presented in Table 3-2. In Yunnan *Scytodes* sp. 3, no nest sharing was observed and all spiders were found to be solitary. Some forms of web-sharing were observed in both *S. gooldi* and *S. rubra*: females were seen sharing the nest with second instar spiderlings only, with a mixture of second instar and later instar spiderlings as well as with later instar spiderlings only. In a few colonies (*S. gooldi*: Table 3-2, rows 12 and 13; *S. rubra*: Table 3-2, row 13), spiderlings shared a nest in the absence of the maternal females. Most nests, however, were occupied by solitary males, egg-carrying and non-egg-carrying females, and solitary juveniles. Males were never found sharing nests with mature females or other conspecifics.

Maternal care

The females practiced maternal care by carrying their egg-sacs in their chelicerae in all three species of ground-dwelling scytodids, and they were never seen to forage during the egg-sac guarding period. That is, females never dropped their egg-sacs to capture prey (Table 3-3).

Table 3-3. The presence and absence of maternal care of egg-sacs and foraging during egg-sac attendance in the three ground-dwelling scytodid species

	Yunnan *Scytodes* sp. 3	***Scytodes gooldi***	***Scytodes rubra***
Carrying egg-sac in the chelicerae	Yes	Yes	Yes
Foraging during egg-sac attendance	No	No	No

Emergent phase and emergent behaviour

The spiderlings of these three species emerged as second instars having undergone one moult within the egg-sacs. Immediately upon eclosing from the egg-sacs, spiderlings of both South African *S. gooldi* and *S. rubra* attached themselves to the broken sacs, and within 24 hours, they clustered in the maternal nests forming a tight congregation. Yunnan *Scytodes* sp. 3 spiderlings, however, mounted their mother’s body (Fig. 3-3). Within 36 hours, they dismounted to form a tight cluster in the maternal nest.

Fig. 3-3. Newly-emerged Yunnan *Scytodes* sp. 3 spiderlings mounting the maternal female's body. Scale bar = 1 mm

Extended maternal care: food provisioning

South African *S. gooldi* and *S. rubra* females, after attacking and wrapping, carried the prey back to the natal nests, and spiderlings fed on these prey captured by their mother. The spiderlings did not assist the maternal female with the prey capture. At times the spiderlings were seen approaching the prey at the capture site to feed, but the mother always transported it back to the natal nest. The mother after biting the prey several times, presumably injecting digestive enzymes into it, then left the prey for her offspring to feed. Most of time, however, the mother fed alongside her brood. Food provisioning, however, was not observed in Yunnan *Scytodes* sp. 3. The female captured prey only for her own consumption.

Natal dispersal pattern

The first individuals to disperse in Yunnan *Scytodes* sp. 3 and *S. gooldi* were at early second instar (approximately 8 days old), and third instars (approximately 33 days old) in *S. rubra* (Fig. 3-4). By day 12, about half the brood dispersed in *S. gooldi*. For *S. rubra*, 50% or more of the spiderlings left the natal nest by day 53 and day 8 in Yunnan *Scytodes* sp. 3.

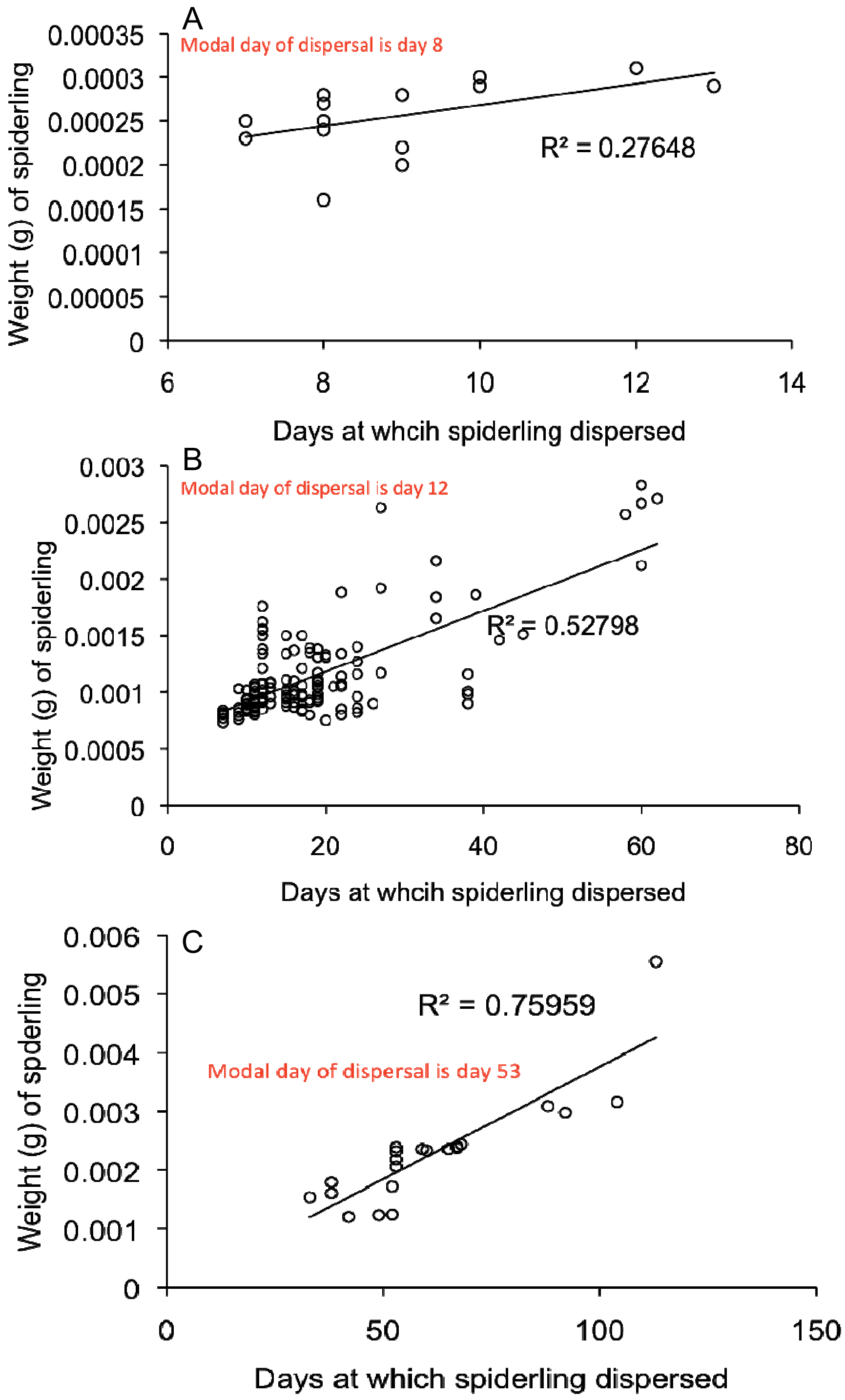

Fig. 3-4. Relationship between the days in which spiderlings dispersed and spiderling mass in three ground-dwelling scytodid species. (A) Yunnan *Scytodes* sp. 3; (B) *Scytodes gooldi* and (C) *S. rubra*.

Group foraging behaviour

Group foraging and food sharing were observed in both South African *S. gooldi* and *S. rubra* (Fig. 3-5), but absent in Yunnan *Scytodes* sp. 3. Both group size and prey type affected the time for paralyzing the prey ($F_{4,26}$ = 33.551, $P < 0.001$), but there was no significant difference between *S. gooldi* and *S. rubra* in the time taken to subdue either the house fly or cricket (Table 3-4). Groups of two, three, four, six and eight spiders did not show significantly higher capture success than a single individual. In fact, regardless of the group size, spiderlings of *S. gooldi* and *S. rubra* were as effective in capturing prey (Table 3-5), but with increasing group size, they became more efficient in capturing prey. The time taken to paralyze either house flies or crickets decreased with increasing number of spiderlings in a group (Fig. 3-6) ($F_{5,26}$ = 79.065, $P <$ 0.001). Within a group, some spiderlings were captors (i.e. those captured prey), some spiderlings did not capture prey but joined to feed on the prey later (i.e. intruders), and others did not capture and feed on the prey at all. There was an upper limit to the number of captors: no more than five captors participated in prey capture. The spiders usually bit an appendage, almost always a leg or the wing, presumably to inject the venom. As soon as the prey was paralyzed, feeding commenced with the captors being always the first that fed. However, in one instance in *S. rubra* I observed a captor moving away after spitting on the cricket, and did not partake in the meal. The captors fed more frequently on the thorax than on any other body part of the prey, although often they started feeding on the head first then proceeded to the thorax where feeding could last up to 6 h. Soon after the captors had settled down on their feeding positions, intruders would join in. Often the captors tried to chase the intruders away by briefly flailing their forelegs at the intruders, but direct attacks on them by spitting were never seen. In most cases, I observed the intruders walking away, but returning later to join in the feeding as was evident from the change in size (and sometimes colour) of their abdomen.

Fig. 3-5. *Scytodes rubra* late second instars feeding communally on a housefly. Black arrows show spiderling abdomen turned bright pink; an indication of consumption of prey.

Table 3-4. Results from three-way ANOVA testing the effects of group size, prey type and species on the time of prey paralysis for *Scytodes gooldi* and *S. rubra*

Source	***df***	**Mean sq.**	***F***	***P*-value**
Group size	5	14304.753	79.065	< 0.001
Prey type	1	184343.273	1018.904	< 0.001
Species	1	1649.974	9.120	0.006
Group size*prey type	4	6070.234	33.551	< 0.001
Group size*species	5	375.072	2.073	0.101
Prey type*species	1	93.091	0.515	0.480
Group type*preysize*species	4	364.832	2.017	0.122
Error	26	180.923		

Table 3-5. Results of Chi-square tests for independence comparing prey capture success rates of singleton, groups of two, three, four and six in *Scytodes gooldi* and *S. rubra*

	Housefly		**Cricket**	
	S. gooldi	*S. rubra*	*S. gooldi*	*S. rubra*
χ^2	3.974	6.429	4.773	2.500
df	5	5	4	4
P	0.533	0.267	0.311	0.645

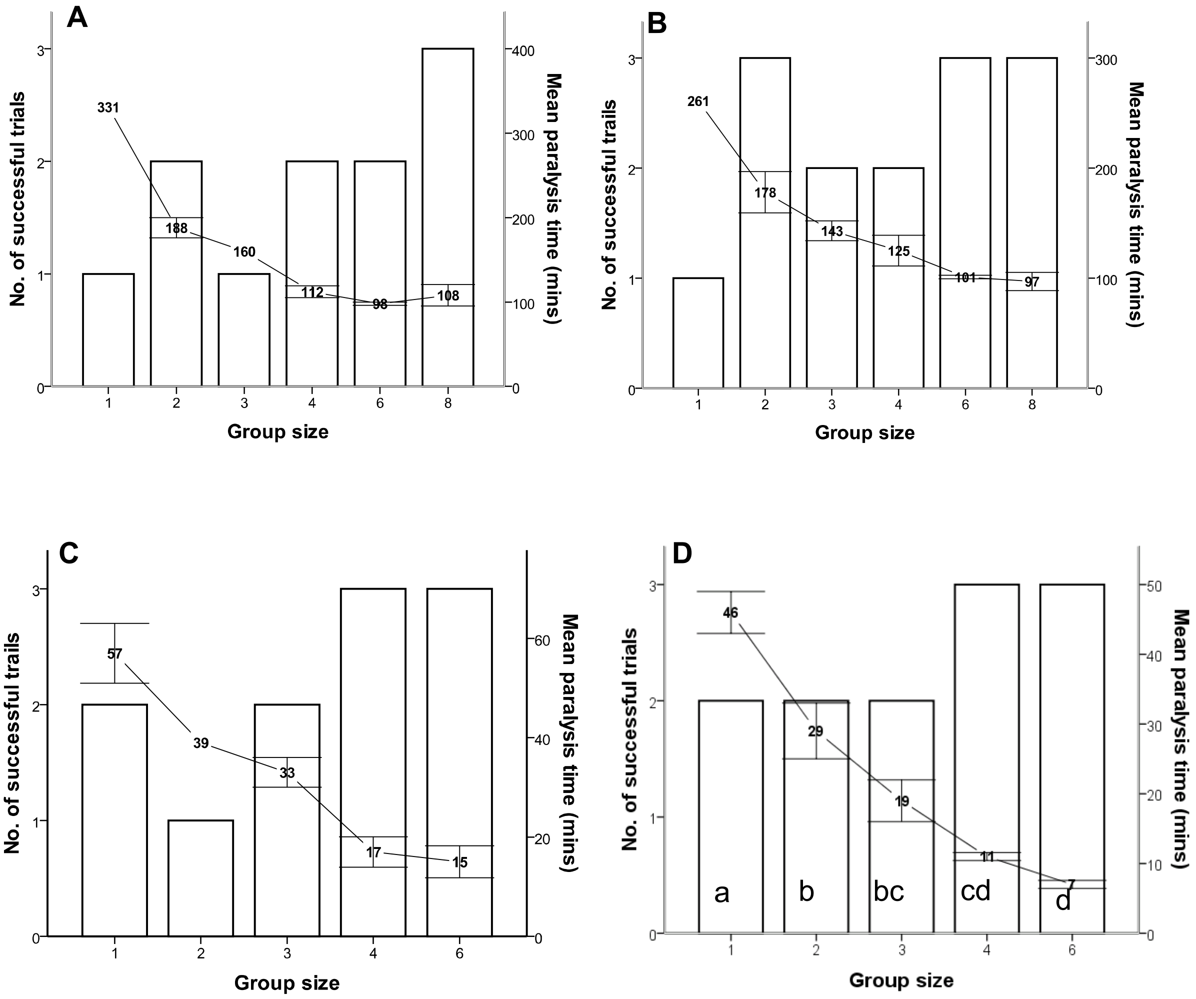

Fig. 3-6. Number of successful trials on the prey (bars) and mean (± SE) time (mins) (line) taken to paralyse house fly by (A) 3-week old *Scytodes gooldi* spiderlings (*n* = 3); (B) 3-week old *S. rubra* spiderlings (*n* = 3); and cricket by (C) 5-week old *S. gooldi* spiderlings (*n* = 3); and (D) 5-week old *S. rubra* spiderlings (*n* = 3). Different lower cases indicate significant differences.

Cannibalism among siblings

Cannibalism commenced from the second instar in Yunnan *Scytodes* sp. 3, third instar in *S. gooldi* and fourth instar in *S. rubra* (Table 3-6). *S. rubra* individuals are especially tolerant of their brood-mates because cannibalism peaked only between the 6th and 7th week, which coincides with the fifth instar stage.

Table 3-6. Total cumulative mortality resulting from cannibalism in groups of spiderlings (three groups, 10 spiderlings per group except for Yunnan *Scytodes* sp. 3 which had 6 spiderlings per group)

	Week 2 (7-13d)	**Week 3 (14-20d)**	**Week 4 (21-27d)**	**Week 5 (28-34d)**	**Week 6 (35-41d)**	**Week 7 (42-48d)**	**Total incidental mortality***
Yunnan *Scytodes* sp. 3	2	6	10	12	13	13	4
S. gooldi	0	0	4	9	14	22	2
S. rubra	0	0	0	2	6	13	2

*Incidental mortality refers to deaths not attributed to cannibalism (e.g. from moulting).

Reproductive traits

MAVONA revealed a significant overall difference in reproductive traits among three species of ground-dwelling scytodids (Wilks' λ = 0.178, $F_{8,18}$ = 3.080, P = 0.023). *S. gooldi* and *S. rubra* produced significantly more spiderlings than Yunnan *Scytodes* sp. 3 ($F_{2,\ 12}$ = 16.101, P < 0.001; Fig. 3-7A). Within each egg-sac, there were also significantly more spiderlings in *S. gooldi* and *S. rubra* than in Yunnan *Scytodes* sp. 3 ($F_{2,\ 12}$ = 17.043, P < 0.001; Fig.3-7F). There were no significant differences in egg hatching time ($F_{2,\ 12}$ = 0.605, P = 0.562; Fig. 3-7B) and the number of egg clutches ($F_{2,\ 12}$ = 1.855, P = 0.199; Fig. 3-7E) among the three species. Multivariate analysis was not performed for interval between successive egg sac and interval between hatching and next egg sac production because *S. rubra* females produced only one egg sac in the laboratory.

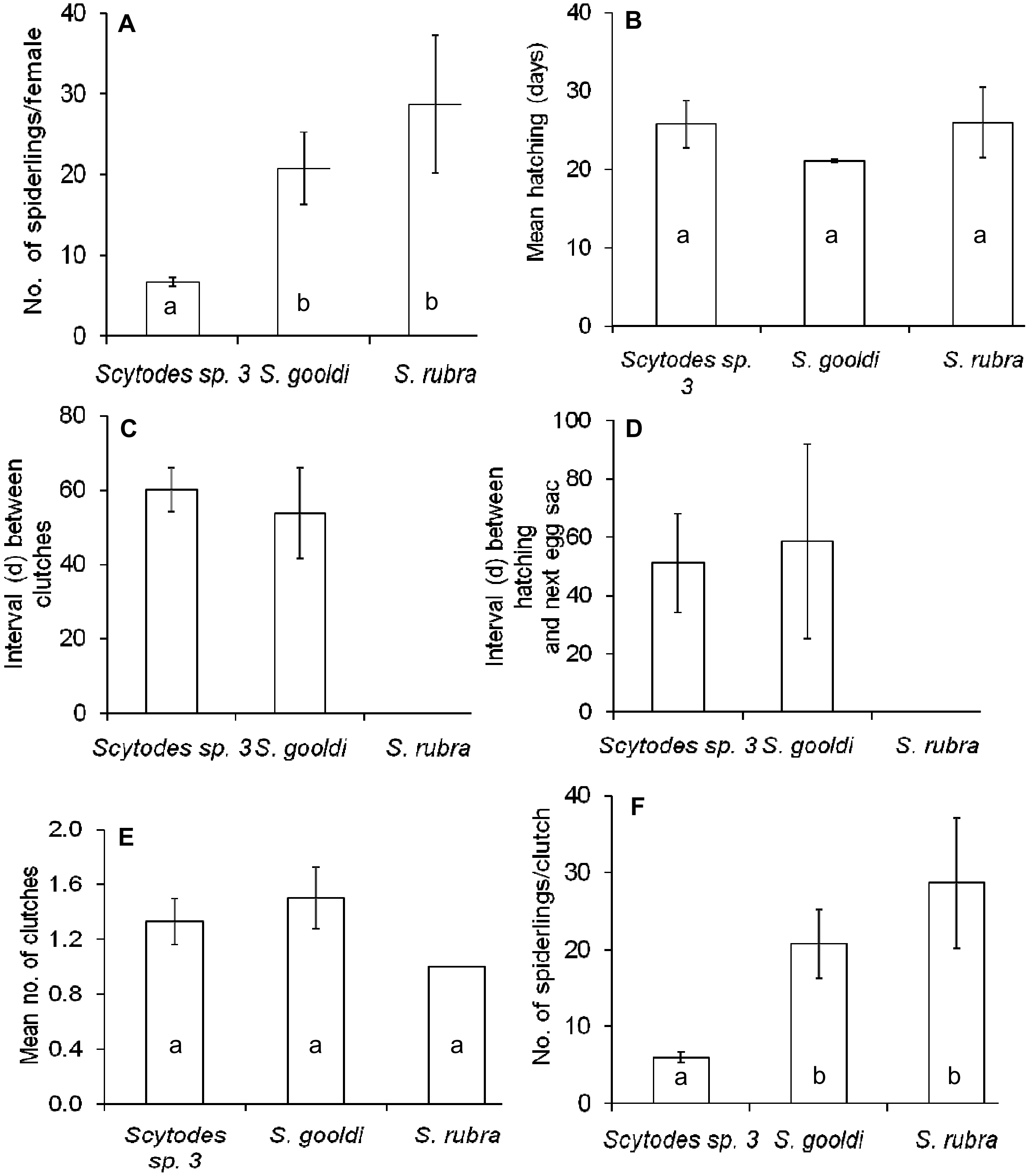

Fig. 3-7. Reproductive traits of three ground-dwelling species of scytodid. (A) Mean (± SE) total number of spiderlings per female. (B) mean (± S.E.) egg hatching time (d). (C) mean (± S.E.) interval (d) between clutches. (D) mean (± S.E.) interval (d) between hatching and the next egg-sac production. (E) mean (± S.E.) number of clutches. (F) mean (± S.E.) number of spiderlings per clutch. Different lower cases indicate significant differences.

DISCUSSION

This study provides evidence that South African *Scytodes gooldi* and *S. rubra* living in logs and under stones are subsocial as they exhibit maternal care of eggs, extended maternal care of offspring by food provisioning, delayed natal dispersal, cooperative foraging among siblings and delayed cannibalism among siblings. However, litter-dwelling *Scytodes* sp. 3 from Yunnan seems to be solitary since they show no extended maternal care in terms of food provisioning by females though females carry egg-sacs and hatchlings mount on female's back. Cooperative foraging and food sharing are also absent in Yunnan *Scytodes* sp. 3. Furthermore, cannibalism among siblings occurs in the early life stage, indicating low level of tolerance among siblings.

Why is *S. gooldi* that live in rotting logs subsocial? There are at least a few reasons for why such a nest site is more favoured. First, the rotting log with its extensive stable preformed cavities tend to maintain uniform and favourable conditions of temperature and humidity (Wilson 1959). Second, the subcortical cavities of the large rotting log which is in the "zorapteran stage" abound with arthropods such as collembolans, mites, isopods and beetles – a ready food source for the spiders (Wilson 1959). Such a microhabitat may be conducive for *S. gooldi* to live in small groups, as was observed rather frequently during the surveys.

Subsociality has been reported in a species of Australian wood-boring cockroach that spend their lives within rotting logs (Billingham et al 2009). Chemical cues seems to be important in group formation and social cohesion, but the potential benefits of subsocial aggregations in *Panesthia australis* is unclear. In *Xyleborini saxeseni*, a fungus-cultivating ambrosia beetle that construct galleries in freshly dead trees, sociality arises from the fact that helping in an already established, productive gallery is likely to be an advantageous alternative to individual gallery foundation (Gadagkar 1990). In *X. saxeseni*, constraints on individual reproduction are probably severe because a risky dispersal flight is required, and finding a suitable host location is uncertain. These factors lead to individuals remaining in the natal brood chamber and a subsequent extension of the gallery resulting in the evolution of delayed dispersal and cooperative brood care (Peer and Taborsky 2007).

For some social spiders, especially web-building species, one of the reasons for not leaving the natal nest is to save on silk production, and so share a commonality with *Xyleborini saxeseni*. By remaining with the group these individuals need only to add on silk threads to enlarge the existing web instead of having to build a web from scratch. The presence of common web attachments enables spiders to save on per capita costs of silk production (Avilés 1997) and lowers the foraging costs of web building and web maintenance (Whitehouse and Lubin 2005) (e.g. *Philoponella oweni*: Smith 1982; *Holocnemus plucheii*: Jakob 1991; *Gastercantha minax:* Lloyd and Elgar 1997). *Scytodes*, however, construct rudimentary irregular webs for shelter and sometimes rely on their webs to communicate to them the arrival of a prey. While the prey is entangled and delayed by silk, the spiders attack by biting and spitting (Miller 2006). A baseball size web similar to those seen in *S. socialis* (Miller 2006) was not observed in *S. gooldi*, hence it is unlikely that the reason for group-living in *S. gooldi* is to conserve silk. Rather, the ecological factor that may be the driving force in *S. gooldi* forming temporary aggregations is possibly the presence of prey that are sufficiently large enough to warrant joint prey capture and sharing.

How and why is *S. rubra* living under stones subsocial? *S. rubra* live in small, fine silk nests attached to the underside of loose stones. These stones occupy the bottom of a stream that was dried due to the preceding winter months. At least one study has found that spiders can select warmer retreat sites because hotter conditions enhance rates of growth and development (Goldsbrough et al. 2004). It is possible that *S. rubra* females select the underside of loose stones as their nesting sites so as to promote the development of their offspring. In addition, invertebrate densities such as adult beetles and corixids (water boatmen) are known to increase in streams during drying (Stanley et al. 1994). In many temporary streams, densities of predators rise sharply during drying (Abell 1984). Predator recruitment in intermittent streams may be timed to coincide with peak prey densities (Boulton and Lake 1992). The macroinvertebrate community that thrives in intermittent streams may also be serving as a ready food source for *S. rubra*. Hence, similar to the case of *S. gooldi* the microhabitat of *S. rubra* may be conducive for these spiders to form small colonies which cooperate in the capturing of prey.

Many amblygids (whip scorpions) and pseudoscorpions live in cryptic environments such as leaf litter, rock crevices, under stones and barks of live trees or rotten trunks (Weygoldt 1969; Harvey 1986). Laboratory observations of two species of captive amblygids have shown that they exhibit prolonged mother-offspring-sibling associations. *Pandinus marginemaculatus* and *Damon diadema* not only tolerate one another, but consistently aggregate and interact frequently with extensive whip contact (Rayor and Taylor 2006). It is also known that manipulation of food availability does not alter aggregation levels of *P. marginemaculatus*, suggesting that the tendency to aggregate may not be directly related to prey capture or hunger level. The amblygids do not hunt cooperatively or share prey, although they may benefit by advanced notice of the presence of prey by their neighbours' heightened whip activity. When frightened, young *D. diadema* move significantly closer to their mother or siblings, and mothers threaten or actively attack aggressors (Rayor and Taylor 2006). The reason for aggregation in amblygids which is probably for group defense is in contrast with that of *S. rubra* and even *S. gooldi*, in which I speculate that food abundance is the promoter of group living and cohesion. In the pseudoscorpion, *Paratemnoides nidificator*, the main functions of sociality could be reproductive and foraging (Del-Claro and Tizo-Pedroso 2009), similar to the *Scytodes*, but differing in the mechanisms. The maternal female and newly emerged nymphs will leave the brood chamber and feed on prey captured by other adult *P. nidificator*. Silk moult chambers built exclusively by cooperating *P. nidificator* nymphs act as a shelter against climatic disturbance, protection against natural enemies, and a place where nymphs and adults can interact (Del-Claro and Tizo-Pedroso 2009).

Subsociality has been reported in a burrowing wolf spider (Lycosidae) (Miller 1989) The colonies of this ground-dwelling spider, *Geolycosa turricola*, live in burrows which take the place of the web for the purpose of communication. Yunnan *Scytodes* sp. 3 is a leaf-litter dwelling wandering spitting spider. My results show that extended maternal care and group foraging are absent in this species. Furthermore, spiderlings dispersed very early from their natal nest. Its phenology and natural history has most likely precluded it from developing sociality. Gregariousness in these spiders may render them conspicuous to predators such as large spiders from the families Lycosidae and Gnaphosidae.

Typically young usually congregate in the mother's web (e.g. *Amaurobius ferox*: Kim et al. 2005; *Scytodes* sp.: Yap and Li 2009) or on her back for a brief period (e.g. *G. turricola*: Miller 1989; *Lycosa punctulata*: Eason and Whitcomb 1965) before dispersal. Yunnan *Scytodes* sp. 3 emergents, however, display an unusual behaviour of climbing onto the mother's body immediately after eclosing from the egg-sac. Such behaviour is known in some species of lycosids and pisaurids, and only in one other species of *Scytodes* – the Los Baños sp. (Li et al. 1999). It is interesting that such behaviour is common in other ground-dwelling spiders such as lycosids. Spiderlings in such aggregations may receive protection from the mother. As Yunnan *Scytodes* sp. 3 were collected by the sieving method, I am unable to determine whether females construct silk nests on the underside of fallen leaves or at the soil surface or if they are wanderers. If they are hunters, then it makes sense for freshly emerged spiderlings of Yunnan *Scytodes* sp. 3 to mount their mother's body.

The effects of maternal care on the young are well documented in social spiders. In *Theridion pictum*, egg-sacs which are left unguarded suffered drastically reduced hatching success (Ruttan 1991). In laboratory experiments conducted on *Scytodes* sp. 1 and *S. pallida*, unattended egg-sacs are prone to fungal attacks, parasitized by mites, and dessicated (Yap, unpublished data; Tang, unpublished data). Although maternal care in spiders includes defense against predators and parasites, maternal care also includes providing food for the spiderlings (Ruttan 1990; Schneider and Lubin 1997; Kim 2000). Although two-week old spiderlings may be large enough to capture their own food (e.g. *Drosophila*), they always left the capturing of prey to the mother if she was around and if the prey was of a large size (e.g. house flies). One would expect that the food provided to them by the mother is obtained at a much lower energy expenditure. In providing food the mother expends energy spitting and attacking the prey, wrapping it, injecting digestive enzymes and carrying the prey back to the nest.

In this study, both *S. gooldi* and *S. rubra* exhibit cooperative prey-capture and food sharing, a characteristic of typical subsocial and social spiders. Interestingly, both subsocial species always use three strategies in cooperative prey capturing: captors, intruders and inactive individuals (neither participating in capture nor in exploitation). One reason for the existence of intrusion or “stealing” is the fact that captors were not able to make full use of the biomass contained in a large prey (Rypstra 1990). Another reason is the risk of injury during captures – captors may be prone to attack by prey (Gonzaga and Vasconocellos-Neto 2001). Indeed I observed such events during my experiments in which spiderlings died as a result of an attack from the crickets. My results show that *S. gooldi* and *S. rubra* are able to subdue prey about two times larger than themselves. Captors usually fed on the head first then proceeded on to the thorax. As captors were always the first to choose and fed for longer duration, they may gain more than the intruders, thereby maximizing their own success whether they fed alone or in a group. Intruders exploited other less suitable parts such as the extremities like the legs and wings, and fed for a shorter time, thereby gaining less. However, further study is needed to determine whether captors are larger and grow faster than intruders and inactive spiderlings.

Spiders as groups also take a shorter time to subdue prey than a singleton. Thus prey capture efficiency as groups may be higher than that as single individual. However, this benefit may not be necessarily because of greater number of cooperative hunters involved in prey capture but rather that in a group the chances of more individuals being prone to hunting are higher. In this respect, spiders behave as solitary hunters with apparent cooperation as an outcome. The fact that group foraging is absent in Yunnan *Scytodes* sp. 3 and even in the five scytodid species that were discussed in the preceding chapter may imply that some form of decision-making must be involved in *S. gooldi* and *S. rubra* to cooperate so that they can reap the benefits of capturing a larger prey, albeit at the expense of incurring high energetic costs. During group foraging, the captors need to inject on average less venom to subdue the prey than when alone. Since venom is a protein, this will save valuable resources (Ward and Enders 1984).

Predatory behaviour and feeding in *S. gooldi* and *S. rubra* begin as a cooperative activity in the maternal nest, but increasing mutual aggressiveness after the second and third moult respectively, and increase in the need for space or resources, resulting in the spiders readily cannibalizing each other, may pressure the individuals to leave. Within a group the food intake per individual may be smaller than that of a solitary predator, but cooperative hunting can increase not only the chance to obtain a larger prey, but also simply the chance of obtaining any prey (Schneider 1996; Ulbrich and Henschel 1999; Amir et al. 2000; Kim 2000; Kim et al. 2005). In subsocial spiders, cooperative hunting not only enables the group members to hunt larger prey, but they do it more quickly and reduce the chances of cannibalism and aggression amongst siblings (Kim et al. 2005a; Whitehouse and Lubin 2005). Nentwig (1985) argued that the ability of groups to exploit large prey may have led to sociality in spiders. However, Packer and Ruttan (1988) argue that for most species, the ability of a group to cooperate in capturing prey is likely to be a resultant advantage of sociality rather than a causal factor.

It is evident that there is some level of tolerance among broods of *S. rubra* and *S. gooldi*, however, cannibalism still occurs and commences at a relatively early life stage in *S. gooldi*. In most types of cannibalism among non-solitary and related spiders, the cannibal kills to obtain food, presumably because of food limitation. The death of the victim, and also the degree to which cannibalism improves the growth, survival, and fecundity of the cannibal, potentially influence the population dynamics of the species. Thus, it is reasonable to hypothesize that spider cannibalism has evolved primarily as a means of foraging for calories and nutrients in limited supply (Wise 2006) This coupled with the fact that size asymmetries exist among the members of the same brood (i.e. some spiderlings/juveniles are larger and heavier than the rest) (Iida 2003; Wise 2006) and that the juveniles begin to exhibit predatory behaviour may be some of the factors that lead to cannibalism in *S. rubra* and *S. gooldi*. It has been suggested that the environment has an important role in determining dispersal and social patterns.

Dispersal from the natal nest in subsocial animal groups is of particular interest because social animal groups may have evolved from subsocial ancestors via the subsocial pathway (Kullman

1972; Krafft 1979), which is a gradual extension of the early social period of the young. Permanent sociality could arise from the young delaying dispersal and remaining within the maternal nest whatever the cost. Dispersal, therefore, is a factor constraining socialization. Gundermann and colleagues (1993) found that if the young of the subsocial species *Coelotes terrestris* are experimentally prevented from leaving the nest, they are capable of living until adulthood. My study does in part support their results because when groups of 10 *S. gooldi* spiderlings were put together in a cage and prevented from dispersing, cannibalism commenced only in the third instar and at low rates. I expected cannibalism to begin from early stages (early second instar) and rates to be very high because more than 50% of the spiderlings disperse before day 20, an implication that nest mates have low tolerance for each other. Therefore, *S. gooldi* are expected not to exhibit group foraging and communal feeding behaviour even until week 5. However, surprisingly, cannibalism only commenced from the third instar in this species. Two related questions emerge from this situation: 1) what determines the timing of dispersal in the more social species; and 2) what promotes tolerance and cooperation. Delayed dispersal benefits juveniles in that they have a greater and steadier intake of resources, but there is an upper limit to colony size (Jones and Parker 2002). High prey availability and presence of large prey may encourage cooperative prey capture and as more nutrition can be gained by remaining in the natal nest this leads to tolerance to be developed among the brood mates. Previous studies have shown that there is a bias towards smaller spiderlings dispersing first when food is unavailable or in low amounts (e.g. Ruttan 1990). The increased distance between individuals as the experimental duration progressed suggests reducing reduction in the group cohesion. Since extended maternal care, communal prey capture and feeding are present in this species, *S. gooldi* is considered a subsocial species. Under favourable conditions such as prey abundance and perhaps a less space-limited system, the duration in which *S. gooldi* juveniles remain in the natal nest may be prolonged. Perhaps this is all it takes for *S. gooldi* to evolve into a permanent-social species.

One other thing that stands out from the results is that *S. rubra* juveniles still continued to participate in group foraging in spite of maternal absence. In two separate colonies in which I experimentally prevented dispersal from occurring, the maternal female died when the juveniles were about 22-23 weeks old. The juveniles were possibly at the penultimate/subadult stage at this time yet tolerance still persisted among 6-8 individuals. These observations lead to the suggestion that *S. rubra* may be a “transition into social” species, and just like *S. gooldi* perhaps all it takes is for ideal environmental conditions such as presence of large prey items to be present that can serve as a positive driving force for juveniles to remain for longer durations in the maternal nest.

In *S. gooldi* and *S. rubra*, the more prey that spiderlings are able to capture in the maternal nest, the more individuals delay their dispersal. Dispersal behaviour in these two species may be influenced by both food availability and internal factors related to development. The individuals that remained in the maternal nest longer were heavier than the individuals that dispersed early (e.g. in *S. rubra* the last individual to disperse weighed almost four times heavier than the first). These early dispersers are likely losers in prey competition involving mutual aggressiveness. The larger spiders will have a better chance of growing than the smaller ones because of their ability to monopolize prey (Schneider 1995). Increased aggression and reduced access to prey may force subordinate spiderlings to retreat from the brood mates to the edge of the maternal web, where they are more likely to lose connection with the maternal web (Kim 2000). When the subsocial behaviour phase ends, spiderlings of *Geolycosa turricola* disperse to construct their first burrow. Whether they are successful in surviving the winter depends on the quality of the burrow. Spiderlings that disperse at larger body sizes construct deeper burrows (Miller and Miller 1984). The South African species, *S. gooldi* and *S. rubra*, also experience winter. It may be that by prolonging the juvenile aggregation, the nutritional gain attained via maternal feeding and cooperative foraging enhances the overall vigour and increased competitiveness in later life stages (Burgess and Uetz 1982). As a result, larger-sized juveniles are better equipped at surviving the winter months. They may be more capable of searching for better retreat sites, hunting the scarce prey or even escaping predators.

Delena cancerides is a social spider different from all other social spiders because its social behaviour evolved in the absence of a heavy reliance on a web structure (Rowell and Avilés 1995). This huntsman spider makes its habitat in the interstitial spaces behind tree bark, unlike the habitats occupied by any other social spiders known so far which usually build their nests on shrubs and branch tips of trees. Females of *D. cancerides* guard their egg-sacs which they attach to substrates, and actively defend their eggs or hatchlings (Rowell and Avilés 1995). Colonies generally consist of a single female and a number of size classes of juveniles with some colonies containing up to twelve adults of both sexes and 300 juveniles. Maternal care, group foraging and prey sharing occur in broods of both *G. turricola* and *D. cancerides*, although in the latter this appears to take the form of independent uncoordinated attacks rather than a concerted effort (Rowell and Avilés 1995). My study has shown that *S. gooldi* and *S. rubra* share similar social behavioural traits as their subsocial ground-dwelling counterparts - *G. turricola* and *D. cancerides* - implying that the evolution of social behaviour does not require the web as a preadaptation. The question of why some individuals disperse earlier than others in arthropod groups is one of the most interesting questions in the evolution of sociality. One hypothesis for spiders is that the maternal web which provides protection against predators and harsh climatic conditions (Seibt and Wickler 1990) could be a desirable inheritable resource for which spiderlings could compete (Wickler and Seibt 1993; Evans 1998).

In conclusion, *S. rubra* and *S. gooldi* from South Africa are subsocial. Although many studies have stressed the importance of an irregular web in the evolution of sociality, I have shown that it is not a necessary prerequisite. *Scytodes rubra*, a species which lives under rocks do not construct an elaborate web yet the mother-offspring-sibling association is more prolonged than any other scytodid species studies thus far. This is the first study to document sociality in scytodids living in the ground substratum. High prey abundance may have promoted group cohesion, delayed dispersal and extended tolerance in *S. rubra* and *S. gooldi*.

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CHAPTER 4

COMPARATIVE STUDY OF SOCIAL BEHAVIOUR OF SPITTING SPIDERS LIVING IN THE AERIAL LEAF ZONE

ABSTRACT

There is considerable variability in degrees of sociality within the animal kingdom. Despite few social species, various forms of sociality have evolved repeatedly in spiders, including some members from the genus *Scytodes*. There are three forms of sociality in this group: subsociality with extended maternal care, communal-territorial species, and non-territorial multiple-adult species. Previous studies have shown that scytodid species exhibiting social behaviour all live in the aerial vegetation microhabitats. In this chapter I examined the social status of 11 scytodid species from two genera (*Scytodes* and *Dictis*) that inhabit the leaves of shrubs and herbs by examining colony structure, eleven behavioural and reproductive traits. Two *Scytodes* species (*Scytodes* sp. 1 and *S. pallida*) were seen to exhibit extended maternal care with food provisioning, joint prey-capture and communal feeding among siblings, low cannibalism rate, and delayed natal dispersal, confirming their status as subsocial species. Other three species of *Scytodes* and all six *Dictis* species were not seen to exhibit extended maternal care, cooperate in prey-capture, or share food among siblings, in addition to starting cannibalism and dispersal from nests early, suggesting they are solitary. I also compare the behaviours of two different populations of *S. pallida* (Singapore and Malaysia) and *Dictis* sp. 2 (Singapore and China). The two populations of *S. pallida* display the same social organization, but there are population variations in the reproduction and cooperative prey-capture efficiency. A hypothesis for the evolution of sociality in *Scytodes* sp. 1 and *S. pallida* is proposed.

INTRODUCTION

There is considerable variability in the degree of sociality within the animal kingdom. Despite few social species, various forms of sociality have evolved repeatedly in spiders, including some members from the spitting spider family Scytodidae. Sociality of spitting spiders ranges from solitary (e.g. *S. longipes:* Dabelow 1958; cave scytodids: Chapter 2) to species with many individuals cooperating in a single web (e.g. *S. socialis*: Miller 2006). In this chapter, I describe two more species that present some characteristics considered as prerequisites to the development of social behavior—retention of juvenile peer tolerance (Agnarsson 2002). Maternal care, considered one of the precursors of social behavior (Agnarsson 2002), has been described for other scytodid species – *S. intricata* (Eberhard 1986) and the Los Baños *Scytodes* sp. (Li et al.1999). Despite being phylogenetically scattered in spiders (Coddington and Agnarsson 2006), subsociality is concentrated in the family Theridiidae, occurring in species of four genera: *Achaearanea* Strand, *Anelosimus* Simon, *Theridion* Walckenaer, and *Helvibis* Keyserling (Agnarsson 2002; Coddington and Agnarsson 2006). Subsociality has evolved independently several times in spiders; here I document yet another genus – *Scytodes* - that has an independent origin of subsociality.

Duffey (1962a, 1962b, 1966, 1968) observed that the proportion of web-spinning species increases with increasing vegetation complexity and that the greatest number of spider species occur in the most floristically diverse habitats. Much of the spiders' prey is made up of Hymenoptera, Diptera and Lepidoptera that are either flower visitors or parasites of flower visitors (Olive 1980; Biere and Uetz 1981; Janetos 1982b). Therefore, the patterns of flowering plants around the web sites will strongly influence the flight patterns of potential prey and in turn the prey supply to spiders (Janetos 1986). A high abundance of prey and provision of larger prey items in the aerial vegetation may explain why most social spiders are found in such habitats. Across the taxonomic spectrum, all but the social huntsman species- *Delena cancerides* that are large non-web building spiders that live under the bark of dead trees in year-round colonies of up to 300 individuals- share key behavioural traits: all known social spiders live in web-based societies in trees or shrubs, even when other members of the family do not build prey capture webs (Avilés 1997).

The aerial leaf zone is defined as the low arboreal stratum, ranging from herbaceous and shrubby ground vegetation to the first several metres of the trunks and branches of larger trees. Spiders are often an abundant component of the canopy fauna (Basset 2001). For example, Majer and colleagues (1994) have shown that spiders are the third most species-rich group in eastern and western Australian eucalypt forest. Gagné (1979) found that in rainforest and savannah spiders are among the four most abundant groups of arthropods. However, to date there are very few records of scytodids in canopy spider community (Sørenson 2004). Nevertheless, scytodids are often found in the aerial leaf zone. I managed to find 11 species of scytodids, including five species of *Scytodes* (Singapore *Scytodes* sp. 1, Singapore *S. pallida*, Malaysia *S. pallida*, Vietnam *Scytodes* sp. 4, Khao Chong *Scytodes* sp. 5, and Cat Tien *Scytodes* sp. 6) and six species of *Dictis* (Thailand *Dictis* sp. 1, Hainan *Dictis* sp. 2, Singapore *Dictis* sp. 2, Singapore *Dictis* sp. 3, Singapore *Dictis* sp. 4, Hanoi *Dictis* sp. 5 and *Dictis venusta*), in the shrubs and herbs of the tropical countries including Singapore, Malaysia, Thailand and Vietnam, an indication that the aerial zone may be species-rich habitats. Does this then correspond to more opportunities of discovering subsocial or social scytodids? Whether the differences in scytodid community composition between sites can be attributed to differences in sampling efforts or differences in habitat complexity remains unclear until studies have been carried out to reveal general patterns.

In the present chapter, I studied the social behaviour of 11 species of scytodids belonging to two genera, *Dictis* and *Scytodes*, that live in the aerial zone. The genus *Dictis* was until now monotypic containing only one species, *D. striatipes*. Dankittipakul and Singtripop (2010) described three new species of *Dictis*, and intend to formally transfer *Scytodes lugubris* and *Scytodes venusta* to *Dictis*. On the basis of two diagnostic characters (i.e. tarsal claws and female genitalia, unpublished data) for this genus, I identified seven species as *Dictis*. Previous to the present study, no information on biology, particularly social behaviour of any *Dictis* spider was available.

Many solitary *Stegodyphus* are ground-dwelling species, but social species colonize higher vegetation and even tree-tops (e.g. *S. mimosarum* and *S. dumicola*) and have entered a new ecological niche. However, their nests are costly to rebuild, this induces extreme philopatry which in turn leads to inbreeding (Wickler and Seibt 1993). Other examples include *Aebutina binotata* that construct webs beneath leaves of tropical forest trees (Avilés 1997) and *Tapinillus* sp. that build a loose, irregular mesh web at the tips of leaves (Avilés et al. 2001). This phenomenon led me to address the question: do scytodids that live in the aerial zone follow a similar trend seen in *Stegodyphus* spiders?

A three dimensional web has been frequently considered as a preadaptation for sociality (e.g. Shear 1970; Krafft 1979, 1982; Buskirk 1981; Cangialosi and Uetz 1987; D'Andrea 1987; Avilés 1997). Webs are important because (1) they are costly to produce; (2) they are discrete physical structures that may hold individuals together in one place; and (3) they facilitate communication (Avilés 1997). Scytodids construct rudimentary webs for the purposes of shelter and also prey capture. Miller (2006) discovered *S. socialis* from Madagascar where webs contain multiple males, females and juveniles. Living in the upper stratum (i.e. aerial zone), scytodids may have many opportunities (e.g. both the space and the purpose to capture larger prey) to construct webs. One of the aims of this part of the study is to investigate whether there exists any social scytodid that construct voluminous webs in aerial vegetation, similar to those in *S. socialis*.

Many solitary species exhibit some maternal care and the distinction between subsocial and solitary species with maternal care is somewhat arbitrary depending mainly on the length of the communal phase after maternal care has ended. The distinguishing characteristic of subsocial species is a prolonged period of maternal care with the potential for interactions among the siblings in the maternal web (Lubin and Bilde 2007). I investigated the behavioural and reproductive traits of six *Dictis* and five *Scytodes* species by examining maternal care, prey capture and feeding, natal dispersal patterns and reproduction. In addition, I also performed comparative studies of two species of scytodids from different populations, *S. pallida* from Malaysia and Singapore as well as *Dictis* sp. 2 from Singapore and China to see if there would be any geographic variations in these behavioural and reproductive traits.

How similar are the different (sub)social scytodids spread among different habitats (i.e. under rocks, in dead logs and in leaves of shrubs) in their behavioural and life-history characteristics? Hailing from tropical areas where not only are there abundant prey year around, but also that much of that prey are large enough to require a group of individuals to capture them, I discuss in this chapter whether these 11 species of scytodids are pre-adapted to living a social lifestyle, with comparisons made to the social *S. socialis*, Los Baños *Scytodes* and *S. intricata*. I also discuss the traits that characterize the social scytodids and the underlying mechanisms that may be maintaining them.

MATERIAL AND METHODS

Colony survey

The procedure for surveying colonial structure of 11 species scytodids/populations was similar as used in Chapters 2 and 3, and only the essential details are given here. Field surveys of colonial structure of each species was conducted along a 2 km line transect. For each colony observed, I counted the total number of adult females, egg-sacs, males and juveniles present in the colonies, as well as estimated the juveniles of different size classes (i.e. instars). Surveys were conducted during the day, between 09 00 and 18 00h. Behavioural observations were conducted on an opportunistic basis. Spiders were examined by un-rolling curled up leaves, by peeling away two leaves that were stuck together or by bush-beating. After surveys, spiders were collected and brought back to NUS laboratory for behavioural observations and experiments.

Table 4-1. Details of localities and microhabitat types of thirteen aerial vegetation scytodids sampled from the field

Species	Locality	Coordinates	Habitat type
Singapore *Scytodes* sp. 1	Singapore: Clementi Woods Park	N01°17'54" E103°46'5" Elevation: 21m	Green leaves of money plant
Malaysia *S. pallida*	Malaysia: Gombak Field Study Centre, Selangor	N03°19’30.9”E101°45’01.7” Elevation: 259m	Green leaves of herbs and shrubs
Singapore *S. pallida*	Singapore: MacRitchie catchment	N01°20′37″ E103°49′52″E Elevation: 18m	Green leaves of *Tectaria* ferns
Vietnam *Scytodes* sp. 4	Vietnam: Ba Vi National park, Hanoi	N21°03'40.9" E105°21'42.7" Elevation: 1042m	Green leaves of herbs and shrubs
Khao Chong *Scytodes* sp. 5	Thailand: Khao Chong National Park, Trang	N07°32'32.4" E 099°47'31.3" Elevation: 78m	Dried, brown leaves of shrubs
Cat Tien *Scytodes* sp. 6	Vietnam: Cat Tien National Park, Ho Chi Minh	N11°25'43" E107°25'40.4" Elevation: 127m	Green leaves of herbs and shrubs
Thailand *Dictis* sp. 1	Thailand: Phuphapet National park, Trang	N07°07'30.8" E99°59'50.6" Elevation: 249m	Brown/Green leaves of herbs and shrubs
Hainan *Dictis* sp. 2	China: Mount Wuzhi, Hainan	N18°46'30.15" E109°31'0.84" Elevation: 1876m	Green leaves of shrubs
Singapore *Dictis* sp. 2	Singapore: Tampines ecogreen wasteland	N01°21’39” E103°56’43” Elevation: 18m	Brown/Green leaves of herbs and shrubs
Singapore *Dictis* sp. 3	Singapore: Bukit Timah Nature Reserve	N01°21′10″ E103°46′42″ Elevation: 74m	Brown/Green leaves of herbs and shrubs
Singapore *Dictis* sp. 4	Singapore: Changi Fairy Point chalets	N01°23'23" E103°58'28" Elevation: 0m	Brown/Green leaves of herbs and shrubs
Hanoi *Dictis* sp. 5	Vietnam: Biodiversity Centre, Hanoi	N21°23'04.9" E105°42'40.7" Elevation: 159m	Dried, brown leaves of shrubs
Dictis venusta	Singapore: Kent Ridge Park	N01°17′1.53″ E103°47′25.98″ Elevation: 37m	Dried pitcher cups

Parameters used in determining social status of the species in the laboratory

Spider collection and maintenance

For laboratory observations and experiments, spiders were collected from study sites and maintained by adopting protocol similar to that used in earlier scytodid studies (Li et al. 1999; Li 2002; Yap and Li 2009) and in Chapters 2 and 3, and only the essential details are given here. Spiders were collected from the field and brought back to NUS Behavioural Ecology & Sociobiology Laboratory (BES Lab). All spiders were housed in a laboratory with controlled environmental conditions (temperature = 24 ± 1°C; relative humidity = 70-90%; light/dark cycle = 12 L : 12 D; lights on at 0800 h). They were fed with fruit flies, *Drosophila melanogaster* Meigen once a week. Life stages were referred to as, for example, egg, first instar (see Whitcomb 1978). 'Penultimate adults' were two moults from becoming adults. Unless qualified, 'male' and 'female' referred to adults. No individual spider was used in formal observations more than once per day. Decisions such as which particular spider was used in a given observation were made randomly. All experiments were carried out between 1100 h and 1800 h.

The procedures for observing social behaviour of scytodid species were as described in Chapters 2 and 3. In order to determine the social status of each species, I measured eleven traits as used in Chapter 2. These include: (1) the presence/absence of maternal care of eggs; (2) the presence/absence of extended parental care (Miller 1989); (3) the presence/absence of group prey-capture; (4) natal dispersal pattern; (5) mother-offspring and juvenile-juvenile interactions; (6) total number of spiderlings/female; (7) number of spiderlings/clutch; (8) the time interval between clutches (egg-sacs); (9) the time interval between the hatching date and next egg-sac production; 10) the time taken for spiderlings to hatch; and (11) number of clutches produced by a female. I also recorded behaviour of the hatchlings immediately upon emergence, i.e. whether they attached themselves the broken egg-sac or mounted the mother's body; or if they aggregated around the mother in the natal nest or scattered around the cage. I predicted that the spiderlings of the social scytodids would cluster as a group staying close to the mother for protection, upon eclosion.

As in Chapter 2, I first checked data for normality using Kolmogorov-Smirnov test and analysed data with parametric or nonparametric procedures when appropriate. Linear regressions were performed to examine the relationships between the timing of juvenile natal dispersal and juvenile size for each species. Multivariate analysis of variance (MANOVA) was run to test the overall difference in six reproductive traits (total number of spiderlings, time taken for spiderlings to hatch, number of clutches, number of spiderlings per clutch, the time interval between clutches, and the time interval between the emergence of spiderlings and the next egg-sac production) among eleven species. If a significant overall difference was detected, one-way ANOVA was then conducted for each reproductive trait, followed by Tukey's HSD. All tests were two tailed and the data are reported as mean ± S.E. of untransformed data. All statistical analyses were conducted using IBM SPSS Statistics 19 (SPSS Inc., USA).

RESULTS

Colony structure

The colonial structures of five *Scytodes* species and six *Dictis* species in the field are presented in Table 4-2 and Table 4-3, respectively. A total 363 records of *Scytodes* sp. 1 (Fig. 4-1 A) were made during a two-month survey in several locations across Singapore (Clementi Woods Park, Bukit Batok Park, Singapore Zoological Gardens and Singapore Botanic Gardens). Except for *Dictis venusta* (Fig. 4-1J) webs which were mostly constructed inside brown and dried-up pitcher cups, webs of the remaining 10 species were found on both living and green or dry leaves. Their webs were three-dimensional, with a small funnel-like opening. The leaf was usually partially curled over so that its top surface was concave. The side of the leaf on which the web was built was usually curled over more than the rest of the leaf so that the web was concealed. Webs of males and juveniles were similar to those of females except somewhat smaller. Presumably, the folded leaf provides protection from adverse weather, affords a safe haven for egg laying, and facilitates concealment from predators.

Several forms of colony structures were observed across 11 leaf-dwelling species (Table 4-2 and Table 4-3), including: (1) the commonest colony structure was that females shared their nest with second instar spiderlings; (2) In all our colony surveys, adult males rarely shared their nests with spiderlings or females except for one instance in Singapore *Scytodes* sp. 1 where a male was seen to be sharing a nest with 2nd instar spiderlings; (3) second instar spiderlings frequently shared a nest without their mother; (4) nest-sharing among later instar spiderlings without adults was rare except in five species: Singapore *Scytodes* sp. 1, *S.*

pallida (two populations: Malaysia and Singapore) (Fig. 4-1C,D), Vietnam *Scytodes* sp. 4 (Fig. 4-1E,F), Cat Tien *Scytodes* sp. 6 (Fig. 4-1B) and Singapore *Dictis* sp. 4 (Fig. 4-1N)*;* and (5) female and males were rarely seen in the same nests except eight cases found in Singapore *Scytodes* sp. 1.

A comparison of two *Dictis* sp. 2 populations (Table 4-3) showed that the colony survey of the Hainan population (Fig. 4-1G) did not find any females with egg-sac, instead I found mature females with newly emerged second instars. On the other hand, I found that the Singapore population of *Dictis* sp. 2 (Fig. 4-1M) had colonies comprising of females and egg-sacs but there were no colonies containing females with second instar spiderlings. These observations imply that the females of *Dictis* sp. 2, like most other scytodids, practice egg-sac attendance and allow newly emerged spiderlings to remain in the maternal web for a short duration since both populations did not have any colonies that contained a mature female and later-instar spiderlings.


A
B
C
D
E
F
G
H

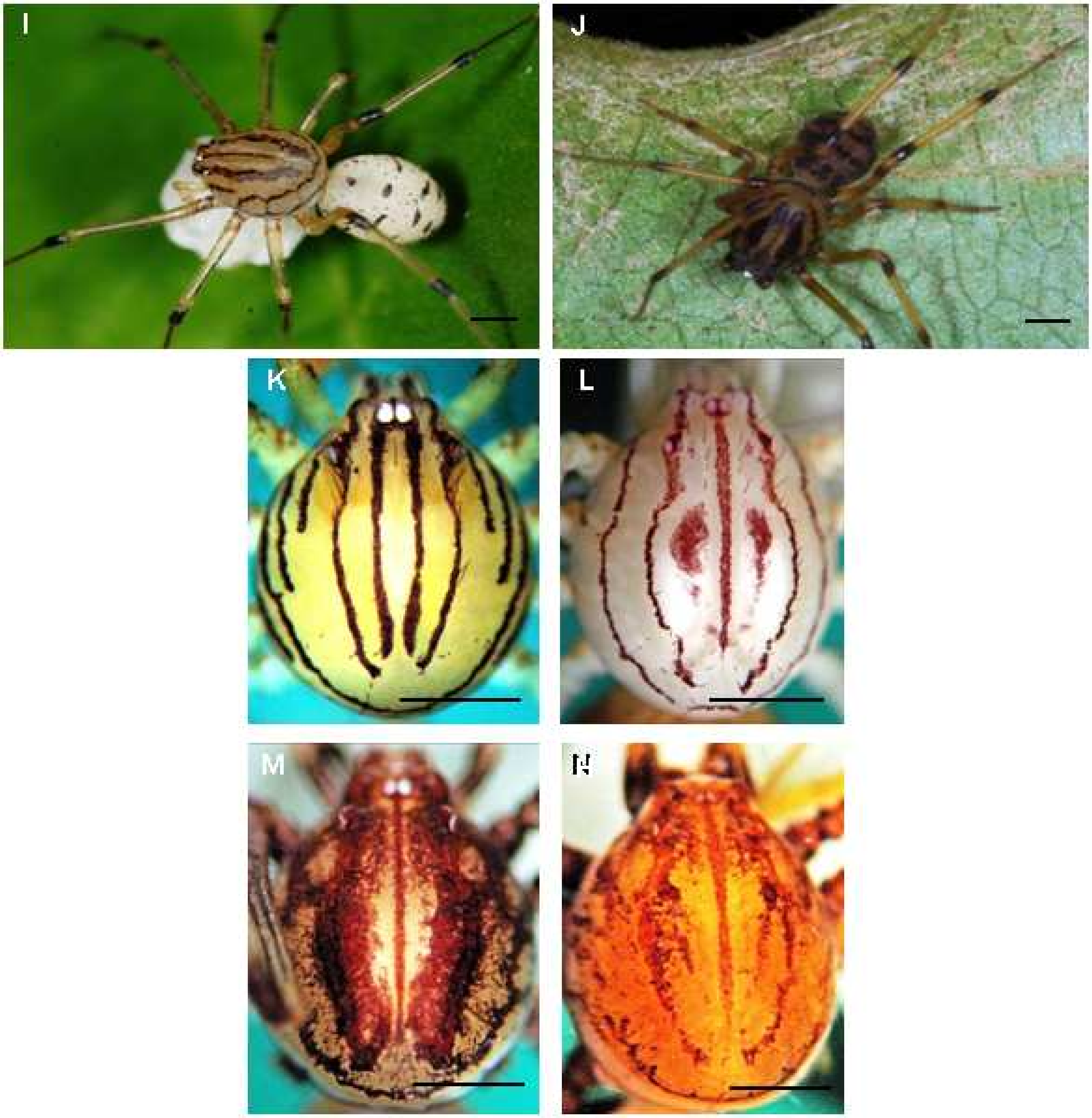

Fig. 4-1. Eleven species/populations of aerial vegetation scytodid spiders. (A) Female *Scytodes* sp. 1; (B) female Cat Tien *Scytodes* sp. 6; (C) male and (D) female *S. pallida*; (E) male and (F) female Vietnam *Scytodes* sp. 4; (G) female Hainan *Dictis* sp. 2; (H) female Singapore *Dictis* sp. 3; (I) female Hanoi *Dictis* sp. 5; (J) female *Dictis venusta*; prosomas of (K) Khao Chong *Scytode*s sp. 5; (L) Thailand *Dictis* sp. 1; (M) Singapore *Dictis* sp. 2 and (N) Singapore *Dictis* sp. 4. Scale bars =1 mm.

Table 4-2. Data on the inhabitants of six species/populations of aerial *Scytodes* nests in the field collected over a 7-day period

Nest inhabitant(s)		**Singapre *Scytodes* sp. 1 (*N* = 363)**	**Malaysia *S. pallida* (*N* = 99)**	**Singapore *S. pallida* (*N*=23)**	**Vietnam *Scytodes* sp. 4 (*N* = 55)**	**Khao Chong *Scytodes* sp. 5 (*N* = 24)**	**Cat Tien *Scytodes* sp. 6 (*N* = 26)**
Mature female	Alone	89	23	1	12	4	5
	Alone with egg-sac	54	4	1	8	2	2
	With spiderlings (2nd instar only)*	49	7	2	1	2	1
	With spiderlings (mix of 2nd instar and later)	12	0	1	0	0	0
	With spiderlings (later instar only)	15	0	0	0	0	0
	With mature male	8	0	0	0	0	0
	With mature male with egg-sac	2	0	0	0	0	0
Mature male	Alone	26	10	4	2	3	2
	With spiderlings (2nd instar)	1	0	0	0	0	0
	With spiderlings (mix of 2nd instar and later)	0	0	0	0	0	0
Groups without adult	2nd instar spiderlings without adult	11	2	2	2	1	1
	2nd instar spiderlings and later without adult	2	0	0	0	0	0
	Later instar spiderlings without adult	10	2	0	1	0	2
Spiderlings	2nd instar spiderling alone	18	16	1	10	4	4
	3rd instar and beyond spiderlings alone	66	35	11	20	8	9

Table 4- 3. Data on the inhabitants of seven species/populations of aerial *Dictis* nests in the field collected over a 7-day

Nest inhabitant(s)		**Thailand *Dictis* sp. 1 (*N* = 16)**	**Hainan *Dictis* sp. 2 (*N* = 33)**	**Singapore *Dictis sp. 2* (*N* = 25)**	**Singapore *Dictis* sp. 3 (*N* = 21)**	**Singapore *Dictis* sp. 4 (*N* = 33)**	**Hanoi *Dictis* sp. 5 (*N* = 37)**	***Dictis venusta* (*N* = 24)**
Mature female	Alone	6	9	7	4	8	6	2
	Alone with egg-sac	1	0	5	1	6	1	6
	With spiderlings (2nd instar only)*	0	4	0	1	1	0	0
	With spiderlings (mix of 2nd instar and later)	0	0	0	0	0	0	0
	With spiderlings (later instar only)	0	0	0	0	0	0	0
	With mature male	0	0	0	0	0	0	0
	With mature male with egg-sac	0	0	0	0	0	0	0
Mature male	Alone	1	10	4	2	3	3	2
	With spiderlings (2nd instar)	0	0	0	0	0	0	0
	With spiderlings (mix of 2nd instar and later)	0	0	0	0	0	0	0
Groups without adult	2nd instar spiderlings without adult	1	0	0	1	1	1	1
	2nd instar spiderlings and later without adult	0	0	0	0	0	0	0
	later instar spiderlings without adult	0	0	0	0	1	0	0
Spiderlings	2nd instar spiderling alone	4	0	2	7	3	18	2
	3rd instar and beyond spiderlings alone	8	10	7	5	10	8	11

Maternal care

Females of all 11 species practiced maternal care by carrying their egg-sacs in their chelicerae (Fig. 4-1D, I). All females of *Scytodes* species and populations (Malaysia *S. pallida*, Singapore *S. pallida*, *Scytodes* sp. 1, Vietnam *Scytodes* sp. 4 and Khao Chong *Scytodes* sp. 5 (Fig. 4-1K)) except for Cat Tien *Scytodes* sp. 6 were never observed to drop their egg-sacs to capture prey during egg-sac attendance. However, females of all *Dictis* species were observed to forage frequently during the egg-sac attendance. Upon having detected the presence of prey, females usually set their egg-sacs aside and then spat (two or three times) at prey to immobilize it; after which, they returned to their egg-sacs. If the prey was struggling with the glue, females returned to the prey, bit it, after 2-3 mins wrapped it up, and transported the wrapped-up prey to the site where they left their egg-sac, and fed on it (see chapter 2, Fig. 2-9).

Emergent phase and emergent behaviour

The spiderlings of all 11 species emerged as second instars having undergone one moult whilst still inside the egg-sac. Immediately upon eclosing from the egg-sac the spiderlings of Cat Tien *Scytodes* sp. 6, Thailand *Dictis* sp. 1 (Fig. 4-1L), Hainan *Dictis* sp. 2., Singapore *Dictis* sp. 2, Singapore *Dictis* sp. 3 (Fig. 4-1H), Singapore *Dictis* sp. 4, Hanoi *Dictis* sp. 5 (Fig. 4-1 I) and *Dictis venusta* attached themselves to the broken sac (Fig. 4-2), and within 24 hours spiderlings of Cat Tien *Scytodes* sp. 6, Thailand *Dictis* sp. 1, Hanoi *Dictis* sp. 5 and *Dictis venusta* would cluster in the maternal web forming an aggregation while in the remaining species, the spiderlings would spread themselves out in the maternal web. Interestingly, the newly emerged spiderlings of Malaysia *S. pallida*, Singapore *S. pallida*, *Scytodes* sp. 1, Vietnam *Scytodes* sp. 4 and Khao Chong *Scytodes* sp. 5 mounted the mother's body and within 36 hours, dismounted to form a tight cluster in the maternal web (see chapter 3, Fig. 3-3).

Fig. 4-2. Newly emerged spiderlings attached to the broken sac.

Table 4-4. The maternal care of egg-sacs and foraging during egg-sac attendance in 11 species of scyodids that live in aerial vegetation.

	Singapore *Scytodes* sp. 1	Malaysia *S. pallida*	Singapore *S. pallida*	Vietnam *Scytodes* sp. 4	Khao Chong *Scytodes* sp. 5	Cat Tien *Scytodes* sp. 6	Thailand *Dictis* sp. 1	Hainan *Dictis* sp. 2	Singapore *Dictis* sp. 2	Singapore *Dictis* sp. 3	Singapore *Dictis* sp. 4	Hanoi *Dictis* sp. 5	*Dictis venusta*
Carrying egg-sac in chelicerae	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Foraging during egg-sac attendance	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Extended maternal care: food provisioning

Food provisioning by females was seen only in Malaysia *S. pallida*, Singapore *S. pallida* and *Scytodes* sp. 1. The females, after attacking and wrapping the prey, usually carried it back to the maternal web. Their spiderlings then fed on the prey, with the mother feeding alongside the brood occasionally (Fig. 4-4A). The second instar spiderlings of these species/populations were capable of capturing small prey such as *Drosophila* on their own, and if an intruder attempted to share the prey a fierce struggle always ensued with the captor eventually consuming the *Drosophila* on its own. For the larger prey such as house fly, the mother was always observed to subdue the prey and drag it back to the web to share with the spiderlings or for the spiderlings to feed on their own. Females of other *Scytodes* species and all *Dictis* species did not provide prey for their young. If the female captured a house fly she usually injected digestive enzymes, wrapped it and fed on the prey on site alone. The offspring were never seen to share the prey with their mother although in several instances scavenging of the prey remains occurred.

Natal dispersal pattern

A spider's dispersal timing was positively correlated with its weight in all the species (although the relationship was weak in Singapore *Dictis* sp. 2 and Singapore *Dictis* sp. 3) except for *Dictis venusta* and Vietnam *Scytodes* sp. 4, which showed a reverse of this correlation: later dispersers tended to be lighter than those dispersed early. However, the negative correlation was not statistically significant (Fig. 4-3). Dispersal started at day 5, day 4, day 3 and day 2 after emergence from egg-sacs in Khao Chong *Scytodes* sp. 5, Thailand *Dictis* sp. 1, Singapore *Dictis* sp. 2, Singapore *Dictis* sp. 3, and Singapore *Dictis* sp. 4, respectively. However, the first occurrence of dispersal in Malaysia *S. pallida* was at day 19. In most of the species, the earliest dispersers were at their early second instars. The earliest dispersers in *Scytodes* sp. 1, Singapore *S. pallida*, Hainan *Dictis* sp. 2 and Hanoi *Dictis* sp. 5 were in their late second instar (Fig. 4-3).

In all 11 species of scytodids, the dispersal was not a simultaneous event for all the individuals within a clutch, rather the spiderlings dispersed over a period ranging from very short durations of 4 days in Thailand *Dictis* sp. 1 to intermediate durations of 18 days as in

Singapore *Dictis* sp. 4 (most species fall within this range) to very long duration of 56 days as seen in *Scytodes* sp. 1 (Fig. 4-3).

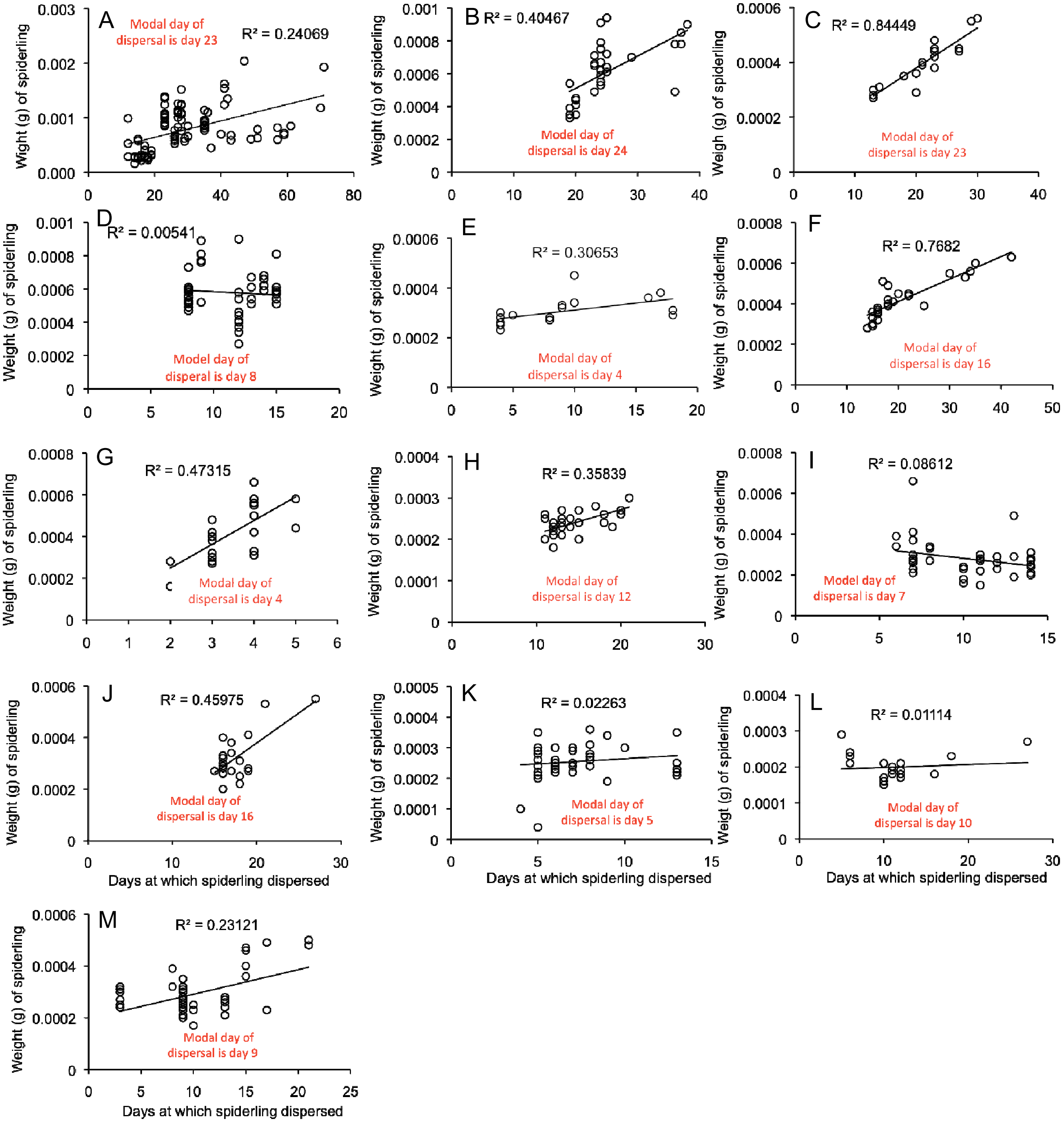

Fig. 4-3. Relationship between the days in which spiderlings dispersed and spiderling mass in 11 aerial vegetation scytodid species. (A) Singapore *Scytodes* sp. 1; (B) Malaysia *Scytodes pallida*; (C) Singapore *S. pallida*; (D) Vietnam *Scytodes* sp. 4; (E) Khao Chong *Scytodes* sp. 5; (F) Cat Tien *Scytodes* sp. 6; (G) Thailand *Dictis* sp. 1; (H) Hanoi *Dictis* sp. 5; (I) *Dictis venusta*; (J) Hainan *Dictis* sp. 2; (K) Singapore *Dictis* sp. 2; (L) Singapore *Dictis* sp. 3; and (M) Singapore *Dictis* sp. 4.

Group foraging behaviour

Group foraging and feeding were observed in 2 out of 11 species: *S. pallida* (from both Malaysia and Singapore) and *Scytodes* sp. 1 (Fig. 4-4B). Overall, group size, prey type and species type affected the time taken to subdue prey (MANOVA: $F_{6,44} = 5.246$, $P < 0.001$; Table 4-5). Groups of two, three, four, six and eight spiders did not show significantly higher capture success than a single individual. In fact, regardless of the group size, spiderlings of *Scytodes* sp. 1 and *S. pallida* from Malaysia and Singapore were as effective in capturing prey (Table 4-6), but with increasing group size, they become more efficient in capturing prey. The time taken to paralyze either house flies or crickets decreased with increasing number of spiderlings in a group (Fig. 4-5; $F_{5,44} = 239.599$, $P < 0.001$). No significant difference was found in the time taken to paralyze prey by groups of spiderlings between *Scytodes* sp. 1 and Singapore *S. pallida*, but Malaysia *S. pallida* took a significantly longer time to subdue the preys when compared to *Scytodes* sp. 1 and Singapore *S. pallida* (Fig. 4-6).

When three, four, six or eight spiderlings were feeding on prey, they were either captors or intruders. In all three species and populations, there was an upper limit to the number of captors: no more than five captors participated in prey capture. The spiders usually bit an appendage, almost always a leg or the wing of the prey, to inject the venom. As soon as the prey was paralysed, feeding commenced with the captors being always the first that fed. I observed that the captors fed frequently on the thorax although often they started feeding on the head first then proceeded to the thorax where feeding could last up to 24 h. Soon after the captors had settled down on their feeding positions, intruders then joined in. The captors often tried to chase the intruders away by briefly flailing their forelegs at the intruders, but direct attacks on them were never seen. In most cases, I observed the intruders walking away but returning later to join in the feeding as was evident from the change in size (and sometimes colour) of their opisthosoma (Fig. 4-4A). The number of spiders feeding on the prey at the same time differed depending on prey type. When prey were house flies, a maximum of four spiderlings fed at the same time, while when prey were crickets, a maximum of six spiderlings were feeding on the prey simultaneously. Cooperative prey-capture and prey sharing was absent in the other 9 species. If the prey was too large for an individual to capture, it was simply ignored.

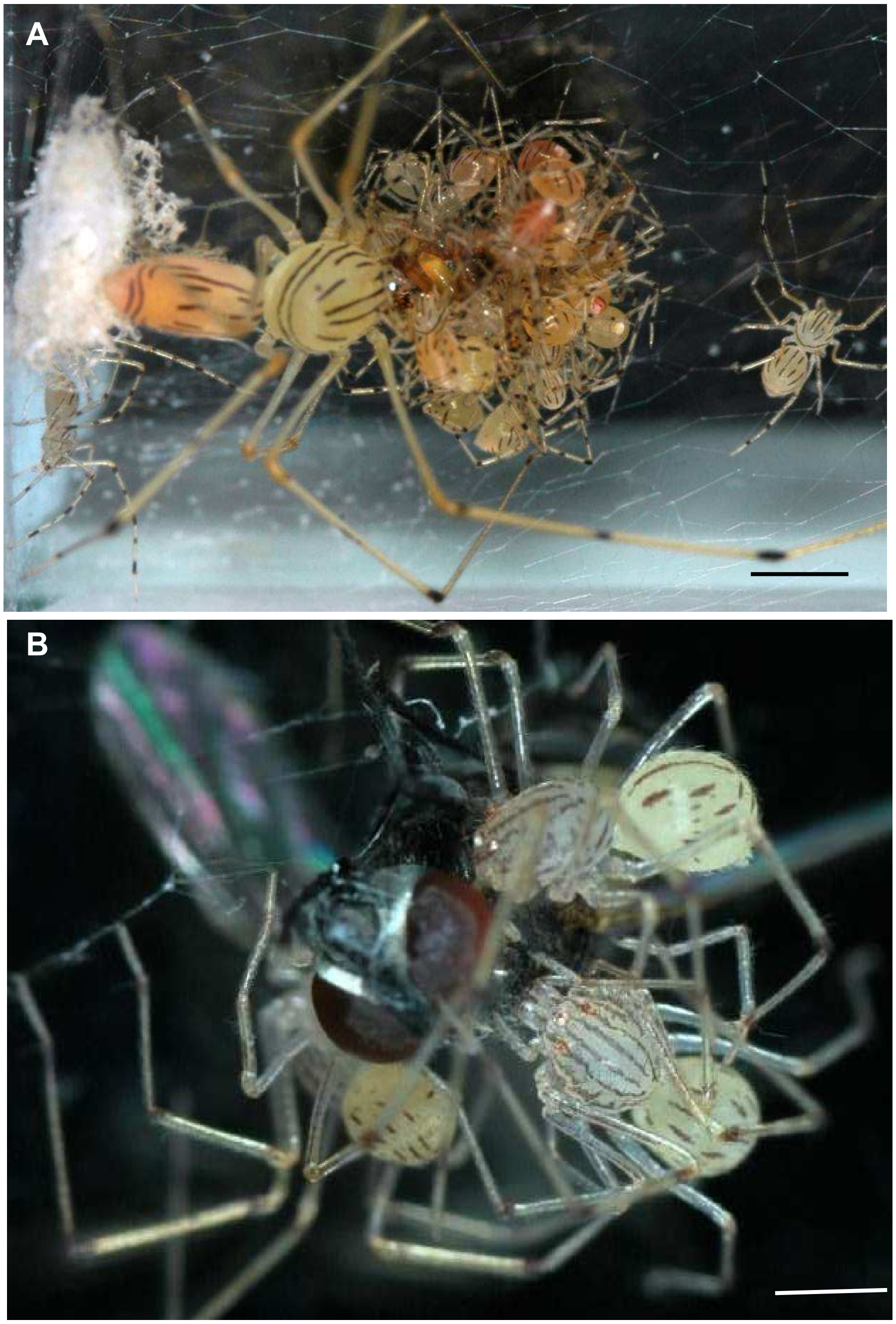

Fig. 4-4. Group feeding of scytodid spiderlings. (A) Female *Scytodes pallida* feeding on the captured prey alongside her offspring. The change in the colour of abdomen from transparent to orangey-pink is an indication that the prey is being ingested, scale bar = 2 mm; (B) third instar *Scytodes* sp. 1 feeding communally on a house fly. Scale bar = 1.5 mm

Table 4-5. Results from three-way ANOVA testing the effects of group size, prey type and species on the time of prey paralysis for *Scytodes* sp. 1, Singapore *S. pallida* and Malaysia *S. pallida*.

Source	*df*	Mean sq.	*F*	*P*-value
Group size	5	764420.400	239.599	< 0.001
Prey type	1	3986649.424	1249.573	< 0.001
Species	2	81192.611	25.449	< 0.001
Error	44	3190.409		

Table 4-6. Results of Chi-square tests for independence comparing prey capture success rates of singleton, groups of two, three, four and six in *Scytodes* sp. 1, Singapore *S. pallida* and Malaysia *S. pallida.*

	Housefly			**Cricket**		
	Scytodes sp. 1	Singapore *S. pallida*	Malaysia *S. pallida*	*Scytodes* sp. 1	Singapore *S. pallida*	Malaysia *S. pallida*
χ^2	8.031	12.390	12.000	3.462	3.462	3.462
df	5	5	5	4	4	4
P	0.155	0.030	0.035	0.484	0.484	0.484

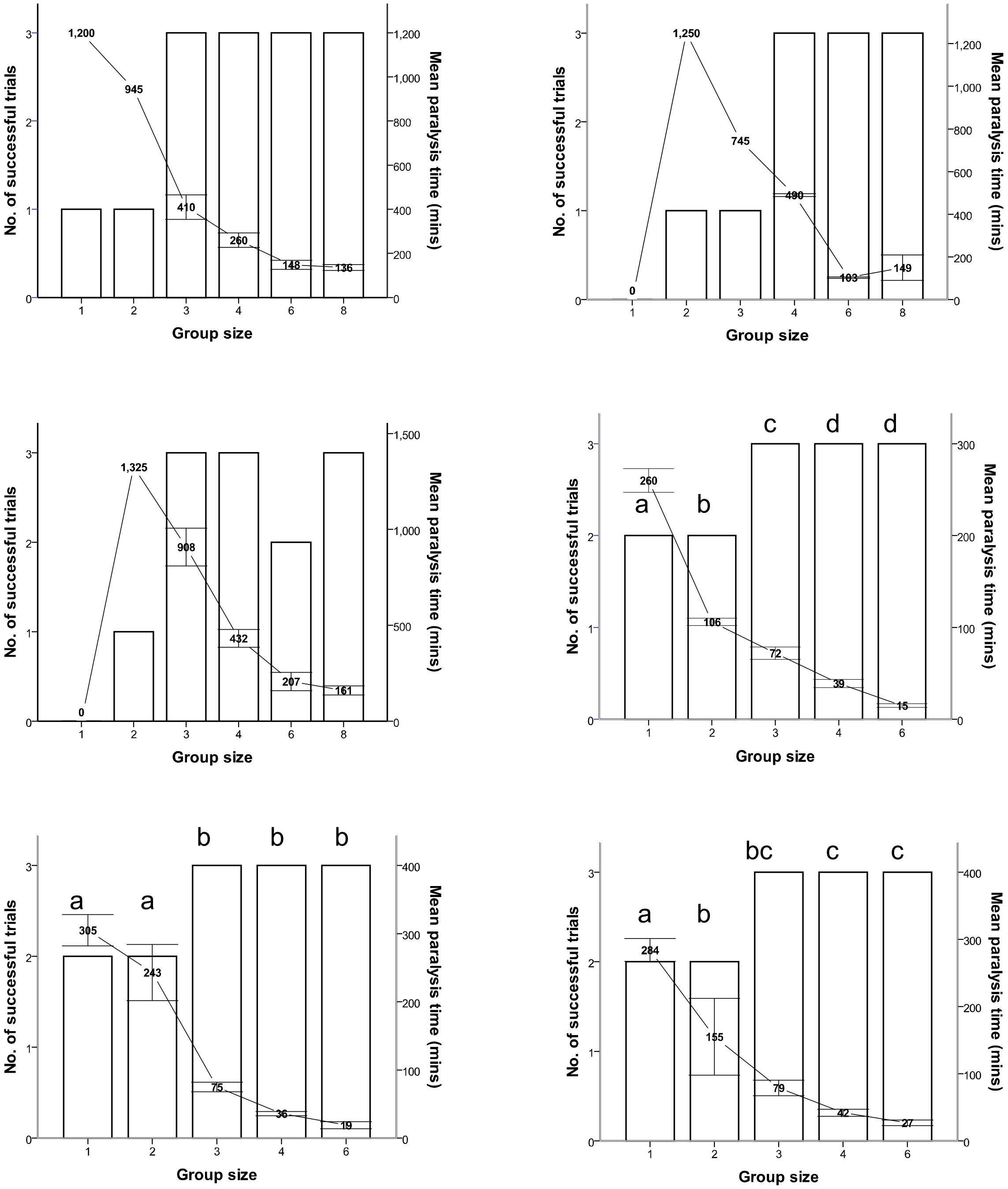

Fig. 4-5. Number of successful trials on the prey (bars) and mean (± SE) time (mins) (line) taken to paralyse house fly by (A) 3-week old *Scytodes* sp. 1 spiderlings (*n* = 3); (B) 3-week old Singapore *S. pallida* spiderlings (*n* = 3); (C) 3-week old Malaysia *S. pallida* spiderlings (*n* = 3); and cricket by (D) 5-week old *Scytodes* sp. 1 spiderlings (*n* = 3); (E) 5-week old Singapore *S. pallida* spiderlings (*n* = 3); (F) 5-week old Malaysia *S. pallida* spiderlings (*n* = 3). Different lower cases indicate significant differences.

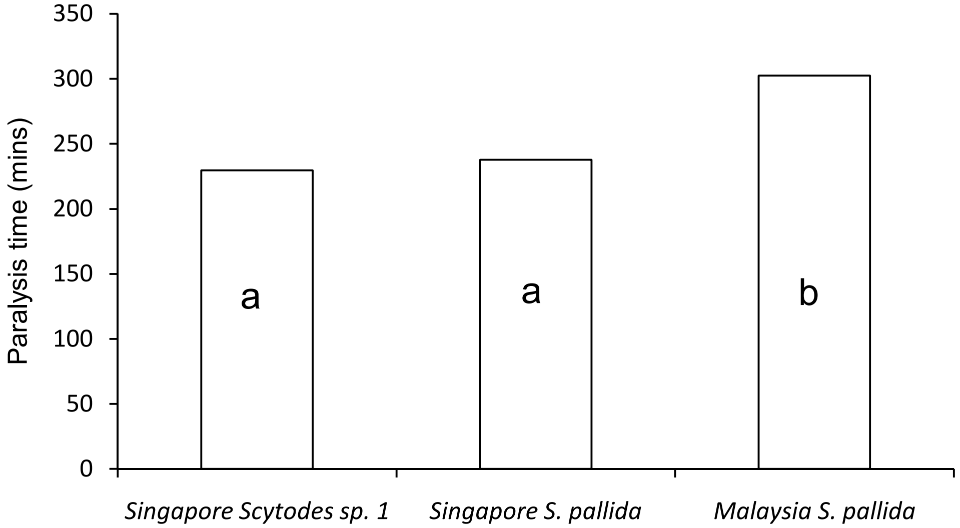

Fig. 4-6. Time taken to paralyze prey (house fly and cricket are pooled) by spiderlings of *Scytodes* sp. 1 (*N* = 26), Singapore *S. pallida* (*N* = 24) and Malaysia *S. pallida* (*N* = 25).

Cannibalism among siblings

Cannibalism among siblings started at the third instars in Malaysia *S. pallida*, Singapore *S. pallida*, and *Scytodes* sp. 1, but cannibalism occurred from the second instars in other *Scytodes* and all *Dictis* species (Table 4-7).

Table 4-7. Total cumulative mortality resulting from cannibalism among siblings (three groups, 10 spiderlings per group except for Hanoi *Dictis* sp. 5 in which there were 8 spiderlings per group).

	Week 2 (7-13d)	Week 3 (14-20d)	Week 4 (21-27d)	Week 5 (28-34d)	Week 6 (35-41d)	Week 7 (42-48d)	Incidental mortality*
Scytodes sp.1	0	1	4	10	16	20	3
Malaysia *Scytodes pallida*	0	0	3	9	12	17	6
Singapore *S. pallida*	0	0	5	10	15	19	5
Vietnam *Scytodes* sp. 4	4	8	13	19	21	23	5
Khao Chong *Scytodes* sp. 5	2	7	12	14	18	22	5
Cat Tien *Scytodes* sp. 6	1	6	11	16	18	20	8
Thailand *Dictis* sp. 1	2	5	10	13	19	23	4
Hainan *Dictis* sp. 2	3	8	12	16	21	24	5
Singapore *Dictis* sp. 2	2	7	13	17	21	24	2
Singapore *Dictis* sp. 3	1	4	9	13	15	19	9
Singapore *Dictis* sp. 4	3	8	10	15	16	20	6
Hanoi *Dictis sp. 5*	2	6	10	12	14	16	7
Dictis venusta	4	9	12	14	16	18	9

*Incidental mortality refers to deaths not attributed to cannibalism (e.g. from moulting).

Reproductive traits

MAVONA revealed a significant overall difference in six reproductive traits among 11 species of scytodids (Wilks' λ = 0.064, $F_{72, 229}$ = 2.096, $P < 0.001$; Table 4-8). One-way ANOVAs revealed significant differences among species in all reproductive traits except for the interval between clutches (Table 4-8; Fig. 4-7F). Singapore *Dictis* sp. 4 produced significantly more spiderlings than the other species ($F_{12, 46}$ = 3.397, P = 0.001; Fig.4-7A). Singapore *S. pallida* females produced significantly more spiderlings than Malaysia *S. pallida* (Fig. 4-7A). The interval between hatching and next egg sac production was longer for Singapore *S. pallida* compared to Malaysia *S. pallida* (Fig. 4-7E). Singapore *Dictis* sp. 2 females had a longer hatching time than Hainan *Dictis* sp. 2 females (Fig. 4-7D).

Table 4-8. Results from MAVONA comparing six reproductive traits among 11 aerial vegetation scytodid species.

Factor	*F*	*df*	*P*-value
Total number of spiderlings	3.397	12, 46	0.001
Egg hatching time (d)	3.280	12, 46	0.002
Interval (d) between successive clutches	1.653	12, 46	0.110
Interval (d) between hatching and next egg-sac production	3.306	12, 46	0.002
Number of clutches (or egg-sacs)	2.082	12, 46	0.038
Number of spiderlings per clutch	2.208	12, 46	0.027

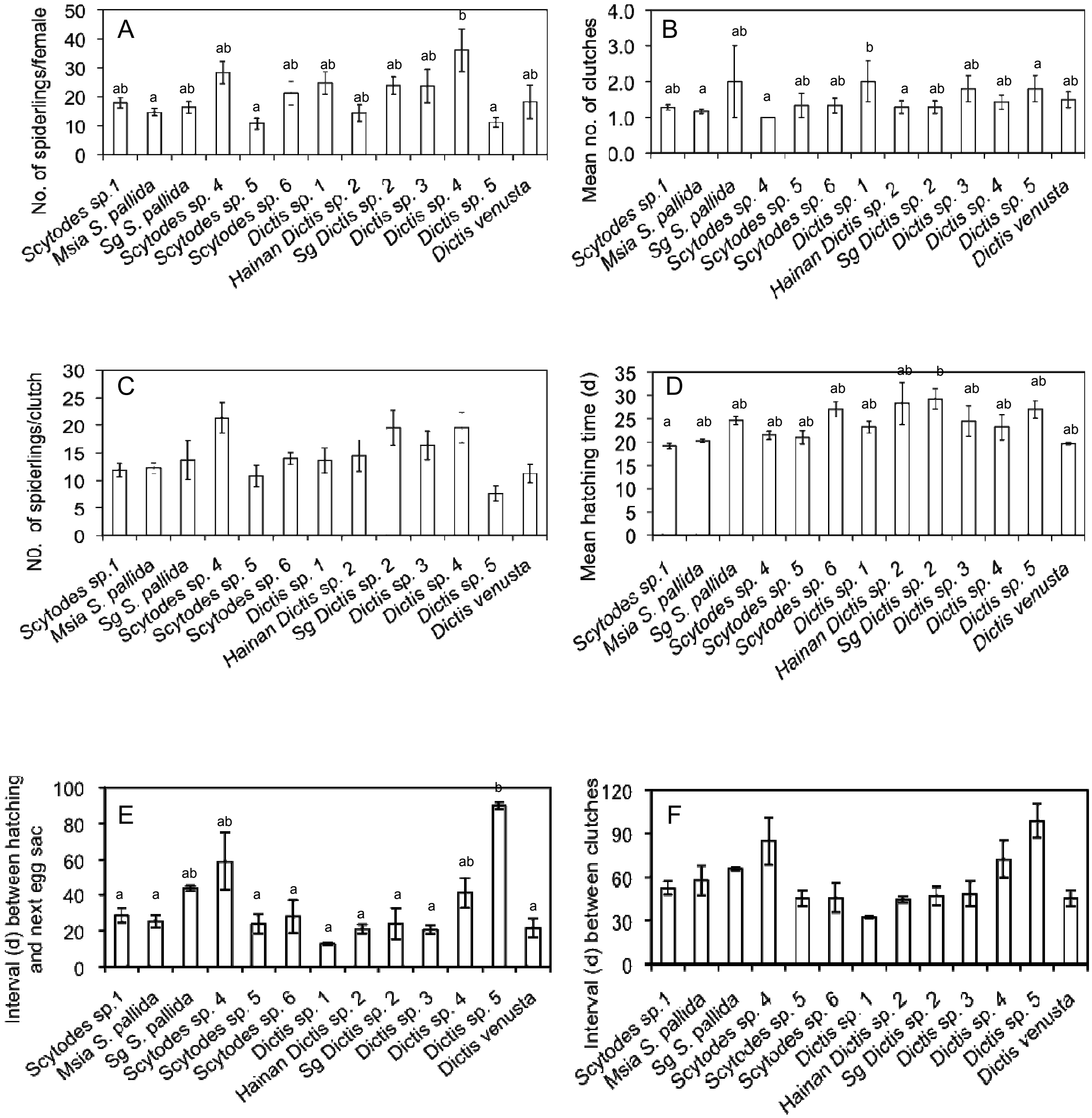

Fig. 4-7. Reproductive traits of 11 species of scytodids living in aerial vegetation habitats. (A) Mean (± SE) total number of spiderlings per female; (B) mean (± SE) number of clutches (i.e. egg-sacs); (C) mean (± SE) number of spiderlings per clutch (i.e. egg-sac); (D) mean (± SE) egg hatching time (d); (E) mean (± SE) interval (d) between hatching and next egg-sac production; and (F) mean (± SE) interval (d) between clutches. Msia = Malaysia; Sg = Singapore Different lower cases indicate significant differences.

DISCUSSION

Although sociality is rare in spiders, 25 social and 18 subsocial species are widely distributed across 18 genera in 10 distinct families (Avilés 1997; Whitehouse and Lubin 2005; Agnarsson et al. 2006; Lubin and Bilde 2007). In Chapter 3, two subsocial species from the genus *Scytodes* have been added to the list. This study shows that although all the 11 species of scytodids display maternal care to a certain extent, only two species possess the traits that would define them as having attained the level of subsociality. Thus, I have documented two additional subsocial species in the genus *Scytodes, Scytodes* sp. 1 and *S. pallida* from Malaysia and Singapore. This is not surprising given five known communal and subsocial species in this genus all live in aerial vegetation habitats (Eberhard 1986; Bowden and Jackson 1988; Li et al. 1999; Li and Kuan 2006; Miller 2006; Yap and Li 2009). These two *Scytodes* species exhibit key traits, including the formation of colonies that consist of a mother and her later instar offspring, extended maternal care, including food provisioning to their young and delayed dispersal of offspring, and cooperative prey-capture and food sharing. Colonies of *Scytodes* sp. 1, Singapore *S. pallida* and Malaysia *S. pallida* are comprising of adult females with second instar spiderlings, and sometimes even with later instars, implying that some spiderlings remain in the maternal web for longer periods. The field observations are corroborated by laboratory observations and experiments. It is evident that members of the brood show tolerance for one another for a brief period in the maternal nest because in nature, I found several colonies made up of spiderlings only (maternal female absent), and in the laboratory I observed siblings participating in cooperative prey capture and prey sharing. In the other three species of *Scytodes* (Vietnam *Scytodes* sp. 4, Khao Chong *Scytodes* sp. 5, Cat Tien *Scytodes* sp. 6) and all six species of *Dictis,* however, maternal care ceases upon the emergence of the spiderlings from the egg-sac. Colony surveys indicate that the spiderlings disperse early from the maternal web, as I found many webs with a single second or third instar spiderling. This trend is further supported with laboratory observations. Spiderlings in these nine species, if kept together in a cage and prevented from dispersing, display high rates of cannibalism. Therefore, all six species of *Dictis* and Cat Tien *Scytodes* sp. 6 can be considered as solitary species. However, as Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 exhibited both solitary and subsocial traits, they can be considered as “transiting into subsocial state” species.

The aerial vegetation supports the development of sociality in spiders because it is a productive habitat with no shortage of flying insect prey all year round (Robinson and Robinson 1973; Lubin 1978). Peaks of insect abundance can be roughly correlated with certain flowering, fruiting, or leaf-flush peaks (Fogden 1972; Smythe 1974b; Buskirk and Buskirk 1976). Most species of social spiders can be found in the tropical regions of the world where insect size and density is highest. Lubin (1978) found that scytodids in the moist tropical habitats of Panama and New Guinea had population peaks corresponding to food supply implying that prey availability is a limiting factor to scytodid diversity. With numerous small and medium-sized flying insects correlated to floristic structures, I predicted more social scytodids can be found in the aerial vegetative zone. Indeed, all the social *Scytodes* documented to date are found in the aerial leaf zones (e.g. the subsocial species of *S. socialis* (Miller 2006), *S. intricata* (Eberhard 1986) and Los Baños *Scytodes* sp. (Li et al. 1999) and the periodic-social territorial *S. fusca* (Bowden 1991)).

By building a communal web, it is thought that the spiders approximately maximize total biomass capture per spider (Yip et al. 2008). Having a larger web and multiple spiders to work together to subdue prey allows them to prey on larger organisms that would be impossible if they led a solitary existence. *Scytodes* construct a rather dense, irregular-mesh, vertical web under leaves, and the spider rests under a leaf or on the surface of the web. The web is not sticky, and may function more to alert this agile spider to the presence of an insect on its surface. Thus, although the webs of scytodids do not serve as a snare web typical of many social spiders such as *Anelosimus* and *Stegodyphus*, it can still serve as a mode of communication among members of scytodid colonies because the vibratory cues elicited by a prey item can signal multiple nest-mates to cooperatively spit at the prey to subdue it, especially if the prey is a large one.

Maternal care of eggs

Maternal care of egg-sacs in the 11 species living in the aerial leaf zone was similar to those of cave (except in the case of *S. magna* and Guangxi *Scyloxes* sp. 1) (Chapter 2) and ground-dwelling scytodids (Chapter 3): females carried their egg-sacs in the chelicerae and also assisted in the emergence of the spiderlings by making an opening in the silken egg-sac. Carrying egg-sac in the chelicerae has also been documented in other *Scytodes* (Bristowe 1958; Eberhard 1986; Bowden and Jackson 1988; Li et al 1999; Miller 2006), but the maternal female aiding the young in the emergence from the egg-sac is the first report in any scytodid species.

Several functions of maternal care in spiders including spitting spiders have been suggested, including protection of egg-sacs against predation and/or parasites (Lubin 1974; Fink 1986; Li et al. 1999), regulation of climatic factors for sac contents (Norgaard 1956; Humphreys 1974), providing food for spiderlings (Kullmann 1972; Burskirk 1981), alerting young to the presence of danger or food (Norgaard 1956), and facilitating emergence of spiderlings from sacs (Eason 1964; Randall 1977). Maternal care of eggs evolved in these 11 species of scytodids because females can guard their egg-sacs and spiderlings against predators and inclement conditions, thus improving egg survival. For example, by carrying egg-sacs in their chelicerae, females of *Scytodes* and *Dictis* practice close egg attendance (Li et al. 1999). In the laboratory, unattended egg-sacs suffered from frequent mould infestation, dessication and mite attacks (Li et al. 1999; Yap unpublished data; Tang unpublished data). In the field, unattended egg-sacs are prone to ant and salticid predation (Li et al. 1999). Females may aid newly-hatched spiderlings to emerge from their egg-sacs. Several hours before spiderling emergence, the females of *Rugathodes aurantius* and *Theridion frondeum* (Theridiidae) repeatedly and vigorously bite at the egg-sac, pulling and stretching the silk to create a hole through which instar II spiderlings soon began to emerge (Stiles and Coyle 2001). Indeed, the opening of egg-sacs appears to be an important component of maternal care in *Scytodes* sp. 1 since spiderlings of only 20% of ‘partially’ guarded egg-sacs (i.e. egg-sacs separated from the females after 14 days) were capable of emerging from egg-sacs (L-M. Y. L. Yap unpublished data). Unguarded spiderlings failed to emerge from 74% of the egg-sacs, and subsequently died within the sacs. Opening the sac is also an important component of maternal care in *Peucetia viridians* (Willey and Adler 1989). Although females of the cave *Scytodes* – *S. fusca*, *S. cavernarum* and Philippines *Scytodes* sp. 2– assist their spiderlings in the emergence, my laboratory experiments have shown that spiderlings of these three species are capable of emerging even without the assistance of their mother. Spiderlings of the 11 species of scytodids that live in the aerial leaf zone, however, are dependent on their mother to make an opening in the egg-sac in order for them to emerge.

Extended maternal care

My results show that out of 11 species of leaf-dwelling spitting spiders, only *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida* exhibit extended maternal care by providing their offspring with food. Providing offspring with food is one of the most important features of extended maternal care, in that it spares the young the many risks related to food supply

(Wilson 1971). The benefits of receiving nutritional maternal care is one of the key factors in the evolution of sociality, because it leads to the retention of young in the maternal nest (i.e. delayed juvenile dispersal). Kullmann (1972) suggested that sociality should arise in clades with extended maternal care; phylogenies of two genera, *Anelosimus* and *Stegodyphus*, confirm the origin of sociality within clades with maternal care (Agnarsson 2006; Agnarsson et al. 2006; Johannesen et al. 2007), thus supporting Kullmann's (1972) hypothesis.

By providing food, spiderlings may gain in weight and advance to a later developmental stage prior to dispersal; this makes delayed natal dispersal a key characteristic of subsociality (Kullmann 1972; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007). The better growth may directly contribute to the offspring reproductive success. In many spiders, since growth is restricted by limited food and time, a higher growth rate is expected to be positively correlated with reproductive success (Vollrath 1987). Larger females tend to produce better quality eggs while in males larger adult size and early maturation allow a higher probability of mating because of competition for virgin females.

Other than food provisioning, extended maternal care may serve another function. High predation risks and the increase in the individual female's fitness may serve as selection factors favouring extended maternal care in *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida*. In defending her offspring against potential predators and preventing them from being eaten, the female increases the survivability factor of the spiderlings which in turn increases her lifetime reproductive success. Presence of extended maternal care is thought to constitute a precursor to more complex forms of social behaviour. This temporary social behaviour, as seen in the subsocial scytodids, could be turned into permanent sociality if tolerance is extended through adulthood leading several authors to suggest a matrifilial route to the evolution of sociality in spiders (Shear 1970; Kullmann 1972). In providing food to her progeny, *Scytodes* sp. 1, Singapore *S. pallida* and Malaysia *S. pallida* maternal females help to promote tolerance and inter-attraction among their offspring by temporarily suppressing their competitiveness.

Foraging during egg attendance

It has been hypothesized that egg-guarding females of species with a high level of sociality have a lower tendency of leaving their egg-sacs to forage due to high predation risks to their egg-sacs. Maternal female foraging during the egg-sac attendance has not been reported in subsocial scytodids studied to date (e.g. *S. intricata*: Eberhard 1986; Los Baños *Scytodes* sp.: Li et al. 1999; *S. socialis*: Miller 2006), Here I also showed that *Scytodes* sp. 1, *S. pallida,* Khao Chong *Scytodes* sp. 5, and Vietnam *Scytodes* sp. 4 rarely leave their egg-sacs to forage, but this is commonly observed in the other scytodids that are solitary. As a result of high maternal investment, social spiders produce fewer offspring than their non-social counterpart, implying that every egg/spiderling is valuable to the female in contributing to her reproductive success. However, my results could not confirm this hypothesis because the number of clutches, number of spiderlings per clutch and total number of spiderlings produced by each female did not differ significantly among species (Table 4-8). In fact, solitary Hanoi *Dictis* sp. 5 and Hainan *Dictis* sp. 2 had lower fecundity yet females were observed to forage during the egg-sac attendance period. Furthermore, laboratory observations of the subsocial *Anelosimus kohi* have shown that females with egg-sacs will forage (i.e. capture house flies) (Yap, pers. comm.). Why the non-social scytodids with low fecundity take the risk of leaving aside their egg-sacs to forage when egg predators such as ants and other cursorial spiders are so common is unknown. Perhaps the silken egg-sacs are laced with chemicals that may deter ants or other predators. Further investigation into this hypothesis is necessary.

Tolerance

Why are the females of subsocial/social species tolerant of the spiderlings that remain in their nest for an extended period? Nyffeler (2000) investigated the question whether in certain species of wolf spiders, maternal females do feed (or not). He found that in both egg sac-carrying and pulli(hatchling)-carrying situations, the females engage in normal hunting activities but to a much lesser extent. As wolf spiders are highly cannibalistic (Hallander 1970; Schaefer 1974; Samu et al. 1999), it has been suggested by some authors that the predatory behaviour of adult females is inhibited during the period of maternal care to avoid the pulli (mistaken as prey) being eaten by their mother (Palmgren 1945; Engelhardt 1964). This explanation may apply to *Scytodes,* especially in *S. pallida* and *Scytodes* sp. 1 since extended maternal care occurs in these two species and females must have tolerance for her spiderlings to avoid cannibalizing them. Since, however, females are still capable of capturing prey to feed her offspring, they must be able discriminate between their own young and similar-sized insects. This implies that predation is inhibited as far as the behaviour of *Scytodes* mothers towards their spiderlings is concerned. It is evident that the maternal instincts of a female are dependent on her reproductive state. For species like *Scytodes* sp. 1 and *S. pallida*, this “instinct” goes beyond the egg-sac phase. In the other non-social species, it terminates upon the emergence of the spiderlings from the egg-sac.

However, in all the studies of scytodid behaviour (this study; *S. longipes*: Nentwig 1985: *S. fusca*: Bowden 1991; *S. intricata*: Eberhard 1986; *S. socialis*: Miller 2006; Los Baños *Scytodes* sp.: Li et al. 1999; and *S. thoracica*: Dabelow 1958), there have been no records of the maternal female cannibalizing on young in her web, implying a general inhibition in scytodid females against consuming her own spiderlings. Although they are solitary species, there is maternal care in Vietnam *Scytodes* sp. 4, Khao Chong *Scytodes* sp. 5, Cat Tien *Scytodes* sp. 6, Thailand *Dictis* sp. 1, Hainan *Dictis* sp. 2, Singapore *Dictis* sp. 3, Singapore *Dictis* sp. 4, Singapore *Dictis* sp. 5, Hanoi *Dictis* sp. 6 and *Dictis venusta*, and any tendencies for the female to cannibalize her young, and for her progeny to cannibalize each other, are suppressed during the period when the young remain with their mother.

In several genera that contain subsocial species (e.g. *Anelosimus*, *Achaearaneae* and *Stegodyphus*), there exist extended maternal care, followed in some species by a variable period of tolerance and cooperation among the orphaned spiderlings (Kullmann 1972; Buskirk 1981; D’Andrea 1987). This temporarily social behaviour could be turned into permanent sociality if tolerance is extended through adulthood (Kraus and Kraus 1988). This scenario may apply to the subsocial *Scytodes* sp. 1, Singapore *S. pallida* and Malaysia *S. pallida*, and even in the other subsocial scytodids both from this and other studies (Eberhard 1986; Li et al. 1999; Li and Kuan 2006; Miller 2006; Yap and Li 2009; Chapter 3). During the colony surveys I found several nests of these two species/populations that contained either the mother and spiderlings of early to intermediate instar or several early to intermediate instars without the mother. Laboratory experiments determined that individuals of these species exhibited a degree of tolerance towards their brood-mates and the extent of cooperation extended to 5-week old spiderlings participating in group foraging.

The level of tolerance in scytodids varies among species. In *S. socialis*, multiple juveniles and adults of both sexes coexist cooperatively (Miller 2006). Young of *Scytodes* sp. 1, *S. rubra*, *S. gooldi*, Singapore *S. pallida* and Malaysia *S. pallida* interact in group foraging communal feeding during the first two to four post-emergence, in the presence of the maternal female. Although *S. intricata* colonies of mother-offpsring associations were common in the field, agonistic interactions within groups of siblings were especially common (Eberhard 1986).

Neotenic retention of juvenile tolerance has been assumed to be the first step toward communal behavior (Kullmann 1968; Buskirk 1981). In many of the social species such as *Agelena consociata*, *Stegodyphus dumicola*, *Tapinillus* sp. 1 and *Anelosimus eximius* direct aggression is generally lacking (Lubin and Bilde 2007). For a socially living individual the cost factor may be most important: attacking will provoke defensive counter-aggression, and the full risk of being severely damaged would fall upon the attacking individual, while costs arising from tolerance would be shared among all community members (Seibt and Wickler 1988). Perhaps this is the reason why high levels of tolerance among nest-mates have evolved in social spiders.

Natal dispersal

Natal dispersal in the subsocial spiders has been linked to loss of cohesiveness (Kullmann 1968; Buskirk 1981). Three major ultimate factors are known to be responsible for natal dispersal in animals: resource competition, mate competition and inbreeding avoidance (reviewed by Powers and Avilés 2003). These factors affect dispersal not only because of their direct fitness consequences, but also because of indirect ones, that is, when there are benefits (kin altruism) or costs (kin competition) to relatives within a group. When relatives compete for the same resources (kin competition), dispersal rates are predicted to be high (e.g. Gandon 1999; Kisdi 2004; Bach et al. 2006). Elevated dispersal rates may contribute to a reduction in the incidence of inbreeding (Gandon 1999; Perrin and Mazalov 1999; Bilde et al. 2005).

In a study conducted by Li and Kuan (2006) on the natal dispersal and breeding dispersal of a subsocial scytodid spider, they proposed that dispersal from the natal nest is most likely driven by resource competition because spiders of both sexes dispersed primarily as juveniles. However, they do not rule out that the mate competition hypothesis may also play a role in explaining a certain phase of the natal dispersal. *Scytodes* females being the exclusive care-givers to their young suggests that males may be limited by the number of females they can fertilize. Males, rather than females, may thus be competing for mates. Their results showed that more males than females tended to relocate their nests, thus supporting the mate competition hypothesis. Although more than 50% of *Scytodes* sp. 1 spiderlings dispersed on day 27, there are many early dispersers that dispersed on day 8. Li and Kuan (2006) found short-distance dispersals in their study of the subsocial *Scytodes*. They suggested that the individuals could be simply moving to the first available site. Short-distance dispersals have also been documented in other subsocial spiders such as *Stegodyphus lineatus* (Johannesen and Lubin 2001) and *Anelosimus cf. jucundus* (Powers and Avilés 2003). The same argument I used on early dispersal of *S. fusca* and the Philippines *Scytodes* sp. 2 may also apply to *Scytodes* sp. 1 in that the early dispersers of *Scytodes* sp. 1 behave as such to avoid competition for suitable nest sites. During our colony survey of this species we found that individuals and colonies could be located with little effort (i.e. found close to each other along a line transect), an implication that 1) individuals disperse more or less in a straight line to the first available site, and 2) this species is abundant and thus to avoid competition from individuals dispersing from numerous neighbouring nests within their local area, some spiderlings choose to disperse early.

Other than *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida*, I found the natal dispersal patterns exhibited by the other *Scytodes* species and *Dictis* species skewed towards an early dispersal. For the solitary spiders, their lives together in the maternal nest are short-lived and they become cannibalistic very early in their ontogeny. Resource competition is likely to be the driving force spurring dispersal from the natal nest, and spiders with predominantly solitary lifestyles consume conspecifics possibly as a means of removing a competitor, as well as to gain nutrition since they do not receive any maternal nutrition like that of the progeny of their subsocial counterparts. Extensive evidence suggests that spiders frequently are food limited, i.e., a relative shortage of prey limits their growth, development, reproduction, and/or survival (Lubin and Henschel 1996; Halaj and Wise 2002), thus it is reasonable to hypothesize that spider cannibalism has evolved primarily as a means of foraging for nutrients in limited supply (Wise 2006), in turn, as a means of avoiding being preyed upon by nest-mates solitary scytodid spiderlings exhibit patterns of early dispersal from the natal nest.

However, increased agonistic behaviour need not be related to food availability, as shown in *Geolycosa turricola*. The cannibalism data presented by Miller (1989) suggest that in *G. turricola* food limitation alone does not lead to a waning of mutual tolerance. Simple size differences which may be a result of normal variation in individual growth rates can increase aggressiveness among nest mates. To determine whether cannibalism is food-dependent, an experiment was performed on *Scytodes* sp. 1 in which two treatments were set up: high-food and low-food treatment (see Yap and Li 2009). Their results show that the timing and the rate of cannibalism among siblings are no different when prey level is high as when prey level is low – a trend similar to *G. turricola*.

Asynchronous dispersal, which continued for as short as 4 days in the Thailand *Dictis* sp. 1 to as long as 56 days in *Scytodes* sp. 1, is another dispersal pattern in scytodids. Asynchronous dispersal has also been reported in the subsocial spiders *Amaurobius ferox* (Kim 2000), *Theridion pictum* (Ruttan 1990), *Coelotes terrestris* (Krafft et al. 1986) and *Stegodyphus lineatus* (Schneider 1995). This asynchronous dispersal may be related with spiderling size. Dispersing individuals showed variance in developmental instar (second or third) and weight: the last dispersing individuals were 2-3 times heavier than the first individuals of the same clutch in all the 11 species of scytodids except *Dictis venusta* and Vietnam *Scytodes* sp. 4. This inverse relationship may be attributed to dispersal behaviour being pre-programmed to a restricted time for all individuals (Plateaux-Quénu et al. 1997). This variability in dispersal time may allow certain individuals to remain in the maternal nest much longer than others. Late dispersers can thus take advantage of this situation and gain more body mass before they disperse. In Vietnam *Scytodes* sp. 4 and *Dictis venusta* in particular, it could be that the spiderlings that remained a few more days in the maternal web were still morphologically immatured and incapable of capturing fruit flies thus as their yolk reserve depleted their body mass also decreased. Spiderlings of these two species dispersed over a period of 8 d which may be too short a time to note any difference in weight between the first and last dispersers.

The defining characteristic of a subsocial species is the termination of its group-living at some point of time, dispersal from the natal nest then follows this termination. Predatory behaviour begins as a cooperative activity in the maternal web but increasing mutual aggressiveness from the mid-second instar stage and increase in the need for space may pressure the individuals to leave before the fourth instar. My results show that in *Scytodes* sp. 1 and *S. pallida*, the group-living phase, which comprised of mother-offspring and sibling associations, lasted for one to five weeks and even up to eight weeks for some groups of *Scytodes* sp. 1. There would always be two or three juveniles remaining at the end of the dispersal phase, one of which will most likely take over the maternal nest. A possible scenario for the evolution of sociality can arise from this: in these subsocial species with extended maternal care, these two or three juveniles may inherit the maternal nest and breed together. Male dispersal at this stage will maintain outbreeding. Care of the young is limited to one's own offspring in the early development stages. Two possible situations could arise: the females' brothers will disperse, while male immigrants will move in with these female such that an outbred system is maintained or due to dispersal costs, the brothers of these females choose to remain in the maternal nest and inseminate their sisters, resulting in an inbreeding mating system.

Cooperative prey-capture and food sharing

Despite its potential relative costs (e.g. presence of free-loaders and cheaters in a group) to the individual, cooperation is an evolutionary outcome that is beneficial to the members of a group (Dugatkin 1997). Benefits from cooperative predation include the capture of larger prey that a single spiderling is unable to capture or that poses greater danger if seized alone. When presented with a cricket, a single spiderling would be attacked if it attempted to capture the cricket single-handedly. I have demonstrated in the subsocial scytodids that the time required by a sibling group to seize the prey decreased with group size. As group size increased, the probability of successfully capturing a large prey also increased, but capture probability did not increase linearly with group size. Rather, doubling the group size from 2 to 4 individuals greatly improved predation success, whereas doubling the group size from 4 to 8 did not have a measurable effect on the ability of siblings to capture prey. In the group of 6 and 8 spiders, the number of captors never exceed five, suggesting that groups of five may be the optimal group size. This could be the result of interacting effects of cooperative attack on one hand and mutual avoidance of group-mates on the other hand (Pekár et al. 2005). At higher densities, spiders would spend more time in agonistic interations which kept them from attacking. This suggests that the larger the prey, the fewer interactions there would be presumably because spiders did not come into contact with one another so frequently. Reduction of the time to paralysis with increasing number of spiders in a group is another benefit for group hunting. It is evidence of cooperation among *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida*. This was particularly apparent when the larger prey were used.

There are population and interspecific variations in efficiency of cooperative prey-capture. Malaysia *S. pallida* spiderlings took a longer time to capture the house flies and crickets when compared to Singapore *S. pallida* and Singapore *Scytodes* sp. 1.

During my community structure survey, a total of 99 Malaysia *S. pallida* nests were found. Nests were very spread out and were observed to decrease in density with increasing distance from the disturbed areas of the forest (personal observations). More importantly, I did not find any colonies comprising of mother and mix of second and later instars association whereas such colonies were seen in Singapore *S. pallida* and Singapore *Scytodes* sp. 1. In addition, 'second instar alone' colonies made up 16% in Malaysia *S. pallida*, but in Singapore *S. pallida* and *Scytodes* sp. 1, it was 5% and 4% respectively (see Table 4-2). These observations may imply that group cohesion in Malaysia *S. pallida* is not as strong as *Scytodes* sp. 1 and Singapore *S.*

pallida, hence spiderlings when kept together in laboratory conditions were not able to coordinate joint prey capture as well as *Scytodes* sp. 1 and Singapore *S. pallida* sibling groups. The spiders' cooperation might be a consequence of group living (see Kim et al. 2005). However, the benefits obtained from cooperation would reinforce mutual tolerance and group cohesion among the members, which are initiated by maternal care, and extended to the post-maternal social period. For the solitary scytodids and even other solitary spiders, the costs of group living may be high (i.e. individuals of the brood face high predatory pressures) and so members do not remain together and reap the benefits of group foraging. Kim et al (2005) emphasize that the developmental homogeneity of siblings established during the maternal social period probably facilitates cooperation. The maternal food care of *Amaurobius ferox* (i.e., trophic egg-laying and matriphagy) provides enough food simultaneously to every spiderling in most of the clutches (Kim and Horel 1998; Kim and Roland 2000). Schneider (1995) observed that when size differences between group members in the subsocial spider *Stegodyphus lineatus* were small, prey were more likely to be shared among the young. In solitary scytodids, food provisioning by maternal female is absent, thus there would not be any developmental homogeneity among these spiderlings.

My study was not designed to measure the feeding efficiency and growth rates of *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida*, but in a separate study conducted on Malaysia *S. pallida* third instars, no significant difference in the prey capture rates between sib and non-sib groups was found (Tang unpublished data). Group foraging which usually involved three juveniles, and communal feeding involving both captors and non-captors, were observed in both treatments with medium-sized prey (e.g. crickets). It is not certain whether the non-captors contributed by injecting digestive enzymes into the prey or not, thus leading to the suggestion that relatedness may not play a significant role in cooperation. Perhaps in the aforementioned experiment, the group size is too small for the effects of relatedness to be apparent, as the largest foraging groups comprise of five spiders. In this study, I found the maximum group-capture size to be five juveniles for all three species. If, in the previous Malaysia *S. pallida* experiment, the number of juveniles was increased from five to ten, there is a likelihood that more cheaters would emerge and as a result the negative effects of feeding competitions would be more obvious.

My results indicate that spiders gain advantage when hunting in groups, but conflict also arise within the groups. Struggles sometimes occurred with spiders pulling the prey item in different directions, probabaly in an attempt to monopolize the prey. The captors had the privilege of freely orienting themselves to all parts of the subdued prey's body, whereas the intruders could only exploit other less suitable parts and fed for a shorter time. In many other spider species (e.g. *Zodarion cyrenaicum*), the preferred feeding place would be the thorax, however, my results showed that the spiders usually fed on the head first before proceeding to the thorax. The head may contain resources which are more valuable than their weight suggests. Also as there are connections between the body sections, it may be possible for the spiders to extract thorax material while feeding on the head (Ward and Enders 1985).

Reproduction

Solitary spiders are known to produce large number of eggs/spiderlings throughout their reproductive lifetime to compensate the presumably low survival rates of their young (Kullmann 1972; Stern and Kullmann 1975; Smith 1982) as a result of low investments in their offspring. Yet my results show that evidently solitary species such as Hanoi *Dictis* sp. 5 and Hainan *Dictis* sp. 2 have lower fecundity (Fig. 4-7A) than subsocial scytodids. Dispersal from the maternal nest is also rather early for the spiderlings of these two species: ≥50% Hainan *Dictis* sp. 2 spiderlings disperse by day 20 and ≥50% Hanoi *Dictis* sp. 5 spiderlings disperse by day 15. Can we assume that with no food provisioning from the mother and having early dispersal patterns resulting in these spiderlings having a low chance of survival after dispersal, coupled with the females' low fertility rates these species are on the road to extinction? I believe not because my colony surveys have hinted that these spiders are not rare in the field. I suggest two hypotheses for this: 1) the spiderlings may have developed survival strategies such as camouflaging with the environment (e.g. since they are brown-coloured they will choose a brown leaf as their resting/nesting site); 2) more elaborate maternal care may be evolving in these species. The data for Khao Chong *Scytodes* sp. 5 led me to suggest the second hypothesis. This species not only has the lowest fecundity (Fig. 4-7A) throughout the female's life, but also displays the earliest dispersal pattern with the first dispersal occurring on day 4. Perhaps to compensate for this, Khao Chong *Scytodes* sp. 5 females rarely or never forage during egg-sac guarding, just like their subsocial counterparts Singapore *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida*, and allow the newly-emerged spiderlings to mount their body, all these to increase the protection levels against predators. Therefore, my data is inconsistent with the prediction that egg production of subsocial species is lower than that of the solitary species (Fig. 4-7).

When I compared the fecundity rates across all 21 scytodids (Chapters 2, 3 & 4), I also found my data not to follow the conventional view that social species are less fecund than their non-social relatives (Riechert 1985; Vollrath 1986; Wickler and Seibt 1993). For example, *S. rubra*, a subsocial species, has one of the highest fecundity, while *S. cavernarum*, a solitary species, has the lowest fecundity. By examining fecundity/fertility of the scytodids, I had wanted to use this as one of the characters in the mapping of the social traits in order to study the evolution of social behaviour. However, it seems that reproductive traits are not good traits for defining sociality in scytodids as seen in other social spiders such as *Stegodyphus* (Kullmann and Zimmerman 1974), *Agelena* (Darchen 1967; Krafft 1970) and *Achearanea* (Kaston 1948; Darchen 1968).

Singapore *S. pallida* females are more fecund than Malaysia *S. pallida*, yet the interval between hatching and next egg-sac production is also longer in Singapore *S. pallida* than in Malaysia *S. pallida.* Again, this also challenges the hypothesis that fewer offspring would mean more parental investment (in this case, longer interval between hatching and next egg- sac due to more time and effort in taking care of progeny). Therefore, these results reinforce the fact that reproductive traits are not useful for defining sociality in scytodids.

Population variation in social behaviour

The level of sociality often varies between species (interspecies), but it also varies within a species (intraspecies). Intraspecific variation is generally habitat dependant, where some populations within a species show all the characteristics of quasisociality, yet a population a mile away may be largely solitary because they inhabit a different environment (Lubin and Robinson 1982). In the colony survey, I found 21.7% of the Singapore *S. pallida* colonies to be social whereas in the case of Malaysia *S. pallida*, only 11.1% of the colonies were social. Malaysia *S. pallida* spiderlings also took a longer time to capture the house flies and crickets when compared to Singapore *S. pallida*, a reflection that perhaps group cohesion is not as strong in the Malaysian species. There were some differences in the reproductive traits. Singapore *S. pallida* females produced significantly more spiderlings than Malaysia *S. pallida* and the interval between hatching and next egg-sac production was longer for Singapore *S. pallida* compared to Malaysia *S. pallida*. However, as discussed earlier reproductive traits are not good indicators of the level of sociality in scytodids. It may be that Malaysia *S. pallida* is “less social” than Singapore *S. pallida*.

A correlation between greater levels of sociality and lower altitude or latitude has been noted both within and among species for a variety of other social organisms, including cooperative breeding birds (Brown 1987), wasps (Reeve 1991), bees (Packer 1990; Eickwort et al. 1996; Richards 2000; Cronin and Schwarz 2001), thrips (Kranz et al. 2002), and the *Anelosimus* spider (Avilés et al. 2007). However, the difference in elevation and latitude between Singapore *S. pallida* and Malaysia *S. pallida* is insignificant and so cannot be used to explain the population variation in sociality. The differences in the architecture of the vegetation substrates between the two populations may account for the less social Malaysia *S. pallida.* While the solitary Singapore *S. pallida* individuals were found mostly under fronds of the *Tectaria* ferns, many of the gravid females, females with egg-sacs and females with second instars were sampled under large fronds of the *Licuala* (*L. ferruginea*) palm in MacRitchie catchment area, in which there were steady streams of tiny insects between the pleats of the fronds (personal observations). *Licuala* palms were also seen in the Gombak Field Study Centre where Malaysia *S. pallida* were found, but they were of a different species i.e. *L. spinosa* in which fronds were smaller and less wide. The colonies comprising of mother-offspring and sibling associations did not occur in these fronds. The vegetation in the MacRitchie catchment area may facilitate the development of cooperative scytodid society better.

Females that exhibit extended care may mitigate some of the costs of competition for resources and conflict among siblings, while increasing inclusive fitness benefits (West-Eberhard 1975; Seibt and Wickler 1987, 1988a, b; Henschel et al. 1995; Lubin and Bilde 2007). Opposing selective forces act on group living in subsocial scytodid spiders: the female's opportunities of producing more clutches and re-mating decreases with investment made on her progeny whereas survival of the young increases with group living due to protection and food provisioning by the mother. Ecological factors and constraints may explain in part why there is group-living in *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida* but not in other species living in similar habitats. Another part may be explained in terms of decision theory.

I have shown that when spiderlings work in a group, they are capable of capturing larger prey such as crickets. *Scytodes socialis* are able to capture prey twice their size (e.g. roaches and moths) when they hunt in a group (Miller 2006). However, a singleton is also capable of capturing albeit one that is smaller in size such as fruit flies. Although other ecological parameters such as the presence of large prey items play a role, predation pressure may be the pivotal reason for the evolution of extended maternal care. By protecting her offspring against predators and providing nutrition to her young, the growth and survival of the spiderlings are increased. As reproductive success is ultimately measured in the number of an individual's offspring surviving to maturation, for species that do not provide extended maternal care, the way of improving reproductive success is to produce a larger number of spiderlings. Females of each species differentially weigh the costs and benefits of extended maternal care hence resulting in different outcomes: behavioural decisions of species such as *Scytodes* sp. 1, Singapore *S. pallida* and Malaysia *S. pallida* are to invest more in the offspring and produce fewer clutches, while those of the other solitary scytodids are to produce more clutches with little maternal investment. The processes underlying the decision to extend the maternal care phase are still poorly understood and can only be answered by performing experiments to measure the costs and benefits of maternal care.

Ants are known to be important predators in terrestrial habitats (Hölldobler and Wilson 1990) and have been observed on numerous occasions to be within close proximity of *Scytodes* webs (Fig. 4-8A). Ants are notorious egg predators (Fink 1987) and will immediately attack unattended scytodid egg-sacs (Fig. 4-8B). This could be one of the main reasons for maternal care, i.e. carrying egg-sac in the chelicerae, to arise in scytodids living in the aerial vegetation. By closely guarding her egg-sacs, egg mortality due to ant predation will be significantly reduced. Ants can either promote or discourage group-living. For example, ants have been shown to reduce the survival of solitary allodapine bees, thus driving an increased cooperative nesting (Zammit et al. 2008). On the other hand, previous studies of social spiders suggest that ants can cause rapid reduction in colony size or colony extinction (Henschel 1998; Purcell and Avilés 2008). In response to ants I never observed *Scytodes* spiders in the field to spit at ants, even when a colony of black ants preyed on a female's eggs (Fig. 4-8B). On two separate occasions, I observed *Scytodes* sp. 1 colonies consisting of an adult female and second instars enclosed in the web with a few black ants at the periphery (Fig. 4-8C). The presence of ants may influence the success of spiders in this system but it is uncertain whether ants disfavour or facilitate extended maternal care. By allowing the spiderlings to remain longer in the maternal nest, females of *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida* offer their young protection from the ants in the web retreat. However, living in groups also renders the spiders more conspicuous to the ant predators such that it hinders the establishment of mother-offspring groups. Further experiments are needed to directly demonstrate the effects of ants on subsociality.

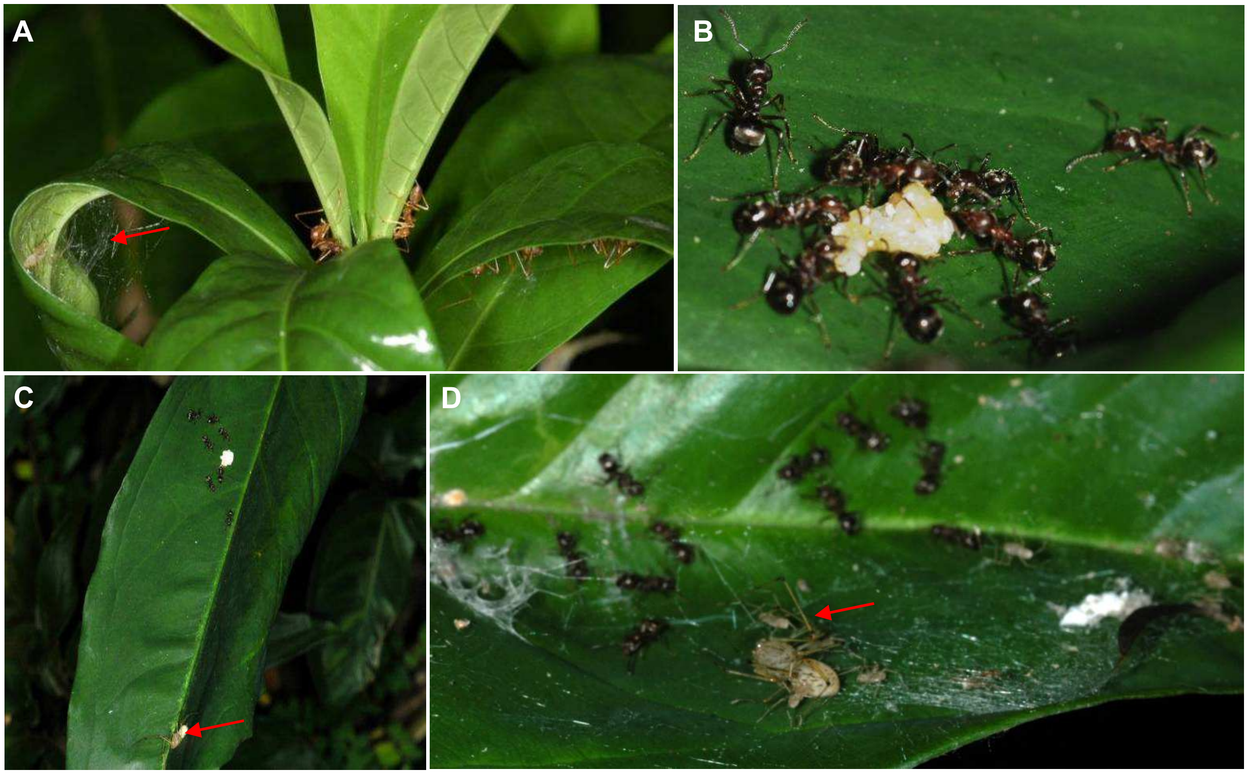

Fig. 4-8. (A) A *Scytodes* sp. 1 juvenile in a web retreat (red arrow) and a group of ants in close proximity; (B) Black ants attacking an unattended *Scytodes* sp. 1 egg-sac. The ants have torn apart the silk egg casing and are removing the individual eggs to transport back to their nest; (C) Black ants attacking egg-sac but female *Scytodes* sp. 1 stays away (red arrow) and does not defend her eggs; (D) Black ants at the periphery of the web containing maternal female and spiderlings. Note the female and a spiderling on her right (red arrow) raising foreleg probably in an attempt to chase away the ant predators

“Transition into subsocial” species

It is evident that Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 are “transiting into subsocial state” (i.e. advancing to become more social) species, exhibiting both solitary and subsocial traits. While extended maternal care is absent in these two species thus warranting them to be solitary, females of Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 rarely leave their egg-sac to forage and allow the newly-emerged spiderlings to mount their body. After dismounting, the spiderlings remain as a tight cluster in the maternal nest before dispersing. The latter three behaviours are characteristic of their subsocial counterparts.

Subsocial behaviour in invertebrates involves parental care given to offspring that have emerged from the egg-sac and which increases the survival of the offspring (Wilson 1971; Tallamy and Wood 1986). However ‘subsociality has been used as a collective term to lump all associations from ephemeral parent-offspring associations (‘‘early parental care’’) to much more complex social groups that comprise of long-lasting mother-offspring-sibling associations extending until or even beyond sexual maturity of the offspring and the death of the mother (Rayor and Taylor 2006). Going by the aforementioned definition, Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 females that allow emergents to climb onto their body can be considered subsocial since “early maternal care is being provided to offspring that have emerged from the egg-sac and which increases the survival of the offspring”. However, since I define a subsocial species as being one that exhibits *extended* maternal care, Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 cannot count as subsocial species. In agreement with Rayor and Taylor (2006) to take into account both the duration and patterns of association among mothers, offspring, and siblings, and that definitions of sociality need to be broadened to more accurately reflect the diversity of social dynamics, I propose the use of the term ‘transition into subsocial species” to describe the social organization of Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4.

Conclusions

My study shows that all the species in the *Dictis* clade seem to be asocial. It is unclear whether all *Dictis* species are genetically pre-disposed to lead a solitary life style, just as how the reverse is true for the genus *Anelosimus* – almost all known species are documented to be subsocial or social. Only with more work will the answer to this question be revealed. In addition, irregular webs that can be shared and extended maternal care are preadaptations that facilitated the evolution of permanent-social behavior in some spider lineages. In my study, I did not find any other scytodid that constructs a web complex similar to *S. socialis* nor did I find the more social scytodids building webs at a higher strata than their solitary counterparts. This implies that web construction may not be a necessary pre-requisite for the evolution of sociality in scytodids since *Scytodes* sp. 1 and *S. pallida* – both subsocial species – do not build irregular ensnaring webs for capturing prey. It has been hypothesized that a high abundance of large-sized prey in the tropics is an environmental factor that may promote sociality. This may apply to *Scytodes* sp. 1 and *S. pallida* since in my laboratory observations, introducing a large prey such as cricket stimulated cooperation among the siblings. The presence of a sufficient supply of large insects may provide the stimulus for group-living. However, this factor certainly does not act alone and it is a combination of factors (including presence of extended maternal care and/or irregular webs) that encourages the evolution of social behaviour. Furthermore, extended maternal care is absent in all *Dictis* species and it may be for this reason that *Dictis* and the other solitary *Scytodes* species are out of the sociality game. While sociality is often discussed in terms of discrete classes, it is really a continuum of behaviors involving increasing levels of maternal investment in offspring and conspecific tolerance. Subsociality and quasisociality can gradually evolve by extension of maternal care to older and older offspring, as exemplified in *S. rubra*. This is known as the "maternal care pathway" to sociality.

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CHAPTER 5

PHYLOGENETIC RELATIONSHIPS OF SPITTING SPIDER FAMILY SCYTODIDAE (ARANEAE) BASED ON DNA SEQUENCE DATA

ABSTRACT

The spitting spiders (Scytodidae) are an assemblage of five poorly defined genera – *Scytodes* Latreille 1804, *Scyloxes* Dunin 1992, *Soeuria* Saaristo 1997, *Stedocys* Ono 1995 and *Dictis* Koch 1872 - among which hypothesized relationships have yet to be studied thoroughly. Understanding these relationships is important because they will serve as a basis for testing the hypotheses concerning scytodid social evolution. In this chapter, I constructed the first molecular phylogeny of the spider family Scytodidae using sequence data from four genes and 44 ingroup taxa from a wide geographic range. Combined analysis of the nuclear 18SrDNA, 28SrDNA and Histone 3 genes and the mitochondrial COI gene did not resolve the most speciose genus *Scytodes* as monophyletic, but my results strongly support the monophyly of Scytodidae. The parsimony analysis (49 taxa) resulted in five equally most parsimonious trees, and six well-defined clades – the tentative '*Magnodes*'; *longipes*; *fusca*; *thoracica*; *pallida*; and *Dictis* groups. At least three other clades (containing *Stedocys*, putative *Scyloxes* and *Stedocys*) remain poorly resolved. Based on their placement on the phylogeny and unique morphologies, I found that scytodid taxonomy is in need of a revision and that erection of new genera may be necessary. This phylogeny may provide a framework for analyzing patterns of evolution in social behaviour of spitting spiders.

INTRODUCTION

Phylogenetic thinking is being applied to wide variety of problems in biology, from systems for classifying the diversity of life on earth to the origin and evolution of behaviour. Phylogenetics can provide a framework for reconstructing the relationships and key evolutionary events that occurred as species diversified. In this chapter, I present an analysis of phylogenetic relationships among some major groups of spiders in the family Scytodidae Blackwall 1864, providing new insights into geographic distribution pattern, and a framework for analyses of the evolution of sociality in this lineage (Chapter 6).

The Scytodidae comprises about 230 described species, with most species being found in tropics and subtropics (Platnick 2010). Scytodids are commonly known as spitting spiders as they usually expel sticky gum through the slit-like opening of their cheliceral fangs onto prey or predators from a distance when foraging or defending, a unique habit among spiders (e.g. Bristowe 1958; McAlister 1960; Gilbert and Rayor 1985; Li et al. 1999). Spitting implies that they are aggressive, yet some members of the genus *Scytodes* are known to practise various forms of sociality, which is rare in spiders (Eberhard 1986; Li et al. 1999; Li 2002; Li and Kuan 2006; Miller 2006; Yap and Li 2009). Although many *Scytodes* species are primarily solitary (Valerio 1981; Eberhard 1986), at least eight species are known to be subsocial, social or communal. *S. fusca* Walckenaer from Queensland lives in colonies (Bowden and Jackson 1988; Bowden 1991), whereas *S. intricata* Banks from Panama (Eberhard 1986) and *Scytodes* sp. from the Philippines (Li et al. 1999; Whitfield 1999), *S. gooldi* and *S. rubra* from South Africa (Chapter 3), *Scytodes* sp. 1 from Singapore (Chapter 4), *S. pallida* from Malaysia and Singapore (Chapter 4) are subsocial. *S. socialis,* which was recently studied in Madagascar, is a non-territorial multiple-adult species (Miller 2006). The variation of sociality within members of this family provides an excellent opportunity for addressing a number of important questions in spider social evolution including whether there are convergences of patterns from asocial to permanent-social via periodic-social species, the question of the number of independent origins of sociality and the causes (i.e. factors that facilitate its origin) and consequences of social evolution in this group of organisms. However, in the absence of a robust phylogenetic tree for scytodid spiders, it has been impossible to reconstruct social trajectories. Recent phylogenetic studies of two major groups of social spiders, *Anelosimus* (Agnarsson 2006; Agnarsson et al. 2007) and *Stegodyphus* (Johannesen et al. 2007), have shed some light on spider social evolution in selected groups. Here I aim to reconstruct a robust phylogenetic tree using molecular data for rigorously testing hypotheses about the evolution of scytodid sociality for the next chapter.

While Scytodidae is a relatively small family (Platnick 2010), surprisingly, no systematic revision or phylogeny of this family has been made (Dankittipakul and Singtripop 2010), and our understanding of phylogenetic relationships within the family is thus limited. Brignoli (1976) may have been the first to provide insights into the taxonomy of the family in his critical review of 46 known species with illustrations. However, he did not propose a classification for the family. Lehtinen (1986) proposed a classification of two subfamilies within Scytodidae, the Scytodinae and an unnamed subfamily consisting *Scytodes leopoldi* (currently *Stedocys leopoldi*) plus two undescribed genera. All scytodid species had been placed in one genus, *Scytodes,* until *Scyloxes* was established by Dunin in 1992 (Dunin 1992) and *Stedocys* by Ono in 1995 (Ono 1995). Saaristo (1997) subsequently revalidated *Dictis* and established the fifth genus *Soeuria*. He also argued that more genera could be introduced in Scytodidae, but again he did not formally propose any classification. In an extensive faunistic and taxonomic study of the scytodids of Thailand, Dankittipakul and Singtripop (2010) divided nine species into four groups based on female genital morphology and argued that *Scytodes* is paraphyletic and should be separated into several genera. Most recently, Lehtinen (2010) suggested the creation of a new asiatic subfamily for *Scyloxes*, *Stedocys* and two undescribed genera from Taiwan and Himalaya. He considered that the majority of the present scytodidids belong to several distinct lineages characterized by good synapomorphies and that they could be treated as new genera. Lehtinen (2010) proposed that more than 25 new genera could be erected.

Scytodidae currently contains five genera, *Dictis* Koch, 1872, *Scyloxes* Dunin, 1992, *Scytodes* Latreille, 1804, *Soeuria* Saaristo, 1997, and *Stedocys* Ono, 1995. As early as from the 1800s Koch recognized that '*Dictis'* was distinct from *Scytodes.* Many arachnologists such as Berland (1942), Schenkel (1953) and Roewer (1960) disagreed with him. However, Saaristo (1997) revalidated the genus *Dictis* L. Koch, 1872 with a species close to its type species (*Dictis striatipes* L. Koch, 1872) recorded from Coetivy, Seychelles. Koch raised the idea of another genus separate from *Scytodes* based on his observations that some "*Scytodes*" had two tarsal claws rather than the usual three claws – prolateral, retrolateral and an inferior third claw. This genus was monotypic until Dankittipakul and Singtripop (2010) documented three new *Dictis* species – *D. elongata*, *D. denticulata* and *D. thailandica* – from Thailand. It is very likely that many of the already described *Scytodes* species belong to *Dictis*, and that there are many more yet to be described, but most of them are still catalogued as *Scytodes* (Platnick 2010). Ono

(1995) established the genus *Stedocys* based on males of *Stedocys uenorum* Ono, 1995 and *Stedocys leopoldi* (Giltay 1935). So far this genus comprises only three species (Platnick 2010). Ono (1995) distinguished *Stedocys* from *Scytodes* by having a peculiar male palp atypical for the family: a tarsus smaller than the copulatory bulb, not prolonged distally, the apical implantation of the copulatory bulb, and a slender, aciculate embolus. Before the establishment of *Stedocys*, Lehtinen (1986) had already suggested that *Scytodes leopoldi* should be placed in a new subfamily and a new genus. The third species, *S. pagodas* Labarque et al. 2009, is described from Yunnan, China. *Scyloxes* and *Soeuria* are relatively unknown. Each contains only one described species (Platnick 2010). *Soeuria soeur* Saaristo 1997, described from the granitic islands of Seychelles, is distinguished by the reduced copulatory pockets which are represented by small, squamous areas (Saaristo 1997). Other than morphological details, the life history and behaviour of *Scyloxes asiatica* Dunin 1992 were not studied. The only mention of its ecology is that *S. asiatica* is found under rocks in hilly semi-desert of Tajikstan. Dunin (1992) distinguished this species from all other scytodids in having a distinct epigynum (i.e. pair of sclerotised brown marks in epigynum, and paired spermathecae) that can also be found in Mygalomorphae and Cribellatae (e.g. *Atypus*, *Calommata*, *Hypochilus*) (Kraus 1978), as well as absence of sclerotised copulatory pockets.

Many widespread and the Old World scytodid taxa have been more often than not incorrectly identified in literature and museum labels (Lehtinen 2010), resulting in much confusion. For example, many species have been wrongly identified as *Scytodes fusca* as this species has a wide range and is even synanthropic (personal observation). The same can be said of *S. thoracica*, a widely spread species common in houses around Europe. Scytodidae is thus in urgent need of taxonomic revision; a modern revision of Scytodidae and phylogenetic analysis from molecular and morphological data would be useful in limiting subfamilies and genera.

Despite much taxonomic attention to the genus *Scytodes*, generic and family-level relationships within this lineage has rarely been addressed (Brignoli 1976; Lehtinen 1986; Platnick et al. 1991; Dunin 1992; Forster 1995; Ono 1995; Ramírez 2000; Brescovit et al. 2004; Dankittipakul and Singtripop 2010). Suggested sister-taxa of Scytodidae includes Sicariidae Keyserling, 1880 (Platnick et al. 1991), Drymusidae Simon 1893 (Platnick et al. 1991; Binford et al. 2008), and Periegopidae Simon 1893 (Forster 1995; Ramírez 2000; Coddington et al. 2004). Simon (1893) placed scytodids as close relatives of sicariids, loxoscelids, drymusids, periegopids and plectreurids. Brignoli (1975; 1978) included families Scytodidae, Loxoscelidae, Sicariidae, Diguetidae, Plectreuridae, Ochyroceratidae, Tetrablemmidae, Pholcidae and Caponiidae in the superfamily Scytodoidea. Brignoli (1975) based his delimitation of Scytodoidea mainly on the similarity of cheliceral structures, especially the presence of the cheliceral lamina. He could not decide whether the latter is a synapomorphy for his Scytodoidea or a symplesiomorphy of a larger group. In 1986, Lehtinen delimited it to include Sicariidae, Loxoscelidae, Scytodidae, Drymusidae and Ochyroceratidae. In recent schemes of phylogeny of haplogyne spiders, Scytodidae is considered as the sister-taxon of the family Drymusidae. Both Scytodidae and Drymusidae form a clade, interpreted as the sister group to Sicariidae (Platnick et al. 1991). Brescovit and colleagues (2004) distinguished Drymusidae from Scytodidae by several plesiomorphic conditions, such as the slightly depressed body, the short and conical cymbium, and the lack of stridulatory pick on the palps and the corresponding ridges on the chelicerae. Ramírez (2000), considering the work of Forster (1995), proposed Periegopidae as a probable sister group to Scytodidae. A recent molecular phylogenetic work has well supported the monophyly of *Scytodes* (Binford et al. 2008). However, taxon sampling within Scytodidae in this study was limited (one *Scytodes* species from 5 populations) and probably inadequate for testing the monophyly of the whole family. Resolving the species, generic and family-level relationships of the Scytodidae is thus important because it is these levels at which scytodid spiders show behavioural variation and sufficient life history information is available for interpreting patterns of social evolution.

Here I provide the first phylogenetic analysis of the family Scytodidae based on mitochondrial DNA and nuclear DNA sequences with extensive taxon sampling. In the present study, I tested the monophyly of the Scytodidae and reconstructed the evolutionary relationships within and between the monophyletic taxa identified.

MATERIALS AND METHODS

Taxon Sampling

Scytodidae is found worldwide and *Scytodes* is the largest genus in this family, comprising of over 190 described species (Platnick 2010). Many of the morphospecies included in my dataset are still awaiting taxonomic description. The scytodid taxa from my dataset spanned a wide geographic range. My dataset comprised 49 species, including 44 ingroup species from four genera, *Scytodes*, *Scyloxes*, *Stedocys* and *Dictis*, of the family Scytodidae, and five outgroup species from two other families, Sicariidae and Drymusidae, that are considered closely related to Scytodidae, (Platnick et al. 1991; Coddington et al. 2004) (Table 5-1).

The five outgroup species included four species from Sicariidae (two species of *Sicarius* and two species of *Loxosceles*) and one species from Drymusidae (*Drymusa capensis*) collected from South America, North America and Africa. The family Sicariidae includes the genus *Loxosceles* (brown or violin spiders) (Heineken and Lowe 1832) and its sister genus, *Sicarius* (Walckenaer 1847) (Platnick et al. 1991). There are approximately 100 described species of *Loxosceles*, the majority of which are distributed in the Americas, West Indies, and Africa (Gertsch 1967; Gertsch and Ennik 1983; Newlands and Atkinson 1988). The 23 described species of *Sicarius* are currently found in Southern Africa, and Central and South America (Gerschman and Schiapelli 1979). Members of the monogeneric family Drymusidae are poorly known, but currently described from Africa, South and Central America, and the West Indies (Valerio 1971; Alayón-García 1981; Goloboff and Ramirez 1991; Platnick 2010).

Table 5-1. Taxonomic and locality information of the specimens included in the molecular analysis

		Genes			
Species	**Locality**	**18S**	**28S**	**H3**	**COI**
INGROUP					
Dictis venusta	Singapore: Kent Ridge park	552 bp	838 bp	344 bp	535 bp
Dictis sp. 1	Thailand: Phuphapet National park, Trang	N/A	801 bp	348 bp	N/A
Dictis sp. 2	Singapore: Tampines ecogreen park	N/A	N/A	255 bp	571 bp
Dictis sp. 2	China: Mount Wuzhi, Hainan	N/A	571 bp	204 bp	601 bp
Dictis sp. 3	Singapore: Bukit Timah Nature Reserve	651 bp	N/A	N/A	491 bp
Dictis sp. 4	Singapore: Changi Fairy Point chalets	N/A	N/A	N/A	400 bp
Dictis sp. 4	Singapore: Pulau Ubin	645 bp	851 bp	348 bp	577 bp
Dictis sp. 5	Vietnam: Biodiversity centre, Hanoi	569 bp	929 bp	349 bp	500 bp
Dictis sp. 6	China: Diaoluo Shan, Hainan	N/A	N/A	345 bp	N/A
Dictis sp. 7	USA: Palm springs, California	642 bp	930 bp	345 bp	510 bp
Dictis sp. 8	Australia: Cairns, Queensland	485 bp	N/A	356 bp	N/A
Scyloxes sp. 1	China: Xi'an caves, Fengshan County, Guangxi	587 bp	930 bp	209 bp	577 bp
Scyloxes sp. 2	China: Swallow cave, Jianshui, Yunnan	608 bp	934 bp	152 bp	607 bp
Scytodes cavernarum	Malaysia: Gunung Senyum, Pahang	605 bp	890 bp	345 bp	505 bp
S. fusca	Malaysia: Batu caves, Selangor	520 bp	875 bp	345 bp	607 bp
S. gooldi	South Africa: Hanglip picnic spot, Limpopo	649 bp	888 bp	283 bp	515 bp
S. longipes	Mexico: Copala, Sinaloa	487 bp	868 bp	348 bp	607 bp
S. magna	Malaysia: Batu Caves, Selangor	503 bp	922 bp	152 bp	577 bp

S. pallida	Singapore: MacRitchie catchment	539 bp	786 bp	348 bp	607 bp
S. pallida	Malaysia: Gombak Field Study Centre, Selangor	663 bp	864 bp	355 bp	588 bp
S. quarta	South Africa: Kai Mouth, Eastern Cape	558 bp	836 bp	345 bp	416 bp
S. rubra	South Africa: Mkuze game reserve, KwaZulu Natal	641 bp	884 bp	346 bp	N/A
S. socialis	Madagascar: Toliara	281 bp	510 bp	355 bp	532 bp
S. thoracica	Bosnia: Medjugorje	221 bp	N/A	161 bp	N/A
S. triangulifera	South Africa: Entabeni Nature Reserve, Limpopo	548 bp	798 bp	204 bp	N/A
S. univittata	Peru: Pisco	636 bp	N/A	356 bp	572 bp
Scytodes sp. 1	Singapore: Clementi Woods Park	637 bp	935 bp	348 bp	604 bp
Scytodes sp. 2	Philippines: Biak-na-bato National Park, Luzon	563 bp	829 bp	336 bp	407 bp
Scytodes sp. 3	China: Xishuangbanna, Menglun, Yunnan	538 bp	786 bp	209 bp	N/A
Scytodes sp. 4	Vietnam: Ba Vi National park, Hanoi	626 bp	560 bp	339 bp	575 bp
Scytodes sp. 5	Thailand: Khao Chong National park, Trang	N/A	932 bp	356 bp	N/A
Scytodes sp. 6	Vietnam: Cat Tien National park, Ho Chi Minh	N/A	919 bp	N/A	605 bp
Scytodes sp. 7	China: Mengla, Xishuangbanna, Yunnan	N/A	N/A	278 bp	N/A
Scytodes sp. 8	Argentina: Corrientes province	540 bp	N/A	345 bp	N/A
Scytodes sp. 9	Panama: Barro Colorado Island	624 bp	871 bp	355 bp	581 bp
Scytodes sp. 10	El Salvador: San Vicente	624 bp	934 bp	N/A	598 bp
Scytodes sp. 11	Dominican Republic: Laguna Salada	626 bp	820 bp	N/A	585 bp
Scytodes sp. 12	Costa Rica: Peninsula de Nicoya cave, Guanacaste	621 bp	N/A	N/A	N/A
Scytodes sp. 13	Nicaragua: Tautepe, Baoco	560 bp	N/A	N/A	606 bp
Scytodes sp. 14	Mexico: North of El Rosario, Baja California	561 bp	781 bp	273 bp	572 bp
Scytodes sp. 15	Panama: Viejo	574 bp	930 bp	347 bp	592 bp
Scytodes sp. 16	Brazil: Sao Paolo	612 bp	865 bp	291 bp	507 bp
Scytodes sp. 17	Peru: Lambayeque	612 bp	866 bp	247 bp	536 bp
Scytodes sp. 18	Guatemala: Santa Elena de la cruz, Peten	573 bp	N/A	138 bp	N/A
Scytodes sp. 19	Namibia: Grootfontein camp, Otjozandjupa	639 bp	N/A	344 bp	N/A
Stedocys leopoldi	Malaysia: Tanah Rata, Cameron Highlands	522 bp	383 bp	357 bp	N/A
Stedocys sp. 1	China: Qiaoxu caves, Dahua county, Guangxi	514 bp	851 bp	347 bp	552 bp
OUTGROUP					
Drymusa capensis	South Africa: Newland's forest, Western Cape	617 bp	570 bp	345 bp	598 bp
Loxosceles arizonica	USA: Arizona	466 bp	N/A	348 bp	599 bp
Loxosceles laeta	USA: Los Angeles, California	612 bp	826 bp	335 bp	607 bp
Sicarius sp. 1	Argentina: Rio Negro Paso Cordoba cliffs	619 bp	930 bp	N/A	601 bp
Sicarius sp. 2	Namibia: Gobabeb research station	624 bp	925 bp	349 bp	606 bp

Spiders used in this study were collected in the field by myself and colleagues (see acknowledgements) from Africa, South Africa, Australia, China, Malaysia, the Philippines, Singapore, Thailand, and Vietnam (Table 5-1). Some of my collection included mature specimens that did not morphologically resemble any described species and are genetically divergent from other taxa in my analysis. I do not describe these here but list them in Table 5-1 as “sp. X” and differentiate them in discussion by collecting locality. Some *Scytodes* individuals had genitalic and somatic characteristics that were similar to current described species; I placed all the scytodids in species groups based on genitalia and morphological characteristics such as tarsal claws. When a species/population was abundant, I preserved a few adult specimens (both males and females if possible) in 75% EtOH for future identification, 1-5 specimens in 100% EtOH and stored at -80°C for molecular work, and also collected other specimens alive for laboratory behavioural observations and experiments. After the completion of the molecular work, all species of spiders were or will be preserved as voucher specimens in 75% EtOH in the Raffles Museum of Biodiversity Research (RMBR) in the National University of Singapore.

DNA extraction

Genomic DNA from whole spiders or two to three legs taken from each spider specimen was extracted using a modified CTAB phenol/chloroform extraction protocol (Shajahan 1995). The specimens were lysed in CTAB buffer, 20 µl Proteinase K was added and the samples were incubated overnight at 55°C. DNA was extracted using phenol: chloroform: isoamyl alcohol (25:24:1) mix and precipitated with 100% ethanol. After washing the DNA pellets in 70% ethanol, they were dissolved in 40 µl of water.

DNA amplification and sequencing

Standard PCR amplifications were carried out with Thai Taq (Department of Clinical Immunology, Isan, Thailand) and Takara Ex-Taq (Takara, Shiga, Japan) using approximately 2 µl of the DNA extractions to amplify the four different gene regions of interest. This included three nuclear markers (18S, 28S and Histone 3) and one mitochondrial marker (COI) (primers in Table 5-2). These genes were chosen because of their universal occurrence, sequence and structural conservation and abundance (Mardulyn and Whitfield 1999), and because these genes have been studied extensively in spider phylogeny (e.g. Arnedo et al. 2004; Su et al. 2007; Agnarsson et al. 2007). I tried out 16S and actin genes but I continuously had trouble amplifying these two genes for several taxa. For 16S, it could have been a problem of evolution of priming sites. With regards to actin, most of the PCRs either failed or resulted in the amplification of multiple PCR products, presumed to represent different actin paralogs. Vink and colleagues (2008) reported that no actin was successfully amplified from genomic samples of haplogynes.

The cycling conditions for the 28S primers included an initial 95˚C denaturation step for 3 mins, followed by 35 cycles of 1.5 mins at 95˚C, 1 min at 52˚C, and 1.5 mins at 72˚C, with a final 3 min extension at 72˚C. The cycling conditions for the other three primers were 35 cycles of denaturation at 95˚C, followed by 55˚C (18S), 46˚C (CO1) and 50˚C (H3) for 1min, and lastly a 72˚C extension for 5 min.

Cycle sequencing reactions were performed on the purified products using BigDye Terminator v3.1 (Applied Biosystems, California, USA). The products were then prepared for direct sequencing by removing the dye-terminator using 5 µl of Agencourt CleanSEQ solution. The purified PCR products were sequenced directly in both directions using automated techniques on the ABI 3100 genetic analyser (Perkin Elmer) (Applied Biosystems, California, USA).

Table 5-2. List of genes and primers used for PCR in this study.

Gene	Primer codes	Primer	References
18S	18S9R	GAT CCT TCC GCA GGT TCA CCT AC	Whiting 2002
	18Sa2.0	ATG GTT GCA AAG CTG AAA C	Whiting et al. 1997
28SRD	28SRD 4.5a	AAG TTT CCC TCA GGA TAG CTG	Whiting 2002
	28SRD 7B1	GAC TTC CCT TAC CTA CAT	Whiting 2002
HISTONE 3	H3aR	ATA TCC TTR GGC ATR ATR GTG AC	Colgan et al. 1998
	H3aF	ATG GCT CGT ACC AAG CAG ACV GC	Colgan et al. 1998
COI	HCO	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. 1994
	LCO	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. 1994
	Chel-R1	CCT CCT CCT GAA GGG TCA AAA AAT GA	Barrett and Hebert 2005
	Chel-R2	GGA TGG CCA AAA AAT CAA AAT AAA TG	Barrett and Hebert 2005

Phylogenetic analyses

For sequence editing and contig (series of contiguous, overlapping cloned DNA fragments) construction I used Sequencher 4.2 (Gene Codes Corporation Inc, Ann Arbor, Michigan, USA). Edited sequences were first put through a BLAST search to confirm that the correct sequences were extracted. The protein encoding genes COI and H3 were aligned using the same program and yielded gap-free alignments. They were unambiguously aligned by eye using MacClade 4.03 (Maddison and Maddison 2001) and neither displayed stop codons when translated to amino acid sequence using MacClade 4.06 OS X (Maddison and Maddison 2003). The ribosomal gene sequences of 18S and 28S were aligned in MAAFT (Katoh et al. 2002), with a gap opening cost of 1.53.

I reconstructed relationships using maximum parsimony (MP) and maximum likelihood (ML) analyses. The maximum parsimony analyses were conducted on TNT version 2.0 (Tree Analyses Using New Technology) (Goloboff et al. 2000). I carried out the analyses using gaps coded as both 'missing data' and as a fifth character state. When coded as fifth character state, gaps were given at most half the weights of the transversions to avoid violations of triangular inequalities (Wheeler 1993). Parsimony searches were heuristic with tree bisection-reconnection (TBR) branch-swapping, using a new tech search, at level 50, finding the minimum length five times. If there were multiple most parsimonious trees, a strict consensus was carried out. Node support was assessed by jackknife re-sampling percentiles (250 replicates, same search options as above) obtained at 36% deletion as recommended by Farris et al (1996).

For likelihood analyses the best model was chosen by using MrModeltest version 2.2 (Nylander 2004). The best fit model based on the Akaike Information Criterion (AIC) was GTR+I+V. The maximum likelihood analyses were conducted with Garli v0.951 (Zwickl 2006). Three independent runs were carried out and node support was assessed using a non-parametric bootstrap with 250 replicates using the automated stopping criterion set at 10,000 generations for each replicate.

RESULTS

The length of the aligned sequences was 23,271 bp for 18S, 31,418 bp for 28Srd, 13,485 bp for H3 and 19,527 for COI. The number of base pairs amplified for each gene for individual species can be found in Table 5-1.

The MP analysis with indels coded as a fifth character state (hereby referred to as 'Indel analysis') yielded five most parsimonious trees with a tree length of 3816, a retention index (RI) of 0.5766 and a consistency index (CI) of 0.3593 (Fig. 5-1 shows the strict consensus tree). The analysis with indels coded as missing data (hereby referred to as 'No-Indel analysis') yielded nine most parsimonious trees with a tree length of 3812, a consistency index (CI) of 0.3597, and a retention index (RI) of 0.5722. Parsimony analysis coding indels as missing data and as a fifth character resulted in trees with identical family-level relationships and sharing approximately 91% of the node support (Fig. 5-2). There were a total of 2591 characters, of which 1671 were constant variables. 240 characters were parsimony uninformative while 680 were parsimony informative. Maximum likelihood tree calculated by heuristic search from the total sequence of 2591 bp. ML most likely tree, -lnL = 20444.721 (Fig. 5-3).

The data support the monophyly of Scytodidae in all analyses, with high Jackknife values of 84 and 88 on the MPT with gaps as fifth character state and as missing data, respectively (Fig. 5-1 and Fig. 5-2). However, patterns of relationships among the representatives of this family are inconsistent and sensitive to data perturbations. The different analyses primarily disagree on the middle part of the tree and *Scytodes* monophyly (Fig. 5-1). On both MP trees the placement of Drymusidae as sister groups to Scytodidae is strongly supported (JK = 99) (Fig. 5-1 and Fig. 5-2).

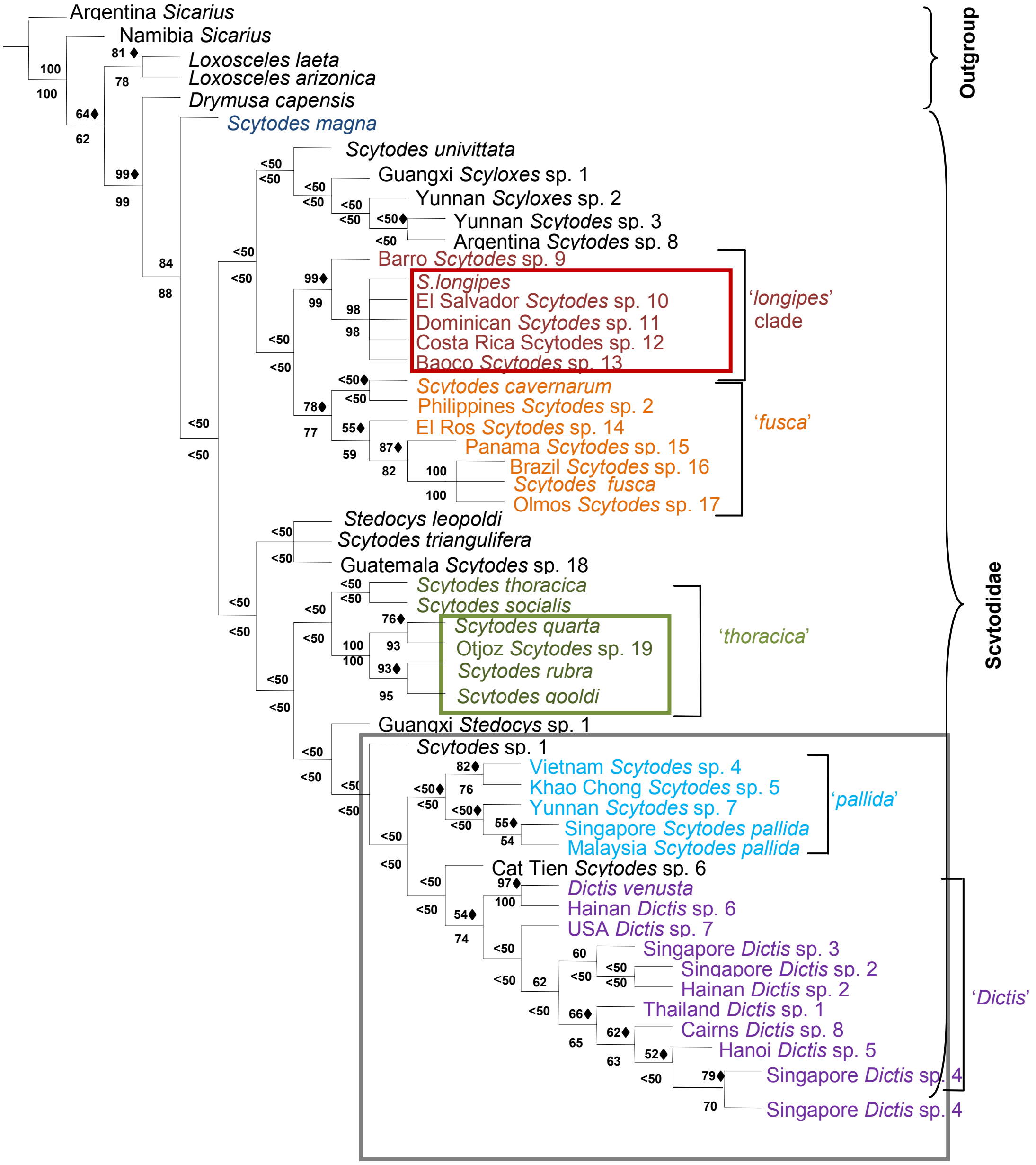

Fig. 5-1. Maximum parsimony tree with indels coded as fifth character states based on combined sequence data from 18S, 28S, H3 and COI. This topology is a strict consensus of five equally parsimonious trees. Numbers above the line are Jackknife support values of MPT = gaps as fifth character state; numbers below the line are Jackknife support values of MPT = gaps as missing data. Nodes shared with ML tree are marked with ♦

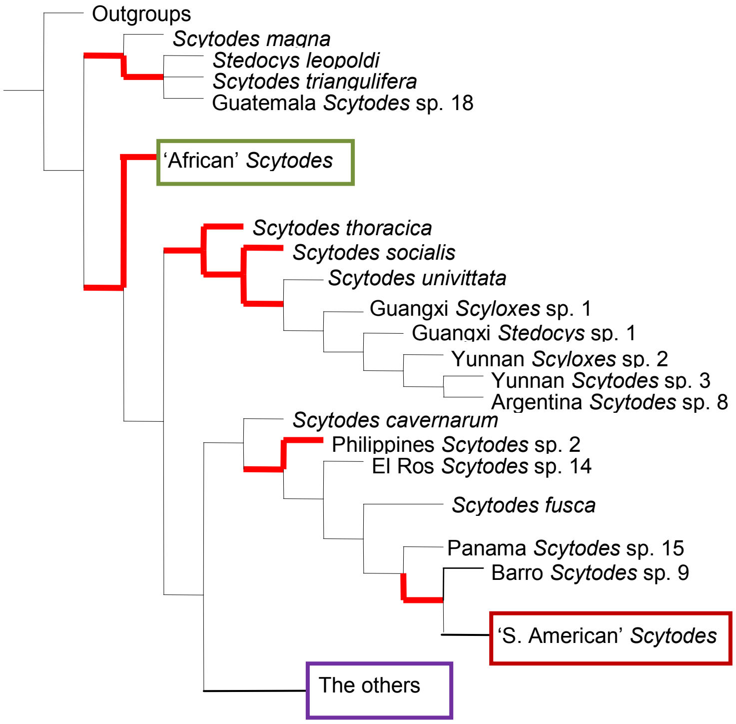

Fig. 5-2. Abbreviated maximum parsimony tree with indels coded as missing data based on combined sequence data from 18S, 28S, H3 and COI. This topology is a strict consensus of nine equally parsimonious trees. Taxa in coloured boxes have the same topologies as those in MPT = gaps as 5th character and are not shown here.

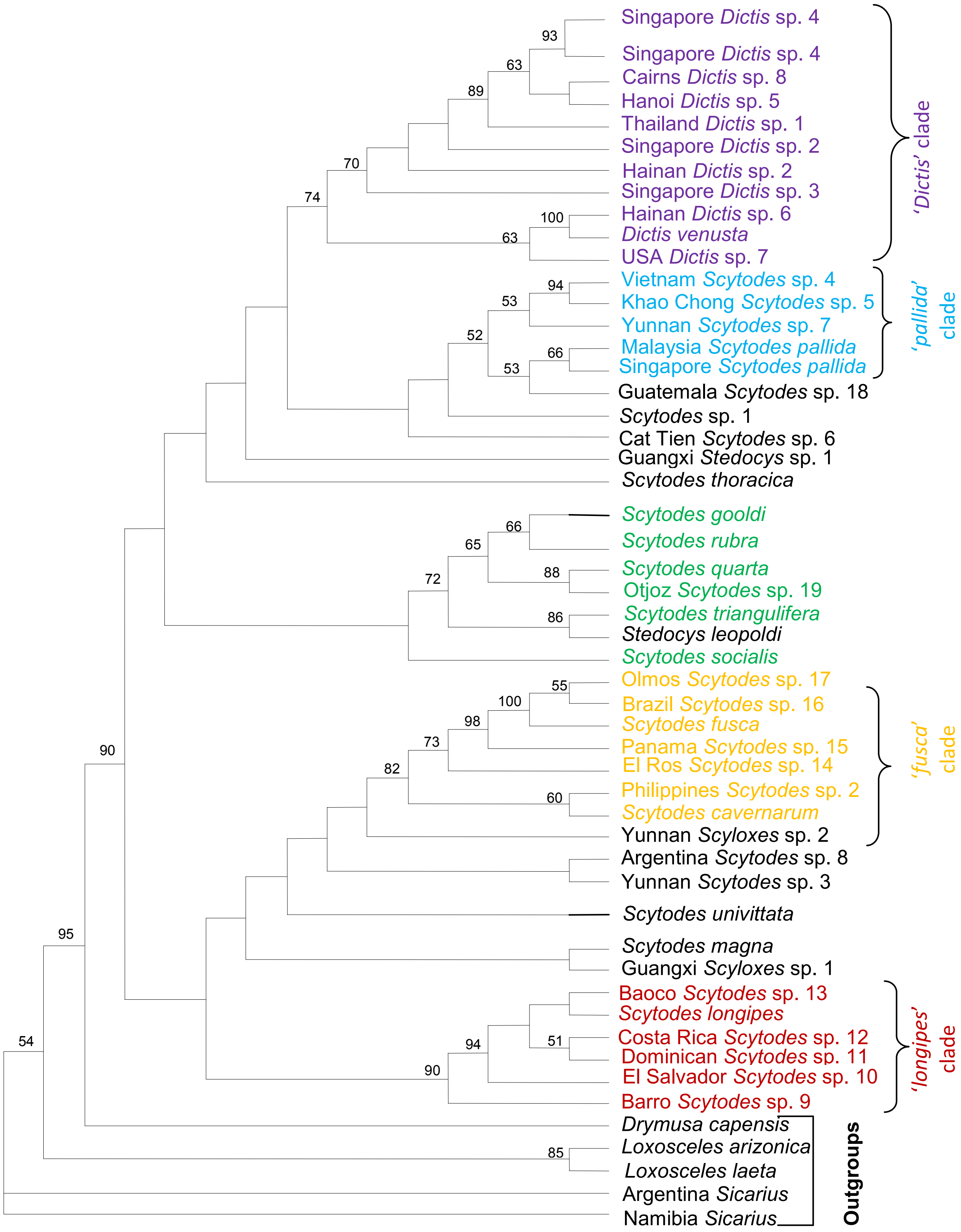

Fig. 5-3. Likelihood tree from ML analysis (Garli) indicating bootstrap values. Colours indicate taxa that fall into the same clades as seen in the MPT = gaps treated as fifth character states (Fig. 5-1)

There are some topological differences, albeit with low support values, within Scytodidae:

(1) *Scytodes magna* and the rest of the scytodids are sister groups on the Indel analysis, but are placed as a polytomy on the No-Indel analysis;

(2) The '*thoracica*' clade is monophyletic on the Indel analysis, but the No-Indel analysis fails to recover it, placing these taxa instead as polyphyletic;

(3) The Guangxi *Stedocys* sp. 1 is nestled between the '*thoracica*' and '*pallida*' clades in the Indel analysis, but between Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2 in the No-Indel analysis;

(4) The '*longipes*' clade and '*fusca*' clade are sister groups on the Indel analysis, but the No-Indel analysis fails to recover this '*longipes*' + '*fusca*' clade, placing these taxa instead as polyphyletic.

The preference of tree used for my phylogenetic discussion and for tracing the evolution of sociality can be debated. Although the two different treatments of gaps resulted in trees of rather different topologies, the support values are not high. I decided, for reasons discussed in the following section, that the Indel analysis should be the tree to be used in the mapping of the social behavior. Jackknife values from both indels' cost analyses were mapped onto the strict consensus tree of the Indel analysis. Although support for many nodes is weak (and a few are unresolved), the consensus tree indicates that at least six generic level clades exist within Scytodidae (Fig. 5-1).

The maximum likelihood tree is shown in Fig. 5-3. Although the topologies of the MPT = gaps as fifth states and ML trees are similar and recover the same family-level relationships, the resolution of the ML tree is more weakly supported. In comparing relationships between clades, the MPT = gaps as fifth states is not congruent with the ML tree. However, composition of individual clades in MPT = gaps as fifth states is very congruent with equivalent groups in the ML tree (see Fig. 5-3). The ML tree managed to recover only three clades that are found on the MPT where gaps were coded as the fifth character states –'*longipes*', '*fusca*' and '*Dictis*'. On the ML tree, the '*thoracica'* clade is not recovered, and furthermore *Stedocys leopoldi* is placed among the 'African' *Scytodes*. Known only from Thailand and Malaysia *Stedocys leopoldi* is a species that was placed into a separate genus from *Scytodes* by Ono (1995) on the basis of having a peculiar male palp atypical for the family: a tarsus smaller than the copulatory bulb, not prolonged distally, the apical implantation of the copulatory bulb, and a slender, aciculate embolus. Hence I am of the opinion that it should not be placed among the 'African' *Scytodes* spiders. The other major difference between the ML and MPT = gaps as fifth states trees is that *S. magna* is not recovered as a sister group to the rest of the scytodids in the ML tree. In fact the placement of *S. magna* is contentious because on the MPT = gaps as missing data tree, *S. magna* is placed as sister group to (*Stedocys leopoldi* + *Scytodes triangulifera* + Guatemala *Scytodes* sp. 18) clade, although it can still be considered a sister group to the other scytodids. Other than the '*Dictis*' clade, none of the genera represented by more than one species is monophyletic in all analyses. No analyses supported *Scytodes*, *Stedocys* or *Scyloxes* monophyly.

DISCUSSION

Impacts of gap incorporation

As methods for coding indels (gaps) have become more prominent and proficient, inclusion of indels as characters in phylogenetic analyses has gained increasing popularity (Kawakita et al. 2003; Johannesen et al. 2005; Řezáč et al. 2008) because gaps have been suggested to be phylogenetically reliable characters (Graham et al. 2000; Simmons et al. 2001). However, the relative utility of gap characters has been a matter of debate. Some have championed indels as reliable phylogenetically (Lloyd and Calder 1991; Ingvarsson et al. 2003) while others suggest indels to be homoplasious or uninformative (e.g. Golenberg et al. 1993; Pearce 2006). Several methods exist for coding gaps as characters. One of these includes the treatment of each indel as a simple binary character (Simmons and Ochoterena 2000). Others treat overlapping indels as multistate characters (e.g. Simmons and Ochoterena 2000). Maximum parsimony will allow a variety of options for dealing with gaps, including treating gaps as missing data, treating gaps as a fifth character state, or including coded gaps as binary or multistate characters. Maximum likelihood currently treats gaps only as missing data. For my study, a maximum parsimony (MP) analysis was conducted for each data set under two different gap schemes: gaps as 'missing' and gaps as 'fifth character state'. In terms of resolution, both data sets resulted in similar resolved topologies (Fig. 5-1 and Fig. 5-2). Although not as straightforward, I found that coding gaps as missing values decreases both topological congruence (e.g. *S. triangulifera* and Guatemala *Scytodes* sp. 18 having a sister-taxon relationship with *S. magna* – wrongly identified as a *Scytodes*) and branch support. There are several nodes within Scytodidae with marginal support from the Indel analysis, but at odds with the No-Indel analysis (see Figs. 5-1 and 5-2, nodes that are coloured red):

1) In the No-Indel analysis, *S. magna* is sister taxon to (*Stedocys leopoldi* + *S. triangulifera* + Guatemala *Scytodes* sp. 18) group, but in the Indel analysis *S. magna* forms a sister group to all other scytodids;

2) The 'African' *Scytodes* is placed at a more basal position in the No-Indel analysis;

3) *Scytodes thoracica* and *S. socialis* fall out of the '*thoracica*' clade and are instead ancestral to *S. univittata* in the No-Indel analysis;

4) The Philippines *Scytodes* sp. 2 is not sister-taxon to *S. cavernarum* in the No-Indel analysis;

5) Panama *Scytodes* sp. 15 is sister to the '*longipes*' clade in the No-Indel analysis but is sister to (Brazil *Scytodes* sp. 16 + *Scytodes fusca* + Olmos *Scytodes* sp. 17) in the Indel analysis.

In summary, my study has shown that gaps can hold considerable phylogenetic signal. The MP analysis with gaps treated as fifth character state benefited from the inclusion of indels both in terms of nodal support and topological resolution. I found that resolution increased with gap coding complexity from treating gaps as missing information through to treating them as fifth character states. In other words, the number of parsimony-informative sites generally increased with gap coding complexity, thus providing more resolution to topologies and support to nodes. Egan and Crandall (2008) in their study of North American Psoraleeae (Leguminosae) in which they analysed gap incorporation also found that gaps can represent a considerable amount of phylogenetic data and enhance phylogenetic studies by increasing resolution and nodal support.

The ambiguity of certain clades could be the result of using an insufficient number of genes, some taxa not having the full set of four genes amplified or some genera, such as *Stedocys*, being represented by only a single confirmed taxon (i.e. *S. leopoldi*) (see Table 5-1). I suggest that for future phylogenetic studies, more mitochondrial markers such as 12S and CYB be used because mitochondrial genes may evolve faster than nuclear genes (about 2-9 times faster in insects), making them very useful for studying closely related taxa that have evolved recently (Lin and Danforth 2004). While my goal was to obtain a thorough overlap of molecular markers for all taxa in my analysis, despite many attempts, I was unable to amplify all genes for all taxa. Therefore, my taxon inclusion varies across genes with 18S and 28S having the most thorough coverage. This is the first investigation of scytodid phylogeny using DNA sequences, and I have discovered that within this family there are numerous taxonomic issues among the five genera. Until recently, taxonomic studies on characters such as genitalia, tracheal systems and tarsal claws for the genus *Scytodes* have been sparse. Receiving weak taxonomic attention, there are no morphological symplesiomorphies or synapomorphies that can confirm the monophyly or paraphyly of the groups within Scytodidae, or even the monophyly of genera such as *Scyloxes* currently represented by only one species, *S. asiatica* (Platnick 2010). It is beyond the scope of this study to document the details of the anatomies of genitalia, spinnerets, chelicerae etc.

Multiple analytical methods are usually performed to assess the degree of congruence among alternative methods. While the relationship between clades on the MPT where gaps are coded as fifth character states are not very congruent with the ML tree, the composition of individual clades in the MPT is very congruent with the equivalent groups in the ML analysis, thus giving confidence in the MP approach. With the scarcity of knowledge of the relationships within this family, there is no *a priori* basis for choosing one analysis over another. One of the goals of this project was to investigate the evolution of sociality in scytodids, that is, I want to find out whether a particular trait is shared-ancestral, shared-derived, uniquely derived or a result of homoplasy. Such information can then be used to infer modes of evolution (i.e. what happened). In line with this goal and for analytical consistency purposes, I chose to map the social behavioural characters onto the MPT instead of the ML tree because the parsimony method is almost universally used to map characters (Bollback 2006) and attains its greatest power for the study of evolutionary patterns and processes (Stewart 1993). Indeed, user-friendly programs that implement the parsimony method are available (e.g., MacClade (2003) and Mesquite (2004)). By the early 1980s parsimony was already well established as the method of choice among phylogenetic systematists. Parsimony assesses the degree to which a tree can account for observed similarities among terminals as the result of inheritance from a common ancestor (Farris, 1983). Because parsimony attempts to minimize the number of changes along a phylogeny in a manner that is consistent with the character states observed at the tips (Bollback 2006), it enables me to find out the parsimonious evolutionary scenario for a particular trait (Grandcolas et al. 1994).

For the reasons as explained above, the results and discussion will be concentrated on the MPT where indels are coded as fifth character state, instead of the MPT where gaps are coded as missing data.

Simmons and colleagues (2007) conducted simulation-based parsimony analyses on indel-coding methods and also found that coding gaps as a fifth character state gives the most accurate tree, but found this to be a function of weighting, a phenomenon inherent in the MPT= gaps as fifth state method. While it was not my intent to subject the dataset to both types of indel treatment and compare indel incorporation methods, a few insights may be drawn from my results. Incorporating gaps as fifth characters coded was in the general sense advantageous over treating gaps as missing data. The Indel analysis provided increased resolution or strengthened nodal support in comparison to the No-Indel analysis. However I acknowledge that treating gaps as a fifth character state produced some topologies that were very different from the other MP analysis (see Results).

There are also some other approaches to dealing with gaps in alignments (e.g. Giribet and Wheeler 1999; Phillips et al. 2000; Wheeler 1995), all showing the importance of including the gap information in phylogenetic analyses. Perhaps more conservative methods such as coding gaps using simple indel coding (sic, Simmons and Ochoterena 2000) and modified complex indel coding (mcic) with gaps longer than 50 bp coded as simple characters (Müller 2006) could be used in future work.

Scytodidae monophyly and outgroups

The monophyly of Scytodidae is strongly supported by all trees, and this result is consistent with the phylogenetic trees of Sicariidae obtained by Binford et al. (2008). The ML tree recovered five out of 7 of the clades seen in the discussed tree (MPT = gaps as fifth state); *S. magna* and the '*thoracica*' clades are not monophyletic on the ML tree. The major conflicting results among the analytical methods are the monophyly and positions of *Scytodes magna*, *Scyloxes* (Guangxi *Scyloxes* sp. 1 + Yunnan *Scyloxes* sp. 2), and *Stedocys* (*S. leopoldi* + possibly Guangxi *Stedocys* sp. 1). In all three analyses, *Scytodes* is never recovered as a monophyletic group as the other genera are always nestled among the taxa of this group although there is no compelling statistical support for this polyphyly. Polyphyly of *Scytodes* was largely unexpected and may be a reflection of poor resolution within the *Scytodes* and/or poor taxon sampling outside of this genus (i.e. *Scyloxes* and *Stedocys*) and insufficient genes utilized. However, herein lies the possibility that Lehtinen's evaluation of Scytodidae has been accurate over two decades; that more than 25 genera need to be erected because '*Scytodes*' has been a genus more often than not used as a "dumping" ground for all spitting spiders with three claws.

At family level both the parsimony and maximum likelihood analyses resulted in trees largely concordant with other published phylogenetic trees (Platnick et al. 1991; Ramírez 2000; Coddington 2005) which are based on morphological data. In all my analyses, Drymusidae is always placed as the sister group to Scytodidae. This interfamilial relationship is uncontroversial regardless of which indel treatment or the analysis strategy is used. In the review of the morphology of the respiratory system of basal araneomorph spiders, the Haplogynae and Entelegynae with female haplogyne genitalia, Ramírez (2000) found that Tetrablemmidae, Pholcidae, Diguetidae and Plectreuridae should be united by the absence of tracheae; and these taxa should be united with Scytodidae, Sicariidae and Drymusidae by the fusion of 3rd entapophyses, thus corroborating Platnick et al's (1991) study. The familial groupings on all my analyses strongly support the phylogenetic studies of Platnick et al. (1991) and Ramírez (2000). Together with the Sicariidae, the Drymusidae shares another character in common with some of the Scytodidae – the males lack a tarsal palp extension. The MPT = gaps treated as fifth character indicates that the tarsal palp extension has evolved several times (e.g. once in '*longipes*' + '*fusca*' clades and again in the '*thoracica*' through to the '*Dictis*' clades). This is a possible but unparsimonious hypothesis.

Phylogenetic analysis – the clades

The combination of data sets with varying rates of evolution (e.g., mitochondrial and nuclear genes) can efficiently accommodate for variation in results of individual analyses (Flook et al. 1999). Here, I present 80.6 Kbp of molecular data for the spider family Scytodidae, and use it to gain a better understanding of intergeneric relationships. I generated nucleotide sequence data from one mitochondrial gene and three nuclear ribosomal gene which I assumed might be informative within a given time scale. These genes were previously used with success in a range of phylogenetic analyses in spiders and other arthropod groups (e.g. Maddison and Hedin 2003; Terry and Whiting 2005; Su et al. 2007; Binford et al. 2008).

The MPT with indels = fifth character can roughly be divided into two tribes, Old World and New World, with the New World tribe comprising of the '*longipes*' and '*fusca*' clades. The former comprises of *Scytodes* from Central America (Panama, El Salvador, Nicaragua and Costa Rica) and North America (Mexico and Dominican Republic), while the '*fusca*' clade consists of spitting spiders from an eclectic mix of continents: North America (Mexico), Central America (Panama), Asia (Philippines and Malaysia) and for one species, *S. fusca*, Pantropical, ubiquitous in Central America (Valerio 1981). The range of '*fusca'* has probably been extended by human transport, becoming widespread and possibly genetically diverse. While *S. fusca* in my dataset was sampled from Malaysia, it appears to be monophyletic with Brazil *Scytodes* sp. 16 and Olmos *Scytodes* sp. 17 (from Peru) (but see ML tree). Detailed morphological investigation revealed that they share similar body patterns and genital structures. Their wide range could be explained by a natural dispersal event from South America to the Old World after the Gondwanan split, as in the case of the *Loxosceles rufescens* lineage (Binford et al. 2008; Duncan et al. 2010). A similar pattern is seen in *S. longipes*. The specimen that was identified as *S. longipes* was from Mexico. After studying their genital structures, body size and patterns, I am able to conclude that El Salvador *Scytodes* sp. 10 and Dominican *Scytodes* sp. 11 may be synonymous with *S. longipes*. Although I suspect the Costa Rican species to also be *S. longipes*, I am unable to make any conclusive remarks at present because the specimen that was sent to me is a juvenile. According to Lehtinen (2010), within his newly limited ‘Scytodinae’ the most plesiomorphic of *Scytodes* is the ‘*longipes’* group. In spite of conspicuous autapomorphies the ‘*fusca’* group is also close to the base of the cladogram together with three undescribed Old World groups, all of which are monotypic. The MPT with indels = fifth character (Fig. 5-1) is in agreement with Lehtinen (2010). The ‘*fusca’* and ‘*longipes’* clades are found towards the base of the cladogram and some or all of “three undescribed Old World groups” may refer to the *Scyloxes* (Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2) and ‘*Magnodes*’ (*S. magna*) species. Although the ‘*longipes’* group falls in the New World tribe in my cladogram, a check with the Raffles Museum of Biodiversity Research (RMBR) reveals that a specimen of *S. longipes* that was collected from Taiping, Malaysia was deposited there. Like *S. fusca*, it is also pantropical (i.e. covers a wide range) (Platnick 2010).

Scytodes cavernarum from Malaysia and the Philippines *Scytodes* sp. 2 are nestled within the New World *Scytodes*. It is possible that although *S. cavernarum* and the Philippines *Scytodes* sp. 2 were sampled from Malaysia and Philippines, respectively, they might have originated from Neotropics. However, from a geographical perspective this is unlikely because millions of years ago, South/Central America and Southeast Asia, specifically Malaysia and Philippines, were never part of the same land mass or composite continent (Dietz and Holden 1970). It is also very unlikely that these two species were accidentally brought over from Central/South America because they are not synanthropic spiders, having been sampled from caves. Their current placement in the cladogram is most likely attributed to the fact that the ‘*fusca*’ clade is internally unresolved given the low bootstrap and jackknife support values of this node.

With the exceptions of the Guatemala *Scytodes* sp. 18 and USA *Dictis* sp. 7 , the Old World tribe is made up of the taxa *Stedocys leopoldi* to Singapore *Dictis* sp. 4 (in a downward sequence). *Stedocys leopoldi* from the genus *Stedocys* was sampled from the highlands of Malaysia; *S. triangulifera* together with the '*thoracica*' clade (except for *S. thoracica*) are found in Africa; *S. thoracica* is common throughout Europe. Guangxi *Stedocys* sp. 1 and *Scytodes* sp. 1, together with the '*pallida*' clade and '*Dictis*' clade, are all found in Asia and Australia (Singapore, China, Vietnam, Thailand, Malaysia and Australia).

The anomalies of the Guatemala *Scytodes* sp. 18 and USA *Dictis* sp. 7 could be explained by the strong possibility that the Guatemala *Scytodes* sp. 18 is a very fast-evolving species as indicated by the extremely long branch in the ML analysis. In both types of maximum parsimony analysis, the Guatemala *Scytodes* sp. 18 is found in the same clade as *S. triangulifera* and *Stedocys leopoldi* (the clade having different topologies), whereas in the maximum likelihood analysis the Guatemala *Scytodes* sp. 18 is found in the same clade as the *S. pallida* species. According to Lehtinen (2010), species belonging to *Dictis* as well as Neotropical three-clawed groups have lost their unpaired tarsal claw in several lines of evolution. With regard to the USA *Dictis* sp. 7, its placement within the '*Dictis*' clade implies that it is very likely a *Dictis* (i.e. one of the five genera in the family Scytodidae). Its position within this clade is consistent in all analysis types. If so, it will be the first record of *Dictis* or a second two-clawed scytodid genus in the New World.

The relationship between the Yunnan *Scytodes* sp. 3 (from Xishuangbanna Tropical Botanical Garden) and Argentina *Scytodes* sp. 8 is uncertain. This is because, although the two are linked in all three analyses, support is low. The Yunnan *Scytodes* sp. 3 is a leaf-litter inhabiting species and, unlike the typical *Scytodes* that are usually large and long-legged inhabiting holes and crevices, Yunnan *Scytodes* sp. 3 is very small measuring only 3.0 mm in body length. Similar to those species that are found in the Amazonian non-inundated rain forest and Atlantic coast forest, Yunnan *Scytodes* sp. 3 also exhibits characteristic hairs covering the cephalothorax and abdomen (Valerio 1981; Brescovit and Höfer 1999). I cannot comment on the Argentinean species because the sample that was sent to me was a juvenile and its natural history is not known.

Scytodes socialis, a species found in Malagasy, is nestled within the '*thoracica*' clade and found grouped together with the rest of the African/South African scytodids in both the MP tree = gaps treated as fifth state and ML tree. However, on the other MP analysis, *S. socialis* is positioned between *S. thoracica* and *S. univittata*. A recent review across multiple lineages (Yoder and Nowak 2006) found that the most common pattern was a sister relationship of Malagasy fauna to taxa from the African continent, and that most Malagasy lineages were too recent to be explainable by vicariance. Examples of taxa that probably colonized via dispersal from Africa include a variety of organisms such as plants (Schaefer et al. 2009), freshwater crabs (Cumberlidge 2008), lizards (Raselimanana et al. 2009) and bats (Weyeneth et al. 2008). While there are also several examples of Gondwanan vicariance lineages in Madagascar (Janssen et al. 2008), Cenozoic (up to 65 Ma) dispersal from Africa appears to have been a prominent biogeographical force shaping the Madagascar biota. The position of *S. socialis* on the MPT = gaps as fifth character state is more likely than in the case of the MPT = gaps as missing data.

Similarly uncertain is the monophyly of the *Stedocys* species with *S. triangulifera* and the Guatemala *Scytodes* sp. 18 in the MP analyses. In the ML tree, *Stedocys leopoldi* is sister-taxon to *S. magna*. I am optimistic that these issues can be resolved by using more appropriate phylogenetic characters and more thorough taxon sampling. My molecular and morphological data for *Stedocys* and *S. magna* contain substantial genetic and genitalic variations that warrant the placement of each of these species groups into separate genera. I do not intend to formally propose any new genus or even sub-family here, but rather to argue that genus delineations in Scytodidae require revision, and that attention to genetic and genitalic differences will be helpful in the process.

Morphological information and characters used in defining clades

Considering molecular phylogenetic trees together with morphological characteristics of the male and female genitalia (Fig. 5-4), cephalothorax patterns (Fig. 5-5), serrula type (Fig. 5-6) and tarsal claws (Fig. 5-7), I further subdivided the tree into seven clades: the outgroups, '*magna*' clade, '*longipes*' clade, '*fusca*' clade, '*thoracica*' clade, '*pallida*' clade and '*Dictis*' clade (Fig. 5-3). The outgroups, '*magna*', '*longipes*', '*fusca*' and '*Dictis*' clades are confidently established with good support, whereas the '*thoracica*' and '*pallida*' clades come out with low support. In studying the morphologies of all the species, I made several interesting observations that solve some issues but also raise other issues which are worthy of further investigation (I first list them as numerical points. Each point will be further elaborated in the following paragraphs).

1) *Scytodes magna* which was described in Bristowe (1952) has been incorrectly placed in this genus;

2) Guangxi *Scyloxes* sp. 1 is a remarkable new scytodid, more closely resembling *Scyloxes asiatica* than any *Scytodes* species;

3) Yunnan *Scyloxes* sp. 2 is another new scytodid, also with some resemblance to *S. asiatica* rather than to *Scytodes*;

4) Guangxi *Stedocys* sp. 1 is a large scytodid with some resemblance to *Stedocys leopoldi* and other *Stedocys* species;

5) *Scytodes* sp. 1 that was originally mistakenly identified as *S. pallida* is a new species that is currently being described. Together with the species in Singapore currently identified as *S. pallida* it will be placed in a new genus;

6) Cat Tien *Scytodes* sp. 6 has palpal organs that are radically different from all other *Scytodes* (except for the rather similar palps of *Dictis thailandica* (fig. 39; Dankittipakul and Singtripop 2010);

7) The *Dictis* genus is verified. Spiders that fall into this clade are characterized by a two-clawed tarsus and a unique female genitalic pattern.

From the cladogram (Fig. 5-4), it is very evident that the palps of *S. magna* lacking the tarsal extension and having a convoluted embolus cannot be a *Scytodes* which is reserved for the possible '*thoracica*' clade, in which *Scytodes thoracica* Latreille 1802 (the type species of *Scytodes*) is placed. Brignoli's (1976: fig. 55) illustration of the very complex female vulva, showing a complexity not seen in the other genera, further supports this. It is in my opinion that based on these characters, *S. magna* should be removed from the genus *Scytodes* and placed into a new genus within the family Scytodidae.

Diagrams of *Scyloxes asiatica* (Dunin 1992; figs 2, 7, 13-16) lead me to hypothesize that Guangxi *Scyloxes* sp. 1 belongs to *Scyloxes* or perhaps, an undescribed sister genus. My hypothesis is based on the following characters: both possessing a convex prosoma with a "tuft" of hair on the prosomal end (near the pedicel) (Fig. 5-5B, C); both having simple palpal organ – bulb and embolus (Fig. 5-4); and both lacking copulatory pockets. It is very unlikely that Guangxi *Scyloxes* sp. 1 is *S. asiatica* because there are differences in the external female genitalia, the length of the palpal segment and embolus details.

From some casual observations in the laboratory, I found that Guangxi *Scyloxes* sp. 1 spiders do not spit as readily as the other *Scytodes* when offered prey such as crickets and houseflies.

Scytodes are easily distinguished by their pear-shaped cephalothorax (Fig. 5-5A). The cephalothorax is high with the highest point well behind the middle. The curved top drops quite steeply to the clypeus and very steeply to the rear margin. This cephalothorax structure is associated with the huge internal pair of glue glands. However, a comparison of Fig. 5-5A – Fig. 5-5E show that the cephalothorax of Guangxi *Scyloxes* sp. 1 and *Scyloxes asiatica* are not pear-shaped. This may indicate that *Scyloxes* possesses relatively smaller glue glands.

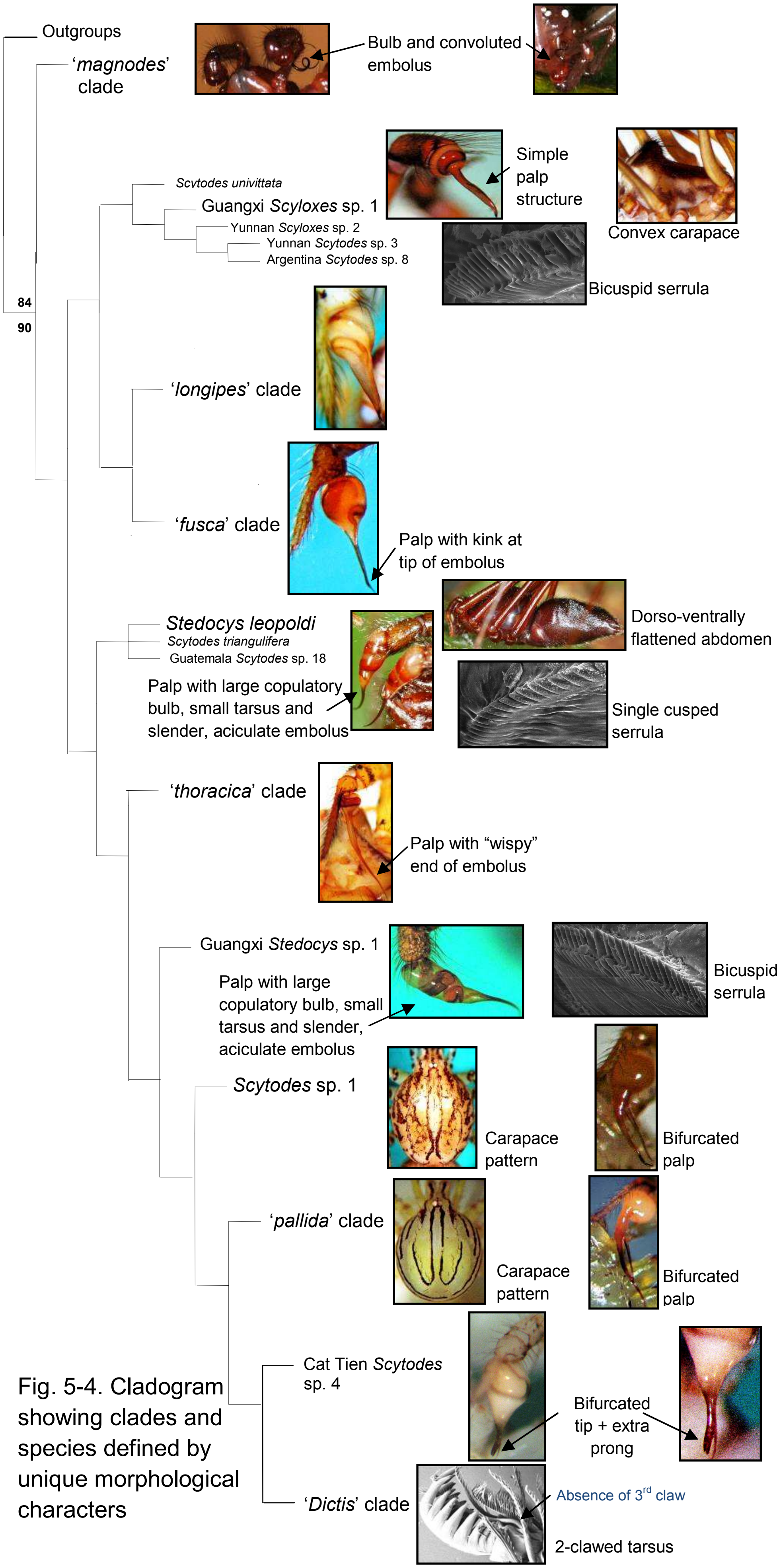

Fig. 5-4. Cladogram showing clades and species defined by unique morphological characters

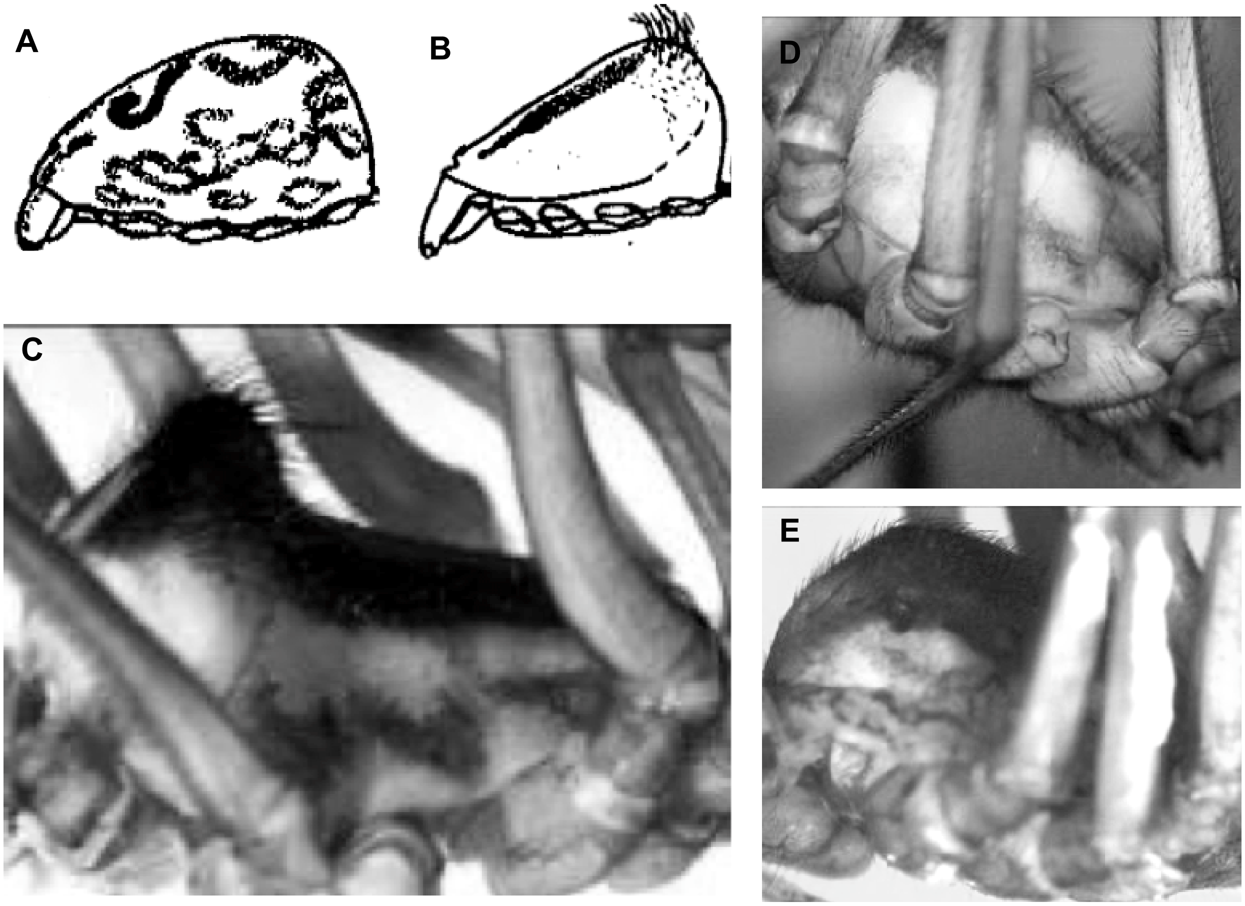

Fig. 5- 5. Comparison of cephalothorax of (A) *Scytodes thoracica*; (B) *Scyloxes asiatica*; (C) Guangxi *Scyloxes* sp. 1; (D) Yunnan *Scyloxes* sp. 2; and (E) Guangxi *Scyloxes* sp. 1.

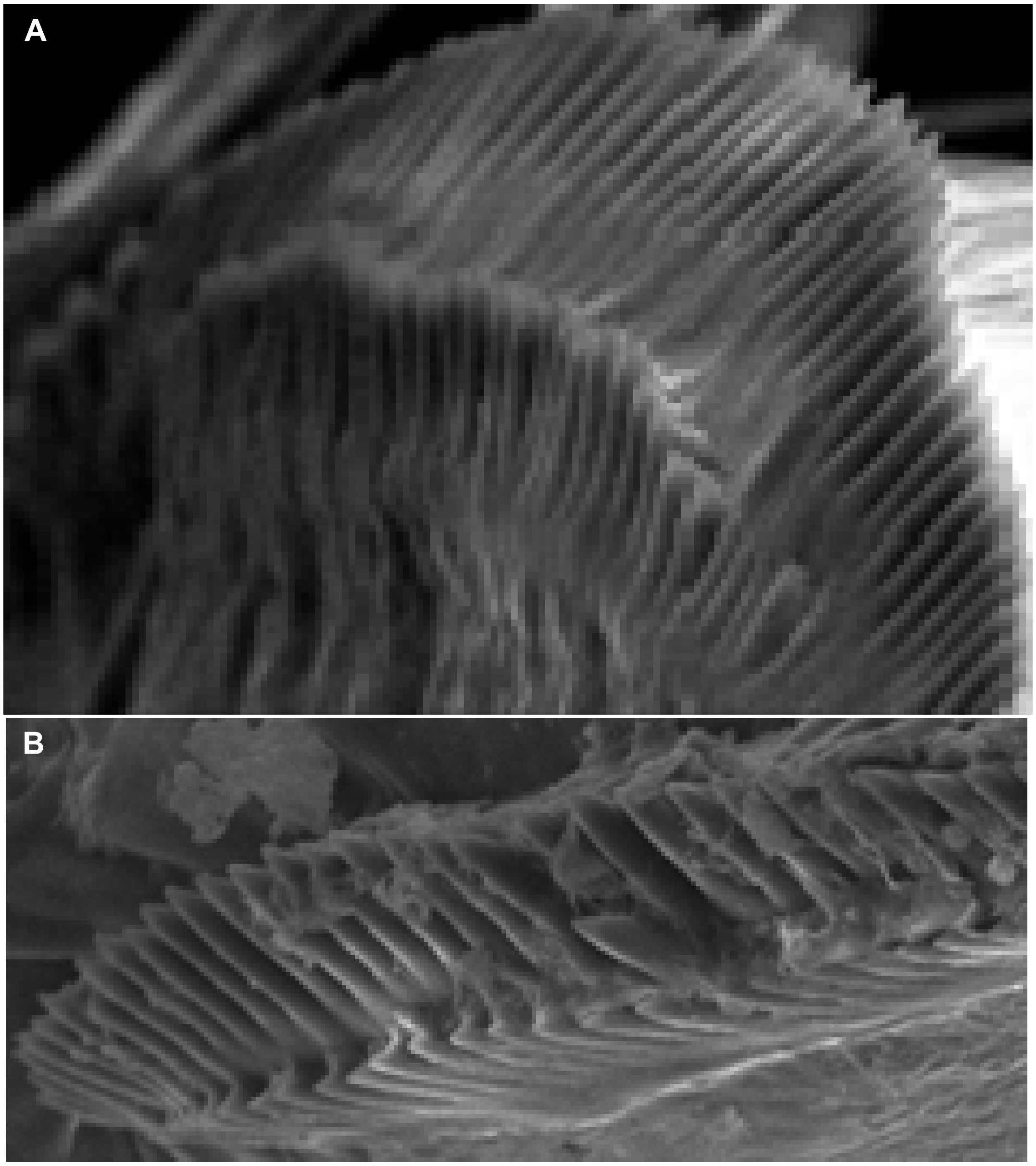

Fig. 5-6. Bicusped serrula of (A) Yunnan *Scyloxes* sp. 2, scale bar = 20 µm and (B) Guangxi *Scyloxes* sp. 1, scale bar = 10 µm.

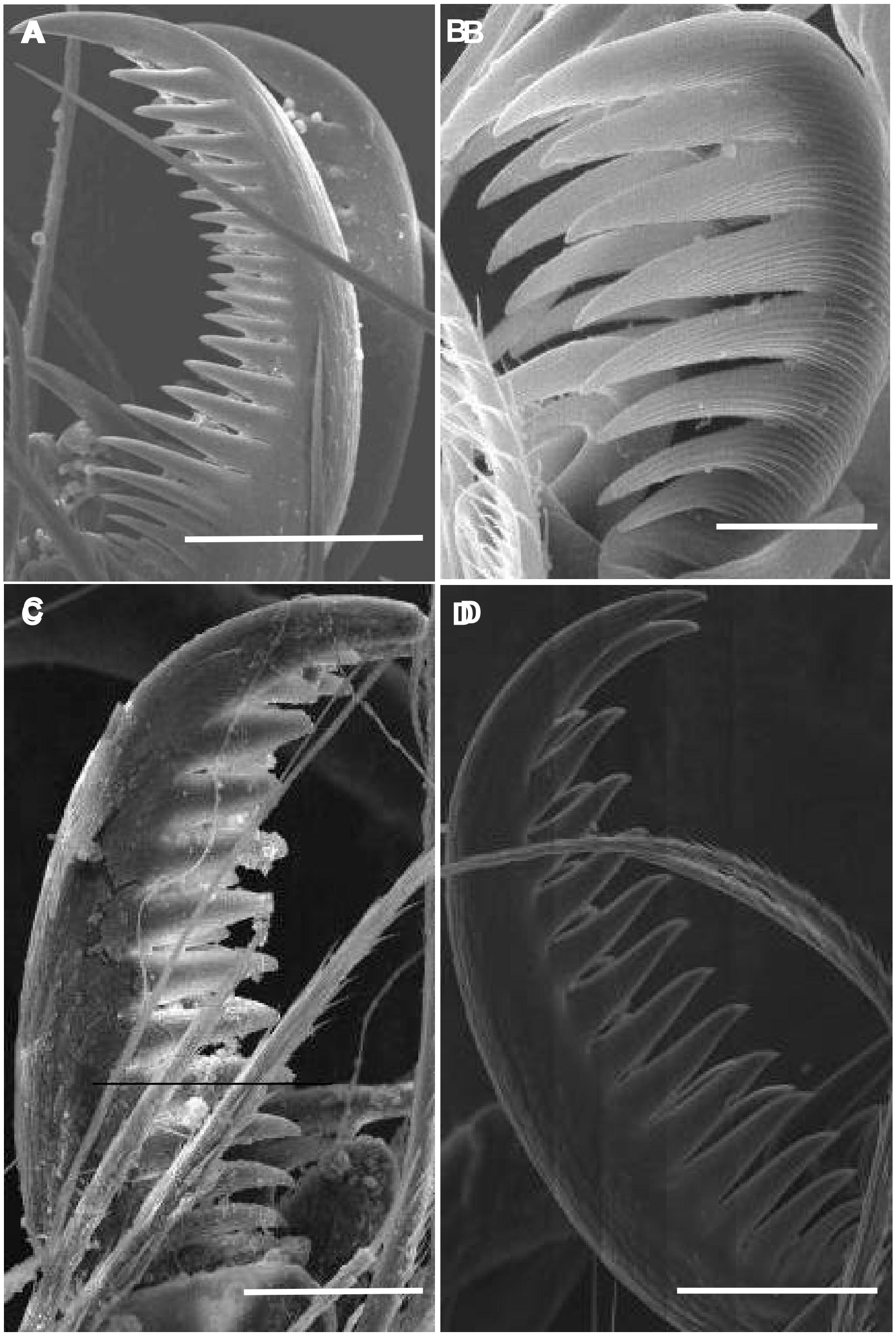

Fig. 5-7. Comparison of tarsal claw structures. (A) Guangxi *Scyloxes* sp. 1 showing 21 teeth on uniserrate claw, scale bar = 50 μm; (B) *Scytodes cavernarum* showing 8 teeth on uniserrate claw, scale bar = 5 μm; (C) *Stedocys leopoldi* showing 12 teeth on uniserrate claw, scale bar = 20 μm; (D) Guangxi *Stedocys* sp. 1 showing 14 teeth on uniserrate claw, scale bar = 50 μm .

It was unfortunate that during both expeditions (one in 2007 and another in 2009) I was unable to collect any males of Yunnan *Scyloxes* sp. 2 from the Swallow caves in Jianshui, Yunnan. Without the males, it is difficult to make further comments. However, images of the cephalothorax of this species (Fig. 5-5D) have led me to consider that Yunnan *Scyloxes* sp. 2 may be closer to *Scyloxes* than to *Stedocys* in terms of the cephalothorax shape – a gentle slope from the top to the clypeus - and presence of large numbers of dorsal hairs similar to those on the female of Guangxi *Scyloxes* sp. 1 (see Fig. 5-5C and Fig. 5-5E). Similarly the serrula of Yunnan *Scyloxes* sp. 2 (Fig. 5-6A) is bicusped like that of Guangxi *Scyloxes* sp. 1 (Fig. 5-6B).

Guangxi *Stedocys* sp. 1 has palps (see Fig. 5-4) and tarsal claw structures (see Fig. 5-7D) that are very similar to those of *Stedocys leopoldi*. However, what is perplexing is that while *S. leopoldi* has single-cusped serrula, Guangxi *Stedocys* sp. 1 has bicusped serrula (Fig. 5-6B). This difference prevents me from making the conclusion that Guangxi *Stedocys* sp. 1 belongs to the genus *Stedocys*. More morphological and even behavioural information are needed to resolve this issue. However, for now I will name it a *Stedocys* species.

This study suggests that the '*pallida*' clade is derived from a close ancestor of *Scytodes* sp. 1. This is highly possible because both *Scytodes* sp. 1 and most members of the '*pallida*' clade share a similar type of palpal structure – a bifurcated embolus (Fig. 5-4). Other than Deeleman-Reinhold's (1989) illustration of the *S. pallida* Ambon habitus, in which the bifurcated palps are shown but not in clear details (Fig. 5-4), this unusual apomorphic character has never been discussed in the literature. All the taxonomic publications of *Scytodes* to date omit such a bifurcated palp. Research is being undertaken to investigate the function of the 'split' embolus and its role in courtship and mating patterns.

Cat Tien *Scytodes* sp. 6 is nestled between the '*pallida*' and '*Dictis*' clades, which may imply that it is an intermediate species between these two clades. Males of this species possess a palp structure – bifurcate embolus tip with an additional prong on the side - not described in any other scytodid (Fig. 5-4), although with a split embolus tip it shares a similarity with the spiders in the '*pallida*' clade. The carapace pattern of Cat Tien *Scytodes* sp. 6 is also similar to that of the '*pallida*' spiders. Other than its radical palp structure, Cat Tien *Scytodes* sp. 6 draws attention because it resembles *Dictis thailandica* described by Dankittipakul and Singtripop (2010) in having a somewhat similar palp structure (i.e. funnel-shaped embolus with a slender subapical embolic spike) and carapace patterns (i.e. slender median band running longitudinally accompanied by two elongated bands, U-shaped bands laterally). However, SEM images from my study have shown that Cat Tien *Scytodes* sp. 6 possess a three-clawed tarsus which implies that they are not *Dictis*, which have a two-clawed tarsus. Dissection and detailed investigation of the Cat Tien *Scytodes* sp. 6 female genitalia is necessary to ascertain if they have genitalia structures like that of *Dictis* species.

In an upcoming publication, Dankittipakul and Singtripop intend to formally transfer *S. venusta* to the *Dictis* genus (Dankittipakul and Singtripop 2010); this species is placed in the '*Dictis*' clade of my cladogram (this study). They used the presence of two tarsal claws as the main diagnostic feature when classifying these spiders in the *Dictis* genus. My molecular and morphological studies support Koch's (1872) proposal and Dankittipakul and Singtripop's (2010) study. Analysis of the tarsal claws indicates that all the members of the '*Dictis*' clade are two-clawed. However, another character that distinguishes *Dictis* from *Scytodes* is the presence of heavily sclerotised copulatory pockets in females, which I did not examine in my present study. A colleague (D. Court) did, however, dissect the female genitalia of a few species from this clade and found that *Dictis* have a different female genital morphology from *Scytodes* (Fig. 5-8). *Dictis* females possess a paired vulva each side comprising of three main parts: atrium, receptaculum and posterior pouch (Fig. 5-8D). These structures have either been modified or are absent in *Scytodes* females. This suggests that female genitalia can be used as another diagnostic character to differentiate *Dictis* from other scytodid genera. I further present another feature that may be diagnostic for *Dictis*: a short tooth-like prong that emerges close to the tip of the male embolus (Fig. 5-4). My molecular analysis is the first to address the possibility of *Dictis* as a sister group to *Scytodes*, and I recommend that a full revision of the genus *Dictis* be made so that this relationship can be more accurately analyzed. The molecular and morphological data cast new light on several contentious issues in scytodid systematics, and they highlight some topics particularly worthy of further research. Placement of some of the species (e.g. Guangxi *Scyloxes* sp. 1, Guangxi *Stedocys* sp. 1 and Yunnan *Scyloxes* sp. 2) treated in this study is provisional pending a generic revision of the family Scytodidae.

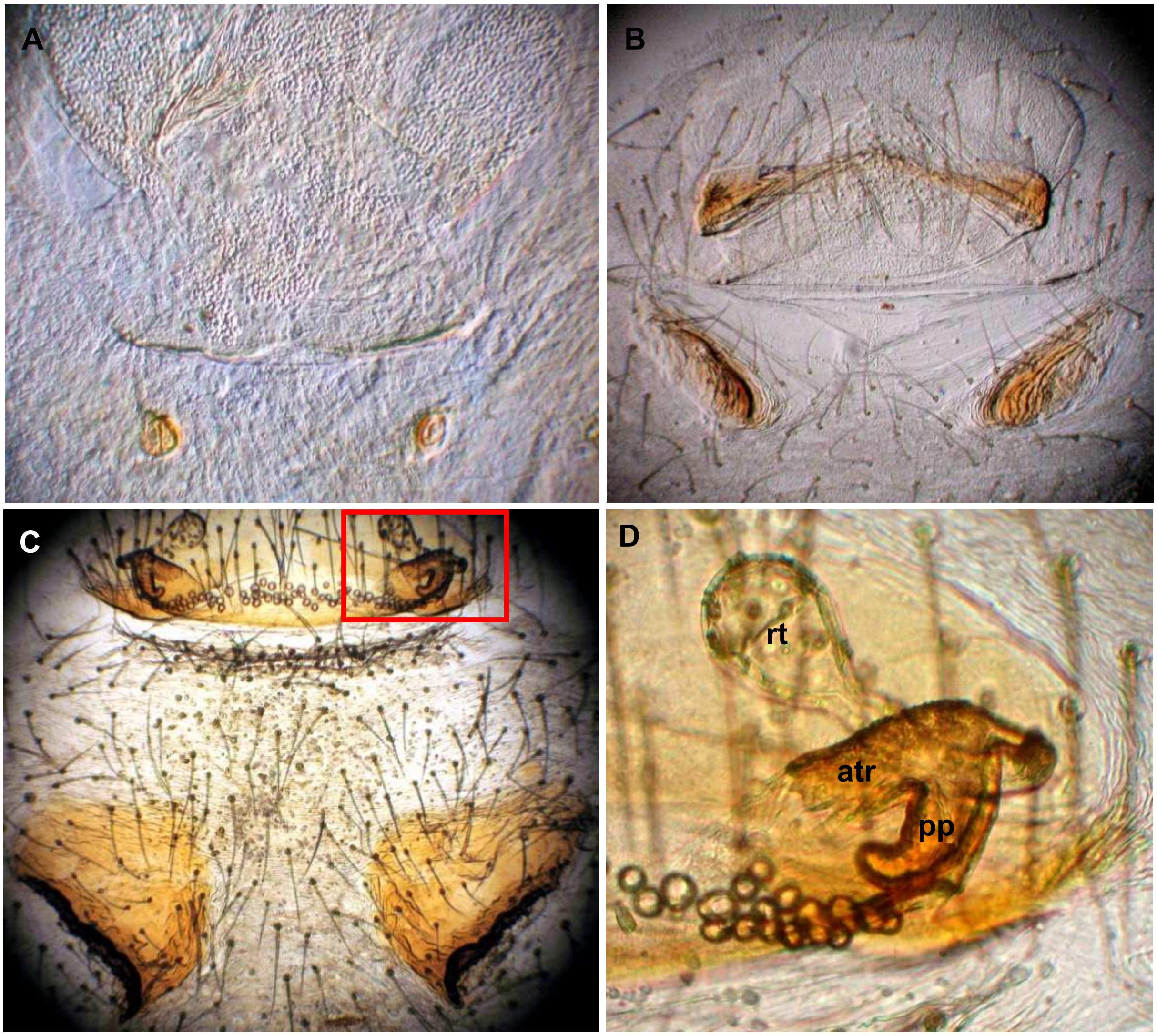

Fig. 5-8. Female genitalia. (A) Singapore *S. pallida*. (B) *Scytodes* sp. 1. (C) Singapore *Dictis* sp. 4. (A-C) Internal genitalia of females, dorsal view. (D) Detail of right part of internal genitalia (inside the red box) of Singapore *Dictis* sp. 4, showing the atrium (atr), posterior pouch (pp) and receptaculum (rt).

Intergeneric relationships

The character that unites all the spiders of the family Scytodidae is their habit of projecting, over considerable distance, a sticky substance from the chelicerae (Gertsch 1979; McAlister 1960). This behaviour is present in all species so far known, but absent in the related families Sicariidae and Drymusidae. According to Lehtinen (1986), the unnamed new subfamily within Scytodidae would be supported by one synapomorphy, the pointed projection of the colulus, while retaining plesiomorphic conditions for other character systems, namely male palp configuration, branched vulva, circular cheliceral spitting orifice, lack of female copulatory pockets, and type of web. The lack of male palpal tarsus extension seems unproblematically plesiomorphic, as it has never been reported in the closely related families (Sicariidae, Drymusidae, Periegopidae; Platnick et al. 1991, Forster 1995). The venom outlet, also functioning as fang spitting orifice, is simple, slit-shaped in *Stedocys leopoldi* (Labarque et al 2009: fig. 42) while it is prolonged in a basal incision in the *Scytodes* species illustrated so far (Suter and Stratton 2005: fig. 11).

However according to Labarque et al. (2009), the remaining characters mentioned by Lehtinen (1986) need further consideration. The lack of a pair of female copulatory pockets behind the epigastric furrow may or may not be plesiomorphic, as they are present in several *Drymusa* species (Valerio 1971: fig. 5; Bonaldo et al. 2006: fig. 5), a putative sister group of Scytodidae (Platnick et al. 1991). Recent progress on the taxonomy of *Scytodes* revealed wide variability in internal female genitalia, including configurations quite similar to those found in *Stedocys* species (e.g., Rheims et al. 2007: fig. 63; Brescovit and Rheims 2001: fig. 30). Along with the male palpal tarsus extension, *Stedocys* males lack the apical blunt macrosetae characteristic of other scytodids (Fig. 5-5; Giltay 1935, fig. 3; Lehtinen 1986, fig. 7; Ono 1995, fig. 4). These macrosetae were illustrated in *Scytodes* (Fig. 5-5; Rheims and Brescovit 2000–2001, 2004; Rheims et al. 2005, 2007) and *Dictis*. The type of web constructed may also not be a good plesiomorphy because all the scytodid species I have studied so far build irregular webs although the size of the web may differ between large and small-sized species. To date no one has been able to examine the other two genera, *Soeuria* Saaristo, 1997 and *Scyloxes* Dunin, 1992, and so on this point the literature is not conclusive (Saaristo 1997).

Despite the above, analyses of morphological data had led Larbarque et al (2009) to suggest that *Stedocys* should be a sister group to all other scytodids. Their paper states, “the extension of the male palpal tarsus bearing blunt macrosetae points to the monophyly of all Scytodidae except *Stedocys*. The remaining characters have a more complex distribution or are poorly known, but they are overall compatible with a placement of *Stedocys* as the sister group of all other genera of Scytodidae". As a member of Scytodidae, *Stedocys* shares with the related families Drymusidae the presence of bipectinate prolateral claws on both legs I and II and a tracheal system with a unique median apodemal lobe (Forster 1995; Ramírez 2000). Its position, however, could not be resolved. Future analyses will have to include additional species, to further test this hypothesis and to test the monophyly of *Stedocys*.

Dunin (1992) stated that *Scyloxes* may be an intermediate genus between *Loxosceles* and *Scytodes* because it shares characters from both genera. Like *Scytodes*, *Scyloxes*' cephlothorax is oval in shape and very high, but there are no parallel and radial stripes on carapace. Like *Loxosceles*, the male bulb is sub-distal. It is interesting to note that on my discussed tree the positions of Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2 are somewhat nestled between the *Loxosceles* and *Scytodes*.

The lack of the tarsal extension on the palps of *S. magna*, *Scyloxes* and *Stedocys* may represent a plesiomorphic character, however, the possibility that they might have some highly derived features is not excluded.

It is clear from some of the unexpected findings that a more extensive taxon sample especially of the genera, *Scyloxes*, *Soeuria* and *Stedocys,* will be needed in order to resolve tribal and generic level relationships. Additional species are needed for a more rigorous test of the monophyly of the genera. Scytodids have received far less systematic attention than other spider groups such as salticids, araneids, linyphiids, and pholcids, and to date there has been no overarching systematic revision of the family. My results reveal preliminary patterns that may inform future detailed systematic work. At present all 228 species are placed in five genera (Platnick 2010). From Brignoli's (1976) many drawings of secondary genital organs, it is apparent that several genera are involved; Brignoli however did not mention the possibility of establishing more genera. Characters such as cephalothorax dimensions, eye distance, and stridulatory tubercles on the medial palpal femur could be helpful for systematic revisions of the family.

CONCLUSION

I have here presented the first detailed phylogenetic hypothesis of Scytodidae. My analyses strongly support the monophyly of Scytodidae and *Dictis* but appear not support the monophyly of other genera, *Scyloxes*, *Scytodes* and *Stedocys*. Scytodids are roughly divided into New World and Old World clades, and the Old World scytodids can be further divided into African and Asian clades. Concurrent with my molecular analysis, morphological analysis of the body shape, size and patterns, genital structures, and tarsal claws were carried out. In my dataset, there were numerous species that I attempted to identify using various papers containing *Scytodes'* descriptions. Despite the efforts, there are still over 25 unidentified species which I am confident are new, undescribed scytodids. These are mostly from Asia. This is not surprising because the sampling of spitting spiders in this region has been limited. In addition, species such as '*Scytodes*' *magna* are in need of re-description and erection of new genera. Reconstruction of the phylogenetic tree should be based on more genes such as 12S and CYTB, as well as morphological data such as genital structures, spinnerets and legs morphology.

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CHAPTER 6

THE EVOLUTION OF SOCIALITY IN SPITTING SPIDERS: A MOLECULAR PHYLOGENETIC PERSPECTIVE

ABSTRACT

Scytodids are primarily known for their unusual prey catching strategy in that they expel sticky spit onto their prey, a predation method not observed in other spiders. Another unique feature of scytodids is that they exhibit various forms of social behaviour, making them an excellent group for studying patterns of social evolution in spiders, only a minority of which exhibit sociality. By mapping social behavioural characters onto molecular phylogeny of the family Scytodidae, I tested the hypotheses about scytodid social evolution. Here I show that sociality may have evolved at least four times in Scytodidae. In the phylogeny, subsocial species appear towards the tip clades and they are not monophyletic, suggesting that the subsocial scytodids are derived. Maternal care as the antecedent behaviour to sociality is thus supported. The dominant trend in the evolution of scytodid sociality is the presence of extended maternal care and tolerance among siblings. Interestingly, a solitary way of life occurred with ancestral habitats - the caves and leaf litter. This could be due to prey scarcity or an anti-predator strategy. The evolution of (sub)social spiders may be due to changing environments. This study should be considered inconclusive because more scytodid species need to be included in the analysis. However, it has significant implications for the exploration of the causes and consequences of social evolution in this group of spiders.

INTRODUCTION

Sociality is a major innovation in the history of life. Understanding how social behaviour evolved and how it is maintained has thus been a focus of evolutionary, behavioural, ecology, genetic and molecular biological research (e.g. Darwin 1859; Hamilton 1964; Wilson 1971; West Eberhard 1975; Costa and Fitzgerald 1996). Sociality is rare in spiders: out of 40,000 described species (Platnick 2010), only about 40 are considered social or subsocial (Avilés 1997; Whitehouse and Lubin 2005; Agnarsson et al. 2006; Lubin and Bilde 2007). Despite being in the minority, social and subsocial spiders are widely distributed in 10 distinctly unrelated families (Agelenidae, Amaurobiidae, Desidae, Dictynidae, Eresidae, Oxyopidae, Scytodidae, Sparassidae, Theridiidae and Thomisidae) and thus differ in many morphological, life history, behavioural, and reproductive traits. Social species are predominantly found in the tropics and subtropics, with their highest concentration being in the neotropic region of South America (Avilés 1997). While most of subsocial species occur in tropics and subtropics, some species extend to temperate regions. Social spiders can occur in diverse habitats (tropical moist forests: Levi et al. 1982; Riechert et al. 1986; dry thornbush and semideserts: Kraus and Kraus 1988; Crouch and Lubin 2000; caves: Machado 2000; Almeida-Silva et al 2009; Chapter 2); social behaviour among spiders is sufficiently variable, ranging from solitary, subsocial to social. Furthermore, sociality has arisen recently and repeatedly as many as 18 times in spiders (Agnarsson et al. 2006). The majority of social species are found in the family Theridiidae, which contains 11-12 social species in three genera (*Achaearanea*, *Anelosimus* and *Theridion*) representing nine independent origins of sociality (Agnarsson 2006; Avilés et al. 2006; Agnarsson et al. 2006). The Eresidae, the second-most abundant group, contains three independent origins of sociality all found within the genus *Stegodyphus* (Kraus and Kraus 1988; Johannesen et al. 2007). Thus, social spiders can be viewed as ideal evolutionary experiment with independent replicates both within and across several families (Lubin and Bilde 2007).

Spiders of the family Scytodidae are well known for their unusual prey catching strategy of expelling sticky spit onto their prey, a predation method not observed in other spiders (Foelix 1996). Another unique feature of scytodids is their social behaviour, which is rare in spiders. Members of Scytodidae exhibit various forms of social behaviour ranging from solitary individuals to subsociality with the degrees of extended maternal care. For example, Australian *Scytodes fusca* is known to exhibit complicated social structure in terms of web sharing and prey capture (Bowden and Jackson 1988). *S. intricata* from Panama (Eberhard 1986), *Scytodes* sp. from the Philippines (Li et al. 1999), *S. pallida* from Singapore and Malaysia (Chapter 4), *Scytodes* sp. 1 from Singapore (Chapter 4), *S. gooldi* and *S. rubra* from South African (Chapter 3) show extended maternal care of eggs and young. *Scytodes socialis* exhibits an even higher level of social behaviour (i.e., many adults sharing a nest)(Miller 2006). The other known *Scytodes* species are solitary (Dabelow 1958; Valerio 1981; Eberhard 1986; Li et al. 1999; Chapters 2, 3, and 4). The variation in sociality among the closely related species within Scytodidae presents a rare opportunity to address a number of important questions in scytodid social evolution.

The evolution of sociality is usually preceded by two adaptations: web-building and maternal care (Avilés 1997). A three-dimensional web has been frequently considered as a preadaptation for sociality (Shear 1970; D'Andrea 1987), and based on Agnarsson's (2004) phylogenies, sociality is concentrated where maternal care and three-dimensional web overlap. Sociality and web-building in scytodids are both rare attributes which give the impression that scytodids do not seem to fit into the current understanding of spider sociality; hence this makes them a very suitable and useful system for studying the evolution of sociality. Scytodids may offer an independent opportunity within spiders to explore the role of maternal care - may be in the form of feeding by regurgitation, offering of prey or the female herself as a food depot (Kullmann 1972; Avilés 1997; Whitehouse and Lubin 2005; Lubin and Bilde 2007; Chapters 3 and 4) - in the transition to sociality, what is known as the 'maternal care or subsocial pathway' to sociality. Using the recorded behaviour and biology of 21 species of solitary and subsocial members (Dabelow 1958; Valerio 1981; Eberhard 1986; Bowden and Jackson 1988; Bowden 1991; Li et al. 1999; Li and Kuan 2006; Miller 2006; Yap and Li 2009; Chapters 2, 3 and 4) and the partial molecular phylogenetic tree for these species reconstructed in Chapter 5, in this chapter, I tested the origin and evolution of sociality in scytodids by mapping the social behavioural traits of these 21 scytodid species/populations onto the molecular phylogenetic tree.

Interestingly, social spiders appear to occupy a set of habitats more restricted than those available to the phylogenetic lineages in which they occur (Avilés 1997; Avilés et al. 2007). Recent studies exploring the ecological factors responsible for the relationship between sociality and latitude or altitude suggest how ecological factors shape the origin and evolution of sociality (e.g. Reeve, 1991; Packer 1990, Eickwort et al. 1996; Richards 2000; Kranz et al. 2002; Avilés and Bukowski, 2006; Jones et al. 2007; Avilés et al. 2007; Powers and Avilés 2007; Purcell and Avilés, 2007). Scytodids are known to occur in a wide range of habitats, including caves (Bristowe 1952; Roewer 1962; Ono 1995; Rheims and Brescovit 2001; Chapter 2), leaf litter, under logs or stones (Rheims and Brescovit 2001; Rheims et al. 2005; Chapter 3), aerial plant leaves (Eberhard 1986; Li et al. 1999; Rheims and Brescovit 2000, 2004; Miller 2006; Yap and Li 2009; Chapter 4) and even on tree trunks (Bowden and Jackson 1988). However, how the sociality of scytodid species is associated with habitats in which sociality occurs is unknown.

The evolution of troglobites is often considered a unidirectional, irreversible, evolutionary dead-end (cited in Predini et al. 2010). Reversal, i.e. the recolonization of endogean or epigean habitats by hypogean taxa, is assumed to be prevented through competitive exclusion by species already occupying, and better adapted to, those habitats (Conway Morris 1995). Endogean or epigean generalists are thought to possess a wider range of features allowing them to adapt to changing environmental pressures better than hypogean specialists, used to environmental stasis. The phylogeny of Typhlochactidae (Scorpiones) challenges the conventional wisdom that ecological specialization (stenotopy) is unidirectional and irreversible, falsifying Cope's law of the unspecialized and Dollo's law of evolutionary irreversibility. Troglobitism is not an evolutionary dead-end: endogean scorpions evolved from hypogean ancestors on more than one occasion (Prendini et al. 2010). Thus, in this chapter, I also examine the relationship between sociality of scytodid species and their habitats by mapping the habitat of each species onto the molecular phylogenetic tree. I predict that the troglobitic condition may be ancestral in Scytodidae (*S. magna*), evolved to a trogloxitic condition (as in *S. univittata*, a species considered occasional visitors to cavernicolous habitats), and then reverted back to the troglobitic/hypogean condition (Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2). The endogean condition then evolved once from the hypogean condition in Yunnan *Scytodes* sp. 3.

The aim of this study is to interpret patterns of evolution of sociality in Scytodidae using a phylogenetic framework by addressing a few important questions. Does the evolution of social behaviour represent a convergent adaptation to ecological conditions, reflect phylogenetic constraints, or both? How often has sociality evolved, and how easily can it be lost once evolved?

MATERIALS AND METHODS

Scytodid species

To test the hypotheses for the origin and evolution of scytodid sociality, I examined the patterns of social behaviour available from 21 species/populations of scytodids (Chapters 2, 3 and 4) from three genera *Dictis*, *Scyloxes* and *Scytodes* collected from China, Malaysia, Singapore, South Africa, Thailand, the Philippines, and Vietnam (Table 6-1) by mapping social behavioural and reproductive characters onto the phylogenetic tree (Chapter 5).

Table 6-1. Information on locality and social status of the 21 scytodid species/populations used in mapping social behavioural traits on the phylogenetic tree.

Species	Locality	Social status	Reference
Scytodes fusca	Malaysia: Batu caves, Selangor	Solitary	This study
Scytodes cavernarum	Malaysia: Gua Angin, Gunung Senyum, Pahang	Solitary	This study
Philippines *Scytodes* sp. 2	Philippines: Biak-na-bato National park, Luzon	Solitary	This study
Scytodes magna	Malaysia: Batu caves, Selangor	Solitary	This study
Guangxi *Scyloxes* sp. 1	China: Xi'an caves, Fengshan county, Guangxi	Solitary	This study
Yunnan *Scytodes* sp. 3	China: Xishuangbanna Tropical Botanic Gardens, Yunnan	Solitary	This study
Scytodes gooldi	South Africa: Hanglip Picnic Spot, Limpopo	Subsocial	This study
Scytodes rubra	South Africa: Mkuze Game Reserve, Kwazulu-Natal	Transition from subsocial to social	This study
Scytodes socialis	Madagascar: Morondava	Subsocial	Miller 2006
Singapore *Scytodes* sp. 1	Singapore: Clementi Woods Park	Subsocial	This study
Malaysia *S. pallida*	Malaysia: Gombak Field Study Centre, Selangor	Subsocial	This study
Singapore *S. pallida*	Singapore: MacRitchie catchment	Subsocial	This study
Vietnam *Scytodes* sp. 4	Vietnam: Ba Vi National park, Hanoi	Transition from solitary to subsocial	This study
Khao Chong *Scytodes* sp. 5	Thailand: Khao Chong National Park, Trang	Transition from solitary to subsocial	This study
Cat Tien *Scytodes* sp. 6	Vietnam: Cat Tien National Park, Ho Chi Minh	Solitary	This study
Thailand *Dictis* sp. 1	Thailand: Phuphapet National park, Trang	Solitary	This study
Hainan *Dictis* sp. 2	China: Mount Wuzhi, Hainan	Solitary	This study
Singapore *Dictis* sp. 2	Singapore: Tampines ecogreen wasteland	Solitary	This study
Singapore *Dictis* sp. 3	Singapore: Bukit Timah Nature Reserve	Solitary	This study
Singapore *Dictis* sp. 4	Singapore: Changi Fairy Point chalets	Solitary	This study
Hanoi *Dictis* sp. 5	Vietnam: Biodiversity Centre, Hanoi	Solitary	This study
Dictis venusta	Singapore: Kent Ridge Park	Solitary	This study

Behavioural and ecological traits

To study the evolution of sociality in the Scytodidae, 19 behavioural characters and one ecological character (Table 6-2) were coded in MacClade version 4.06 (Maddison and Maddison 2000) and mapped onto the most parsimonious tree reconstructed in Chapter 5 (see Figure 5-1). The following 13 behavioural characters were coded and mapped as discontinuous variation:

Character 1: Carrying egg sac in the chelicerae: absent (0); present (1).

Character 2: Foraging during egg-sac guarding: never (0); rarely (defined as once or twice out of 10 times) (1); frequently (defined as 7 or 8 times out of 10) (2).

Character 3: Emergent phase: first instar (0); second instar (1).

Character 4: Behaviour of hatchlings immediately upon emerging: attach to broken sac (0); attach to mother's body (1).

Character 5: Emergents clustering in the natal nest: all spread out in the cage (0); some cluster (1); all cluster (2).

Character 6: Food provisioning by female (i.e. female subdues the prey, but may not necessarily transport prey back to the natal nest): absent (0); present (1).

Character 7: Mother dragging prey back to the natal nest: absent (0); present (1); irrespective of whether she wraps the prey first.

Character 8: Mother feeds alongside brood: absent – the female bites the prey several times, presumably injecting digestive enzymes into the prey, then moves away for her brood to feed (0); present (1).

Character 9: Aggression (i.e. waving/circling of forelegs) from mother towards spiderlings when she is feeding: present – female raises the first pair of legs and waves them about (0); absent – female allows the spiderlings to share in her prey (1).

Character 10: Instar in which natal dispersal first occurred: early second instar (0); late second instar (1); third instar (2).

Character 11: Cooperative prey capture among siblings (i.e. individuals acting together to physically restrain and capture the prey, followed by communal feeding): absent (0); present (1).

Character 12: Food-sharing by captors: absent – the captors “chase” away the non-captors via leg-waving or making sudden jerking motions towards intruders (0); present (1).

Character 13: Stage when cannibalism first occurred: second instar (0); third instar (1); fourth instar (2).

The following six characters were mapped as continuous data:

Character 14: Duration of egg sac guarding. This is also known as the (mean) number of days the spiderlings take to emerge from the egg sac.

Character 15: Mean number of clutches each female produces.

Character 16: Mean total number of spiderlings per species.

Character 17: Mean number of spiderlings per clutch.

Character 18: The time interval between two successive egg-sac production.

Character 19: The time interval between the emergence of spiderlings and the next egg-sac production.

In addition, one ecological character was mapped as discontinuous data:

Character 20: Habitat types: leaf litter (0); under rocks (1); aerial leaves (2); caves (3).

Table 6-2. Data matrix assembled for 21 scytodid species/populations. Numbers 1-13 and 20 represent the characters mapped as discontinuous variation (see text for details)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**20**
Scytodes fusca	1	2	0	0	1	0	0	0	0	1	0	0	0	3
Scytodes cavernarum	1	2	0	0	1	0	0	0	0	2	0	0	0	3
Philippines *Scytodes* sp. 2	1	2	0	0	1	0	0	0	0	0	0	0	0	3
Scytodes magna	0	1	0	0	0	0	0	0	0	2	0	0	0	3
Guangxi *Scyloxes* sp. 1	0	1	0	0	0	0	0	0	0	2	0	0	0	3
Yunnan *Scytodes* sp. 3	1	1	1	1	2	0	0	0	0	0	0	0	0	0
Scytodes gooldi	1	0	1	0	2	1	1	1	1	0	1	1	1	1
Scytodes rubra	1	0	1	0	2	1	1	1	1	2	1	1	1	1
Scytodes sp. 1	1	1	1	0	2	1	1	1	1	1	1	1	1	2
Malaysia *Scytodes pallida*	1	1	1	1	2	1	1	1	1	2	1	1	1	2
Singapore *Scytodes pallida*	1	1	1	1	2	1	1	1	1	1	1	1	1	2
Vietnam *Scytodes* p. 4	1	1	1	1	2	0	0	0	0	0	0	0	0	2
Khao Chong *Scytodes* sp. 5	1	1	1	1	2	0	0	0	0	0	0	0	0	2
Cat Tien *Scytodes* sp. 6	1	2	1	0	1	0	0	0	0	1	0	0	0	2
Thailand *Dictis* sp. 1	1	2	1	0	2	0	0	0	0	0	0	0	0	2
Hainan *Dictis* sp. 2	1	2	1	0	0	0	0	0	0	1	0	0	0	2
Singapore *Dictis* sp. 2	1	2	1	0	0	0	0	0	0	0	0	0	0	2
Singapore *Dictis* sp. 3	1	2	1	0	0	0	0	0	0	0	0	0	0	2
Singapore *Dictis* sp. 4	1	2	1	0	0	0	0	0	0	0	0	0	0	2
Hanoi *Dictis* sp. 5	1	2	1	0	1	0	0	0	0	1	0	0	0	2
Dictis venusta	1	2	1	0	1	0	0	0	0	0	0	0	0	2

Phylogenetic tree

Data for the social behaviour and ecological traits of the 21 taxa were compiled from this study (Table 6-2). Before mapping, the most parsimonious tree with gaps coded as fifth character states was reduced by pruning all outgroups and terminals lacking detailed social behaviour information (i.e. kept only the 21 taxa of interest), and this yielded one strict consensus tree. The characters were mapped onto the most parsimonious tree using different character definitions (e.g. absent/present; never/rarely/frequently) and “trace character” in MacClade version 4.06 (Maddison and Maddison 2003). Parsimony reconstruction methods find the ancestral states that minimize the number of steps of character change given the tree and observed character distribution. For discontinuous data (categorical characters), the ordered states assumption was used. In the case of multiple optimizations, I inspected all equally parsimonious mappings. The number of most parsimonious reconstructions (MPRs) was calculated using the "number of MPRs" item in the Trace menu (Maddison and Maddison 2003). For continuous characters, the squared change assumption was applied.

RESULTS

Behavioural traits

The phylogenetic tree with the social organization information of each scytodid species obtained either from this study or literature is presented in Fig. 6-1. There are at least four independent origins of (sub)sociality indicated on the tree. To date, the subsocial species have been found to only occur within the Old World tribe, 2 origins in African and 2 origins in Asia. The pruned cladogram with the social behaviour traits mapped on is presented in Figs 6-2 and 6-3, respectively. Mapping the discontinuous data yielded a total of 50 optimized trees. A continuum of social habits were found: solitary, quasisolitary (a term used to describe species in which juveniles remain in the maternal nest for a longer duration compared to solitary counterparts (Agnarsson 2006)), subsocial and transition to social. Subsociality is observed only in the genus *Scytodes*. Furthermore, a solitary life style might be the ancestral condition for Scytodidae, and information at this time suggests that the subsocial system evolved from an antecedent condition of maternal care, followed by an elaboration via extension of maternal care through food provisioning and delayed dispersal of the young from the maternal web.

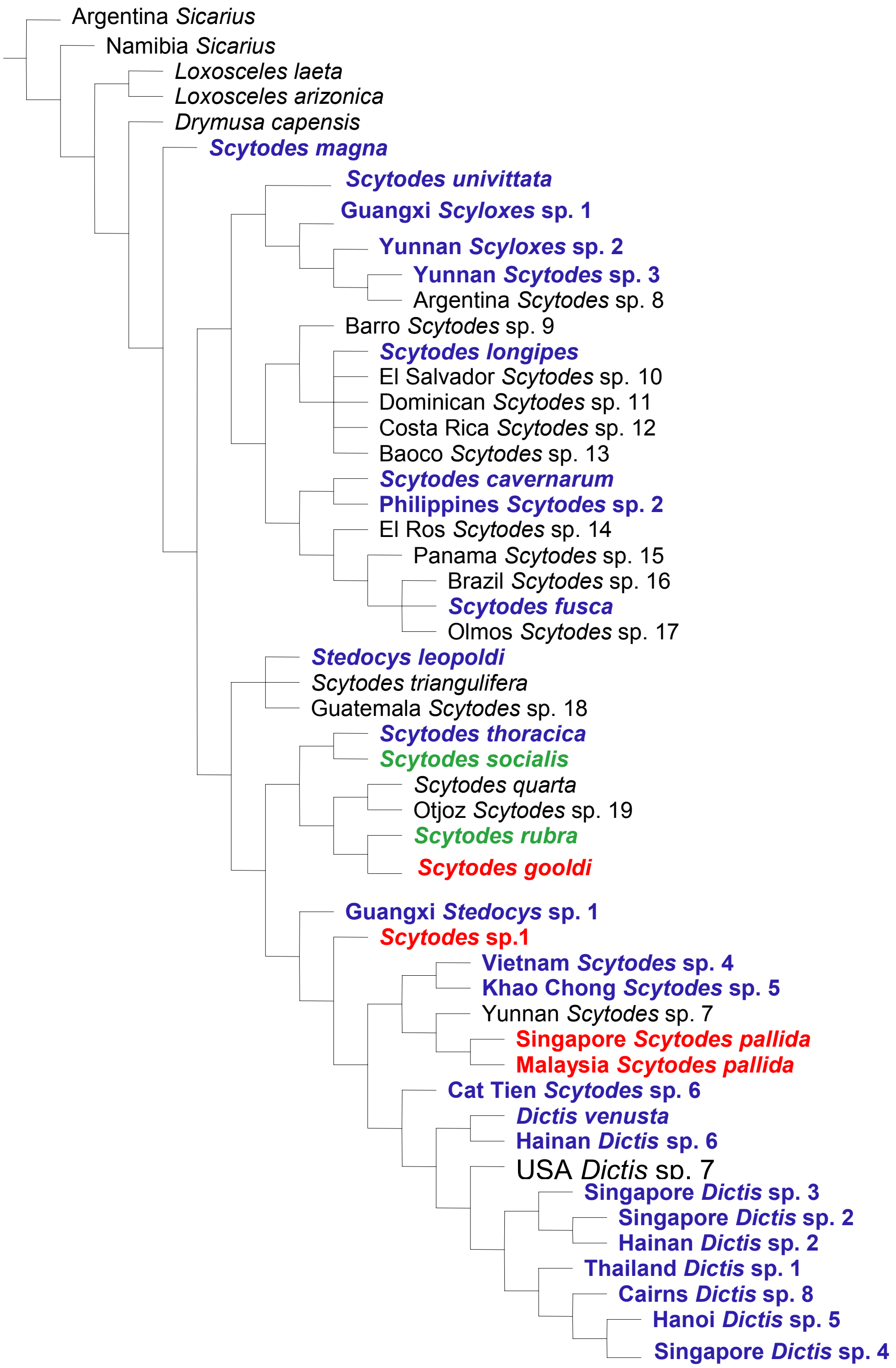
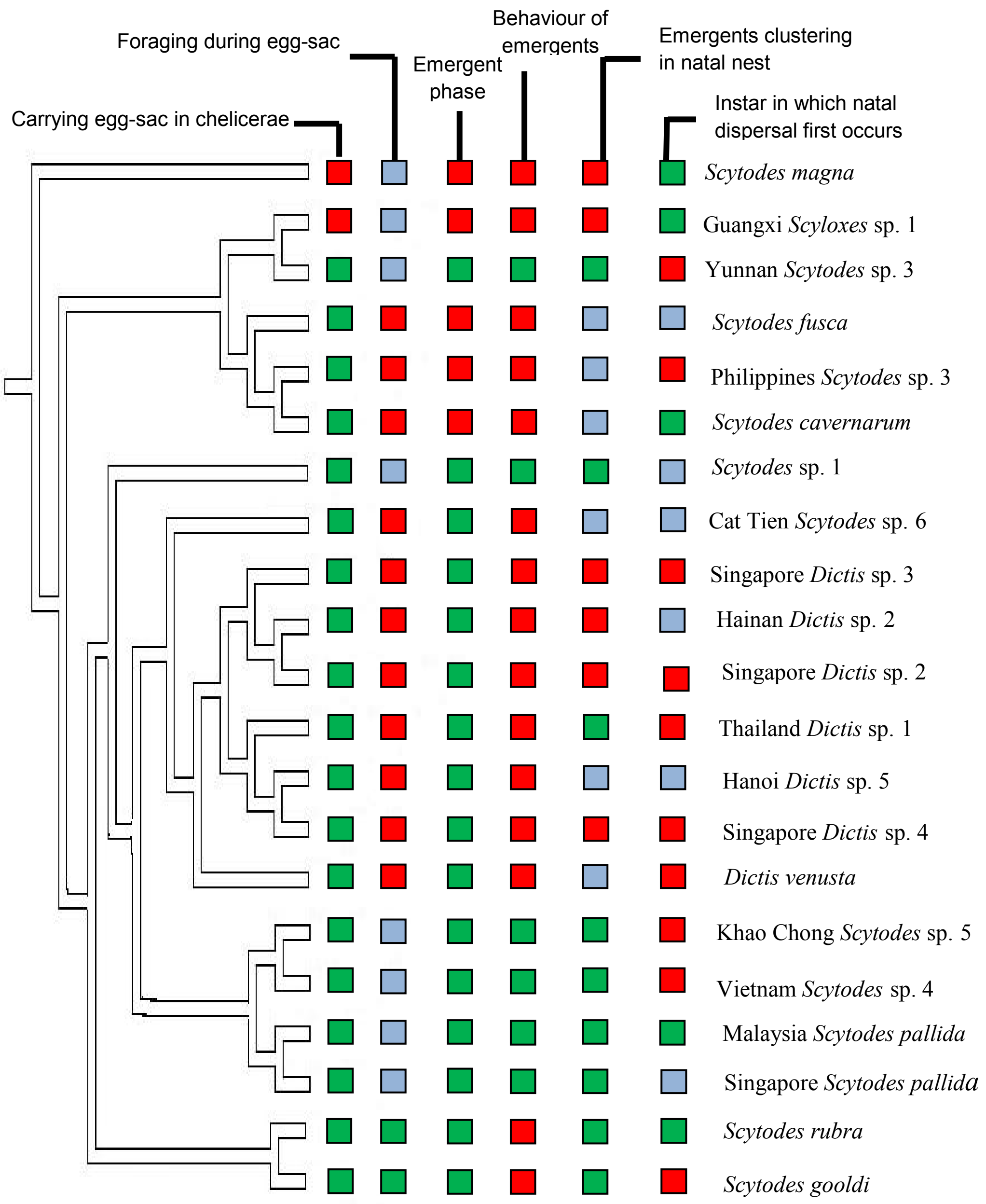


Fig. 6-1. Cladogram showing social status of scytodids from this study and literature. Species in blue are solitary; species in red are subsocial; species in which social state is unknown is in black. *S. rubra* and *S. socialis* (the only two species in green) are termed 'transition to the social state' species.



	Carrying egg-sac in chelicerae	Foraging during egg-sac	Emergent phase	Behaviour of emergents	Emergents clustering in natal nest	Instar in which natal dispersal first occurs
Scytodes magna	🟥	🟦	🟥	🟥	🟥	🟩
Guangxi *Scyloxes* sp. 1	🟥	🟦	🟥	🟥	🟥	🟩
Yunnan *Scytodes* sp. 3	🟩	🟦	🟩	🟩	🟩	🟥
Scytodes fusca	🟩	🟥	🟥	🟥	🟦	🟦
Philippines *Scytodes* sp. 3	🟩	🟥	🟥	🟥	🟦	🟥
Scytodes cavernarum	🟩	🟥	🟥	🟥	🟦	🟩
Scytodes sp. 1	🟩	🟦	🟩	🟩	🟩	🟦
Cat Tien *Scytodes* sp. 6	🟩	🟥	🟩	🟥	🟦	🟦
Singapore *Dictis* sp. 3	🟩	🟥	🟩	🟥	🟥	🟥
Hainan *Dictis* sp. 2	🟩	🟥	🟩	🟥	🟥	🟦
Singapore *Dictis* sp. 2	🟩	🟥	🟩	🟥	🟥	🟥
Thailand *Dictis* sp. 1	🟩	🟥	🟩	🟥	🟩	🟥
Hanoi *Dictis* sp. 5	🟩	🟥	🟩	🟥	🟦	🟦
Singapore *Dictis* sp. 4	🟩	🟥	🟩	🟥	🟥	🟥
Dictis venusta	🟩	🟥	🟩	🟥	🟦	🟥
Khao Chong *Scytodes* sp. 5	🟩	🟦	🟩	🟩	🟩	🟥
Vietnam *Scytodes* sp. 4	🟩	🟦	🟩	🟩	🟩	🟥
Malaysia *Scytodes pallida*	🟩	🟦	🟩	🟩	🟩	🟩
Singapore *Scytodes pallida*	🟩	🟦	🟩	🟩	🟩	🟦
Scytodes rubra	🟩	🟩	🟩	🟥	🟩	🟩
Scytodes gooldi	🟩	🟩	🟩	🟥	🟩	🟥

Code key for character states

Carrying egg-sac in chelicerae:
- Present 🟩
- Absent 🟥

Foraging during egg-sac guarding:
- Never 🟩
- Rarely 🟦
- Frequent 🟥

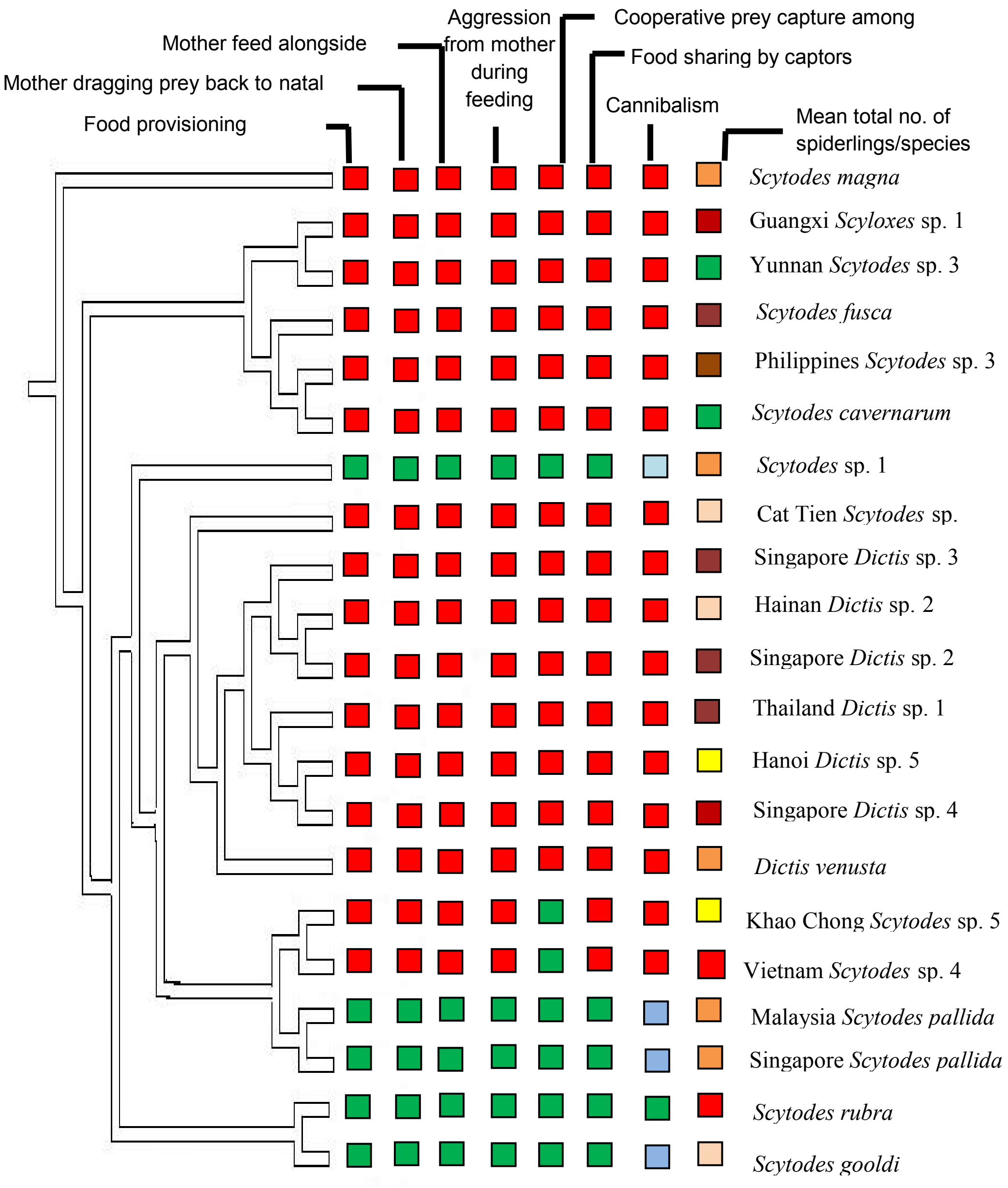
Emergent phase:
- 1st instar 🟥
- 2nd instar 🟩

Behaviour of emergents:
- Attach to broken sac 🟥
- Attach to mother's body 🟩

Emergents clustering in natal nest:
- All spread out 🟥
- Some cluster 🟦
- All cluster 🟩

Instar in which natal dispersal first occurs: Early 2nd instar 🟥 Late 2nd instar 🟦 3rd instar 🟩

Fig. 6-2. Summary of the social behavioural traits exhibited by each of the 21 species/population



Code key for character states

Food provisioning by mother:

Present 🟩

Absent 🟥

Mother dragging prey back to natal nest:

Present 🟩

Absent 🟥

Mother feed alongside brood:

Present 🟩

Absent 🟥

Aggression from mother during feeding :

Present 🟥

Absent 🟩

Cooperative prey capture amongst sibs:

Present 🟩

Absent 🟥

Food sharing by captors: Present 🟩 Absent 🟥

Stage when cannibalism first occurs: 2nd instar 🟥 3rd instar 🟦 4th instar 🟩

Mean total no. of spiderlings/sp.: 7.0 - 10.7 🟩 10.7 – 14.4 🟨 14.4 – 18.1 🟧 18.1 – 21.8 ⬜
21.8 – 25.5 🟫 25.5 – 29.2 🟥 32.9 – 36.6 🟥 40.3 – 44.0 🟫

Fig. 6-3. Summary of the social behavioural traits exhibited by each of the 21 species/population

Two character states were assigned to egg-sac guarding behaviour. Scytodids that do not carry their egg-sacs in the chelicerae maintain close body contact with their eggs. Tending to their egg-sacs is universal among scytodids. However, cavernicolous scytodids (*Scytodes magna*, Guangxi *Scyloxes* sp. 1 and Guangxi *Stedocys* sp. 1 (from personal observations)) that inhabit the dark zones of caves do not carry egg sacs in their chelicerae. In contrast all the other *Scytodes* and *Dictis* females carry the egg sac. Two optimizations arose from the mapping of this trait (Fig. 6-4). One tree shows the possibility that carrying egg-sac in the chelicerae may be plesiomorphic (Fig. 6-4B), while the other represents an apomorphy (Fig. 6-4A). Mapping of the character 'foraging during egg-sac guarding' yielded one tree (Fig. 6-5). Present numerous times (either rarely or frequently) across the 21 taxa, it may be a result of either parallel evolution or simply a behaviour shaped by environmental factors (e.g. high predation pressure). Results show that foraging during the egg guarding phase may be a plesiomorphic behaviour.

In the course of this study, I found that spiderlings of a few species emerged as first instars instead of the usual second instars. I also observed that emergents of a few species would mount their mother's body immediately upon eclosion, as opposed to aggregating (form a tight cluster) in the maternal nest. Species with emergents as first instars might occur only in solitary species because the maternal female might be manipulating the egg-sac so that the spiderlings would emerge sooner and the adult female would then be able to cease caring for the eggs. Spiderlings mounting the female's body may be for protection purposes, thus the more social species that practiced extended maternal care beyond the egg stage would be more likely to evolve this behaviour. With the view that these three behaviour traits should be useful, I included them in my study of evolution of sociality of scytodids. To find whether an evolutionary trend exists in the emergent phase of the spiderlings, two character states were defined: first and second instar. The single optimization showed that only spiderlings of the cave species emerge as first instars (Fig. 6-6). An independent origin in Yunnan *Scytodes* sp. 3, a leaf-litter dwelling species, in which spiderlings evolved to emerge as second instars is hypothesized. 'Behaviour of the emergents' yielded two optimizations (Fig. 6-7). One optimization represented three separate origins for the behaviour in which spiderlings would immediately climb onto the mother's body upon emerging from the egg-sac (Fig. 6-7A) and the second represented two origins with a loss in Cat Tien *Scytodes* sp. 6 + *Dictis* clade (Fig. 6-7B). The character 'emergents clustering in natal nest' similarly yielded two optimizations (Fig. 6-8). All emergents forming a tight cluster upon eclosion is an ancestral state with three losses in one optimization and four in the other.

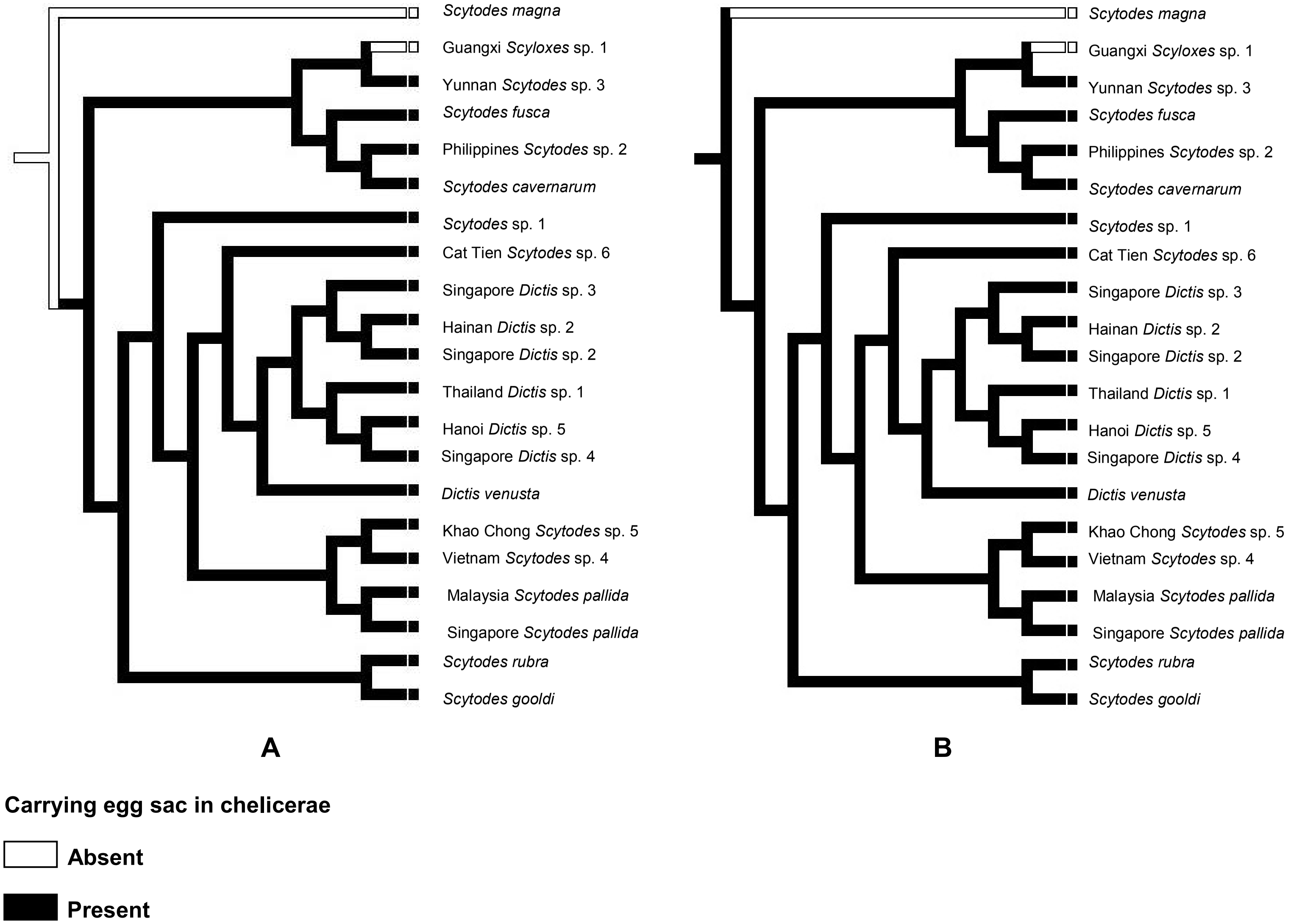


Fig 6-4. Two optimized trees (A and B) of the character: carrying egg-sac in chelicerae; mapped onto the MPT = gaps as fifth character state for 21 taxa

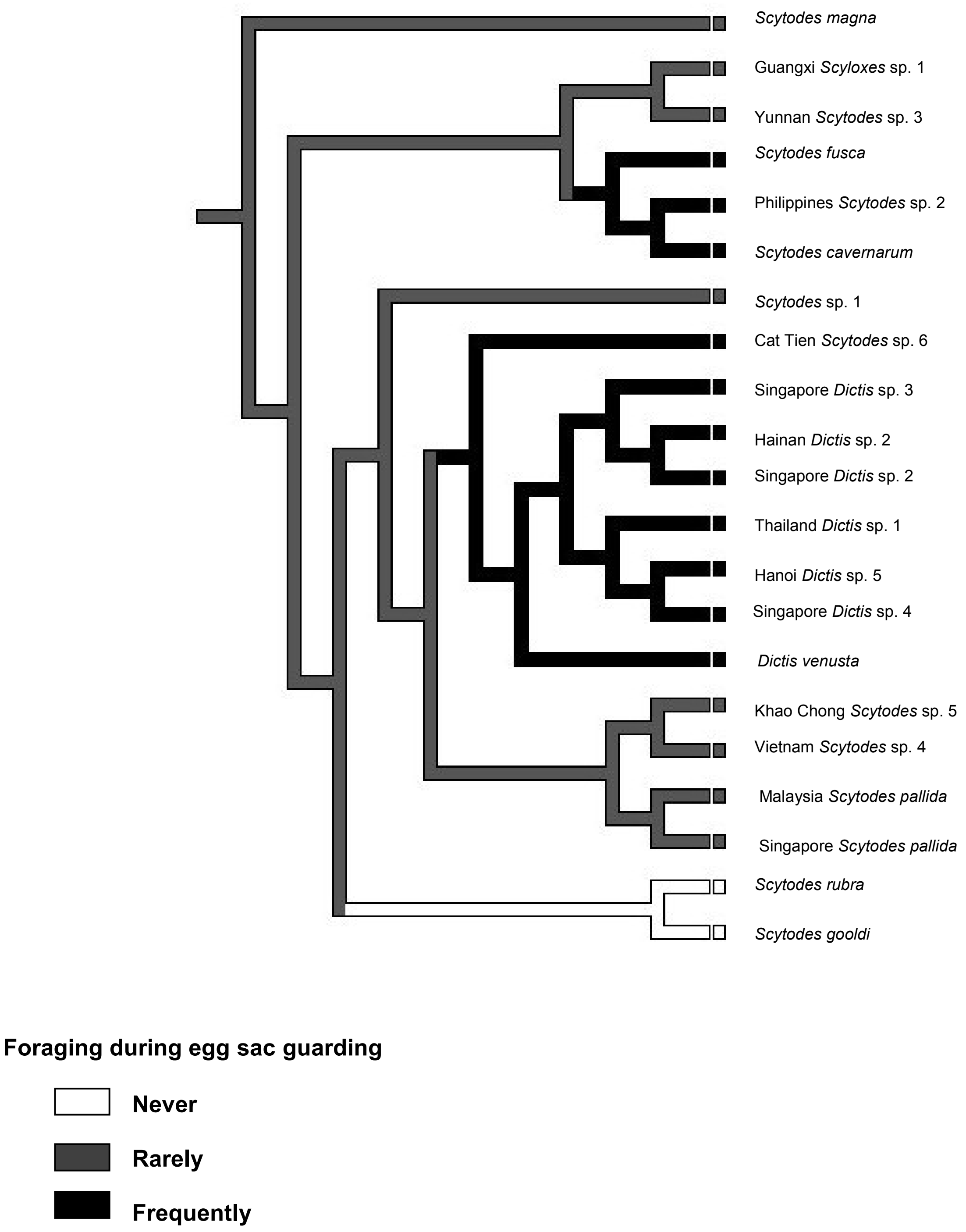


Fig 6-5. Optimized tree of the character: foraging during egg-sac guarding; mapped onto the MPT = gaps as 5th character state for 21 taxa

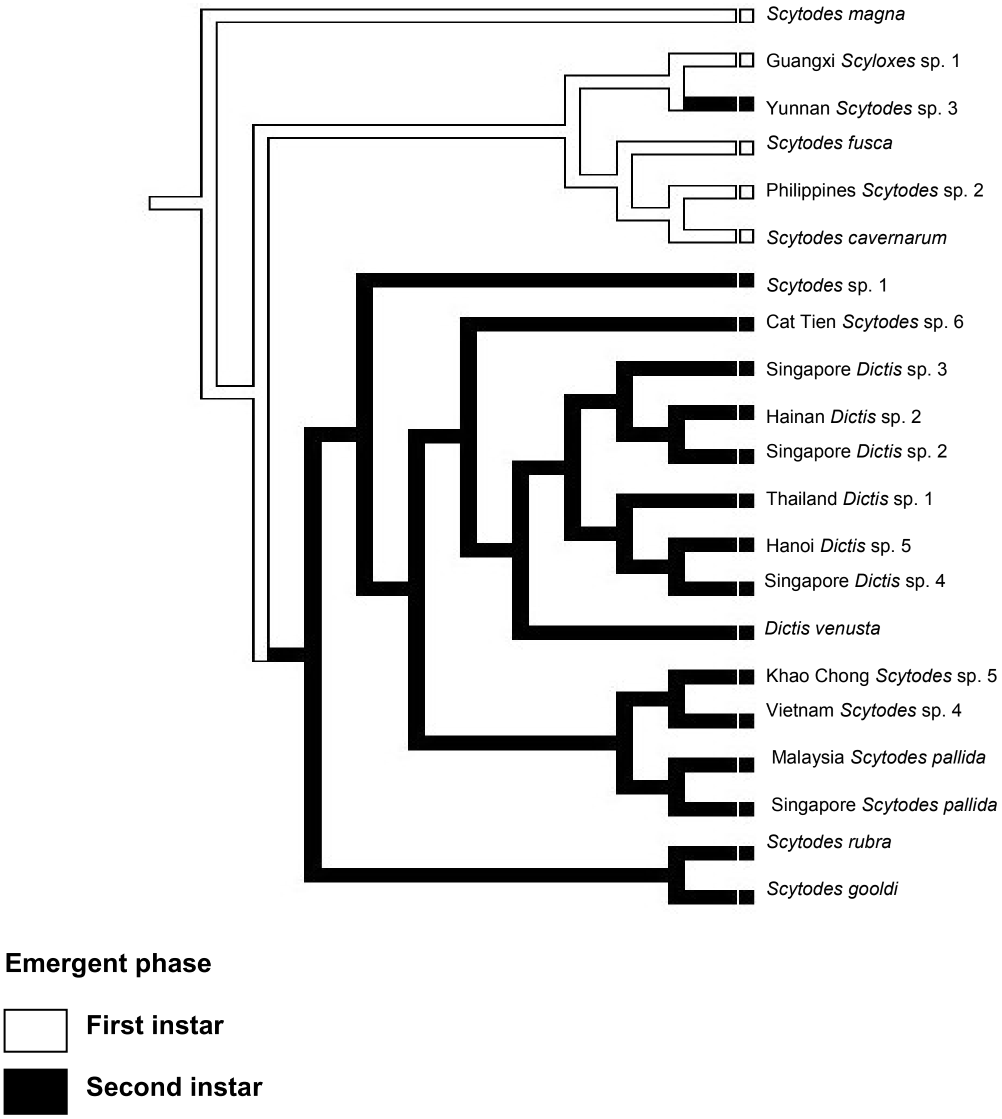


Fig 6-6. Optimized tree of the character: emergent phase mapped onto the MPT = gaps as 5th character state for 21 taxa**.**

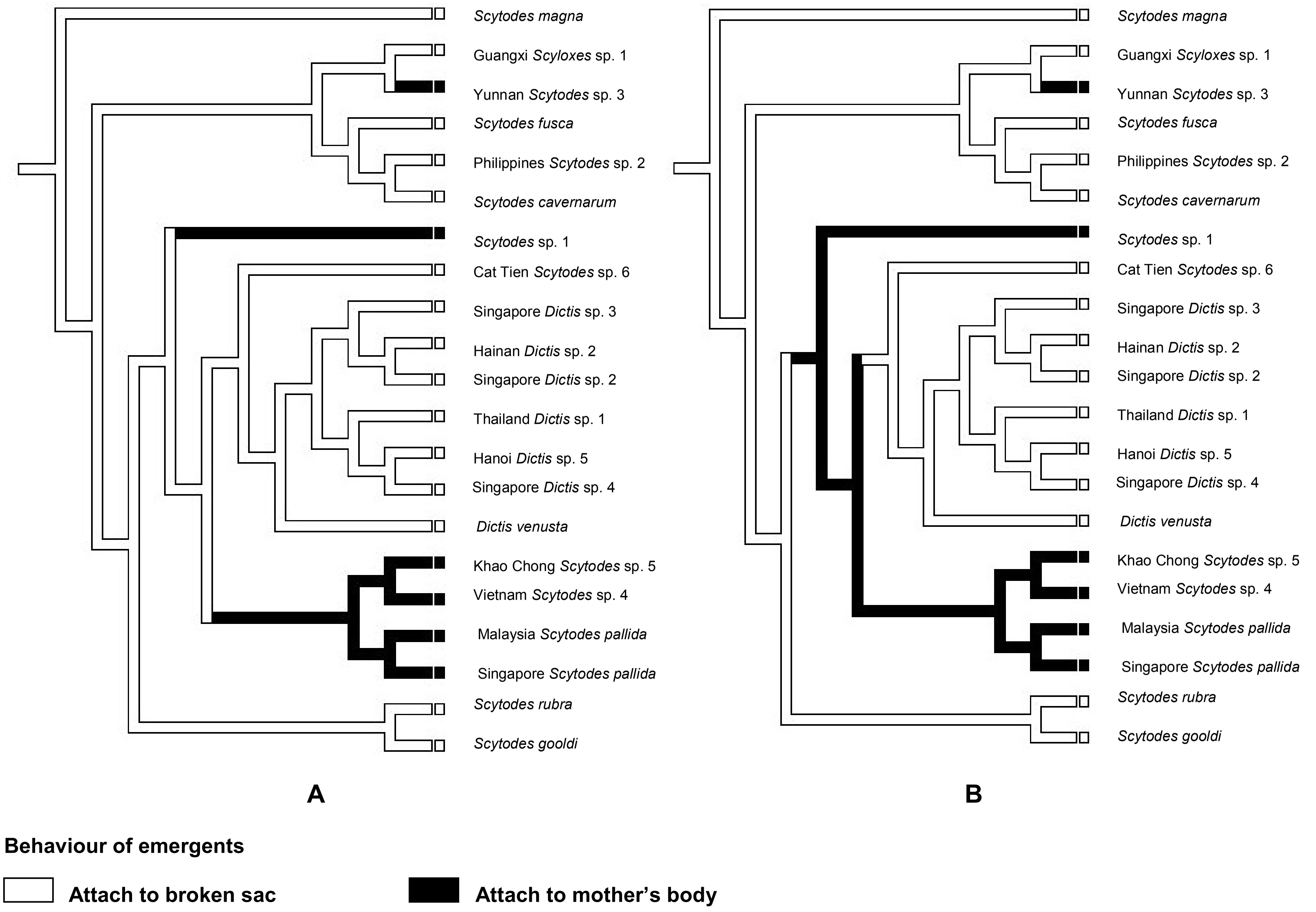


Fig 6-7. Two optimized trees (A and B) of the character: behaviour of emergents; mapped onto the MPT = gaps as 5th character state for 21 taxa.

Fig 6-8. Two of six optimized trees (A and B) of the character: emergents clustering in natal nest; mapped onto the MPT = gaps as 5th character state for 21 taxa.

‘Food provisioning by maternal female’, ‘dragging prey back to natal nest’, ‘mother feeding alongside brood’, and ‘aggression from mother towards spiderlings during feeding’, the traits pertaining to extended maternal care yielded three optimizations (Fig. 6-9). As did the characters ‘cooperative prey capture’ and ‘food-sharing by captors’, which are traits characteristic of tolerance among siblings. Two character states: absent and present were coded. One of the optimizations implied three separate origins; while the other two implied gains and then reversals back to the ancestral state, which is a secondary loss of these six social behavioural traits.

Low rates of cannibalism or cannibalism occurring in the later instars were hypothesized to be traits of the more social species because there should be intraspecific tolerance among group-mates. Three character states, i.e. second, third and fourth instar, were defined for the mapping of ‘stage when first cannibalism first occurred’, and which resulted in five optimizations (Fig. 6-10). Three of them are less parsimonious because they represent loss or reversal back to the ancestral state of early cannibalism or little tolerance among siblings.

To determine evolutionary patterns in the natal dispersal, ‘instar in which natal dispersal first occurred’ and ‘instar in which 50% of spiderlings dispersed’ were mapped. The former which had three character states – early second instar; late second instar; and third instar - yielded one optimized tree (Fig. 6-11), while the latter with four character states, i.e. early second instar; late second instar; third instar; and fourth instar, yielded 12 optimizations (Fig. 6-12). No clear pattern arose but I hypothesize that late dispersal from the natal nest is a derived character that changed from one in which spiderlings started to practice early dispersal. As scytodids undergo fewer numbers of instars as compared to most other spider species (e.g. male *Scytodes* undergo six instars to reach sexual maturity whereas male *Peucetia viridians* undergo nine) throughout their lifetime, I considered dispersal at the third instar stage and beyond to be late (delayed) dispersal. High costs of group-living may have attributed to spiderlings leaving the natal web.

I studied the fecundity and fertility rates because social spiders are known to produce fewer spiderlings than their non-social counterparts due to heavier maternal investment in the brood. I predicted that the more social scytodids would produce overall fewer spiderlings and hence characters 14 to 19 would be useful key indicators of studying the evolution of social behaviour. It turned out that these characters were labile and it was not possible to score them into discrete categories since the values were continuous. The mapped trees of character 14 to 19 are presented in the appendix (Appendices A).

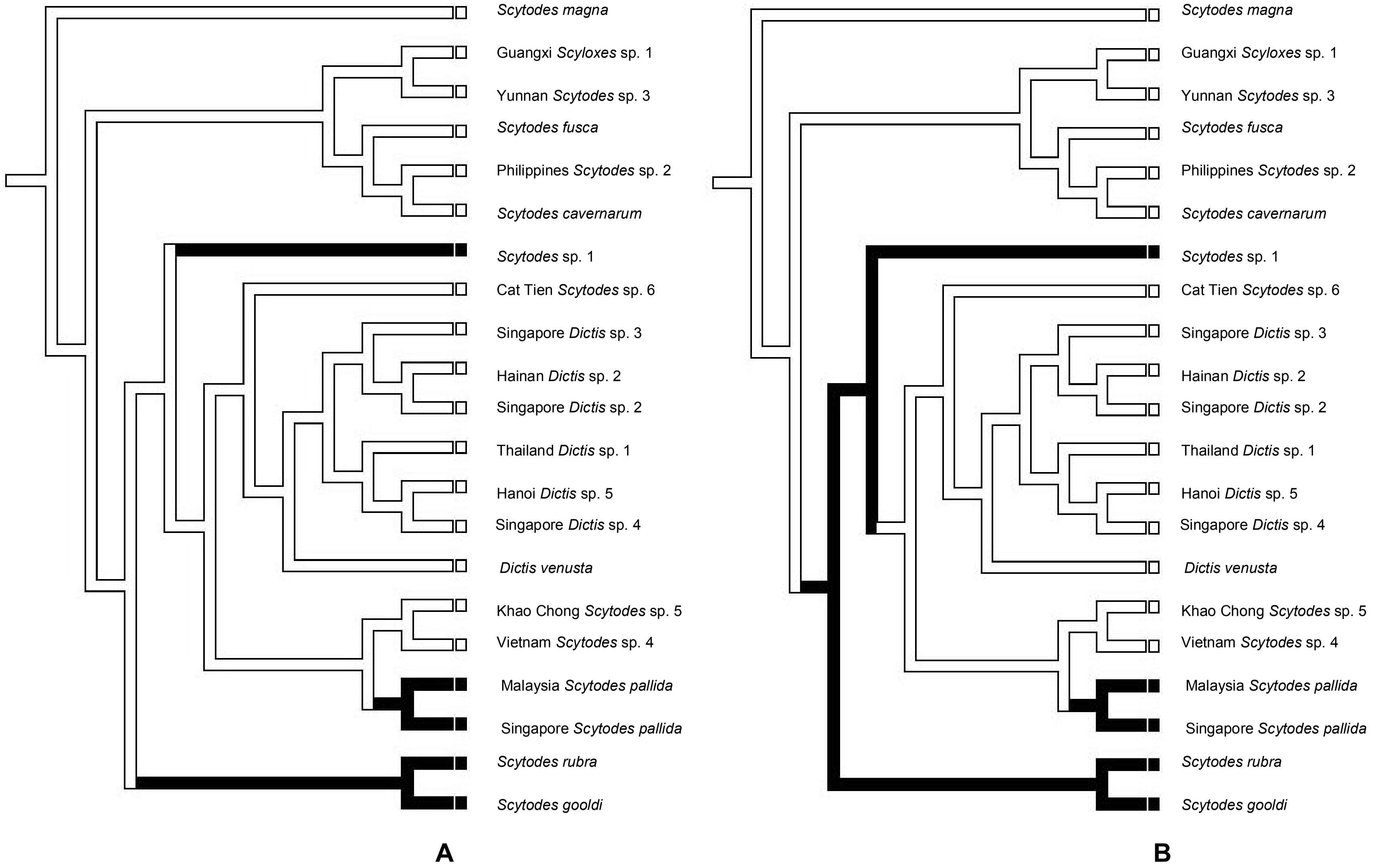
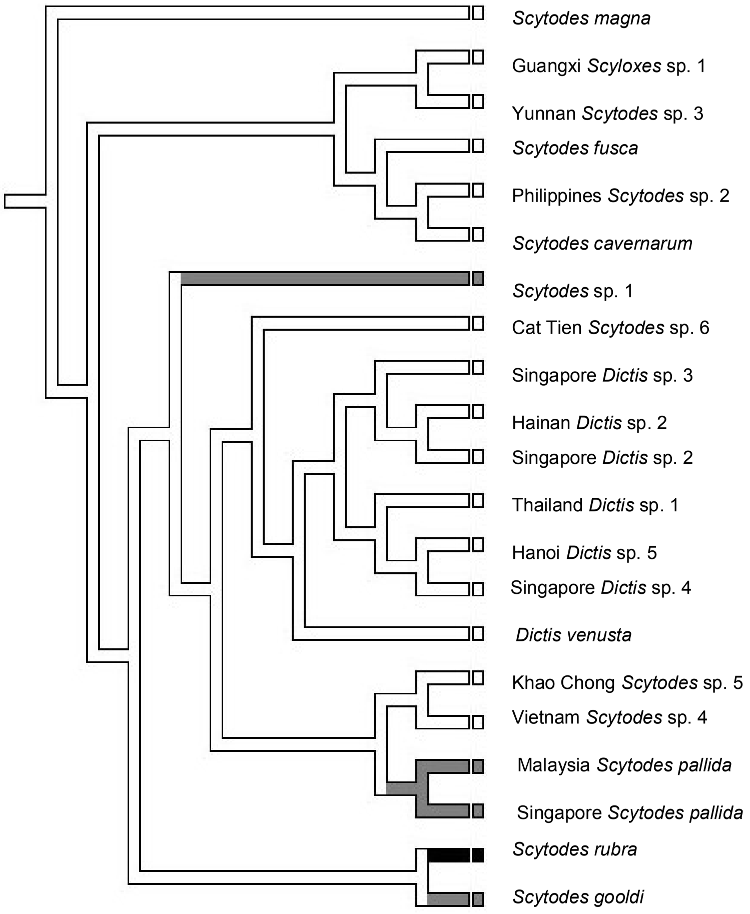

Scytodes magna
Guangxi *Scyloxes* sp. 1
Yunnan *Scytodes* sp. 3
Scytodes fusca
Philippines *Scytodes* sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien *Scytodes* sp. 6
Singapore *Dictis* sp. 3
Hainan *Dictis* sp. 2
Singapore *Dictis* sp. 2
Thailand *Dictis* sp. 1
Hanoi *Dictis* sp. 5
Singapore *Dictis* sp. 4
Dictis venusta
Khao Chong *Scytodes* sp. 5
Vietnam *Scytodes* sp. 4
Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi
A
Scytodes magna
Guangxi *Scyloxes* sp. 1
Yunnan *Scytodes* sp. 3
Scytodes fusca
Philippines *Scytodes* sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien *Scytodes* sp. 6
Singapore *Dictis* sp. 3
Hainan *Dictis* sp. 2
Singapore *Dictis* sp. 2
Thailand *Dictis* sp. 1
Hanoi *Dictis* sp. 5
Singapore *Dictis* sp. 4
Dictis venusta
Khao Chong *Scytodes* sp. 5
Vietnam *Scytodes* sp. 4
Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi
B

Fig. 6-9. Three optimized trees (A, B and C) of the character: cooperative prey capture amongst siblings; mapped onto the MPT = gaps as 5th character state for 21 taxa. These trees apply to the following characters: Food sharing by captors; food provisioning by maternal female; dragging prey back to natal nest; mother feeding alongside brood; and aggression from mother towards spiderlings during feeding.

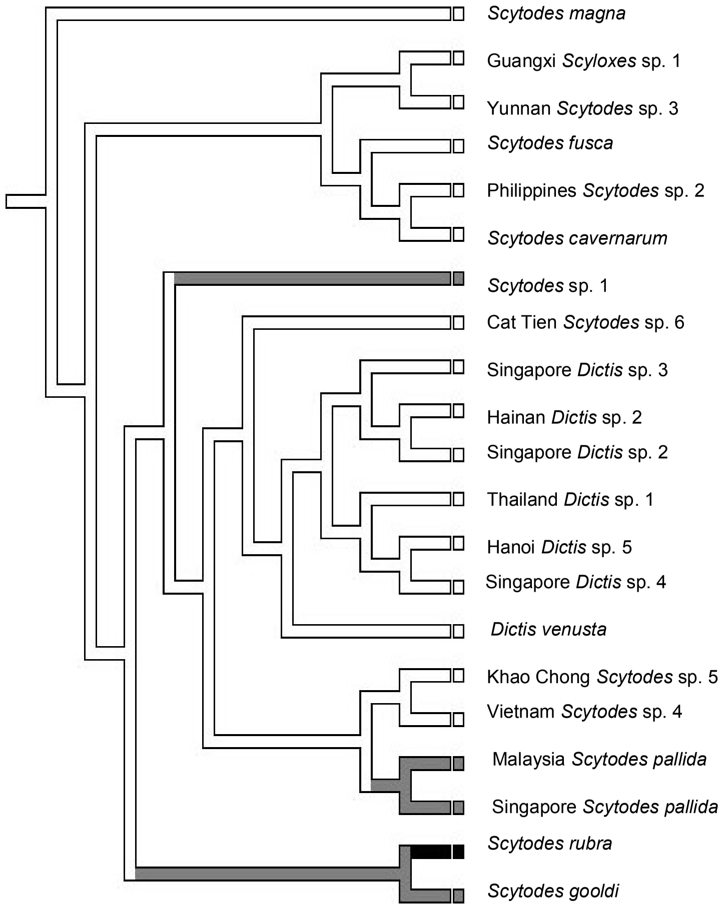
Habitat

Three optimizations arose from mapping the different habitat types – leaf litter, under rocks/logs, aerial leaves and caves. They show that the endogean (e.g. Yunnan *Scytodes* sp. 3 and *S. rubra*) and epigean scytodids (e.g. those that are found in herbs and shrubs) may have arisen from the cave scytodids. While two optimizations are plausible, I argue that one of the optimized trees (i.e. Fig.6-13B) is least parsimonious because it represents a transition from the caves to aerial vegetation and then a "downward" migration to the ground stratum.

Fig. 6-14 is a pictogram summarizing the relationship between habitat type (which is correlated to prey availability) and sociality type. The general pattern to emerge from this qualitative comparison is that cave species are likely to be solitary compared to related species from ground and aerial vegetation habitats. The association between habitat and social behavioural traits observed here support the hypothesis that evolution of sociality in spiders may be tightly linked to environmental factors.


Scytodes magna
Guangxi *Scyloxes* sp. 1
Yunnan *Scytodes* sp. 3
Scytodes fusca
Philippines *Scytodes* sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien *Scytodes* sp. 6
Singapore *Dictis* sp. 3
Hainan *Dictis* sp. 2
Singapore *Dictis* sp. 2
Thailand *Dictis* sp. 1
Hanoi *Dictis* sp. 5
Singapore *Dictis* sp. 4
Dictis venusta
Khao Chong *Scytodes* sp. 5
Vietnam *Scytodes* sp. 4
Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi

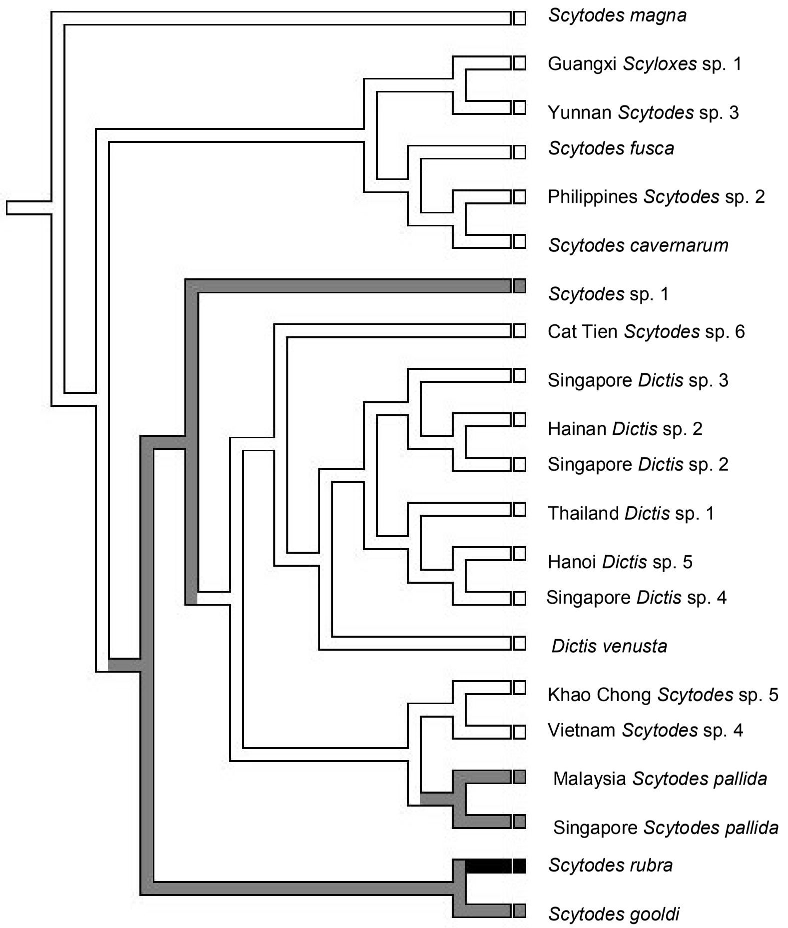
A


Scytodes magna
Guangxi *Scyloxes* sp. 1
Yunnan *Scytodes* sp. 3
Scytodes fusca
Philippines *Scytodes* sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien *Scytodes* sp. 6
Singapore *Dictis* sp. 3
Hainan *Dictis* sp. 2
Singapore *Dictis* sp. 2
Thailand *Dictis* sp. 1
Hanoi *Dictis* sp. 5
Singapore *Dictis* sp. 4
Dictis venusta
Khao Chong *Scytodes* sp. 5
Vietnam *Scytodes* sp. 4
Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi

B


Scytodes magna
Guangxi *Scyloxes* sp. 1
Yunnan *Scytodes* sp. 3
Scytodes fusca
Philippines *Scytodes* sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien *Scytodes* sp. 6
Singapore *Dictis* sp. 3
Hainan *Dictis* sp. 2
Singapore *Dictis* sp. 2
Thailand *Dictis* sp. 1
Hanoi *Dictis* sp. 5
Singapore *Dictis* sp. 4
Dictis venusta
Khao Chong *Scytodes* sp. 5
Vietnam *Scytodes* sp. 4
Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi

C


Scytodes magna
Guangxi *Scyloxes* sp. 1
Yunnan *Scytodes* sp. 3
Scytodes fusca
Philippines *Scytodes* sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien *Scytodes* sp. 6
Singapore *Dictis* sp. 3
Hainan *Dictis* sp. 2
Singapore *Dictis* sp. 2
Thailand *Dictis* sp. 1
Hanoi *Dictis* sp. 5
Singapore *Dictis* sp. 4
Dictis venusta
Khao Chong *Scytodes* sp. 5
Vietnam *Scytodes* sp. 4
Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi

D

Fig. 6-10. Five optimized trees (A to E) of the character: stage when cannibalism first occured; mapped onto the MPT = gaps as 5th character state for 21 taxa

DISCUSSION

From egg-sac guarding to the timing of natal dispersal, evolutionary trends in the scytodid social behaviour can be identified. The maximum parsimony tree with gaps treated as fifth character used for estimating ancestral conditions from characters mapped on the phylogenetic trees yielded interesting hypotheses about the evolution of social behaviour in Scytodidae. These results do depend on taxon sampling and tree pattern, and should be considered with caution since, as discussed in the previous chapter many of the relationships are not well supported. However, the general congruence between the ML and MPT= gaps as 5th character state trees strengthens the confidence I have in the results. My results suggest that the cave scytodids may be primitively solitary and social behavioural traits appear towards the base of the Old World clade. On the preferred phylogeny, the subsocial species are also scattered, implying sociality has been acquired at least four times in this family (Fig. 6-1). Agnarsson et al. (2007) had not expected multiple origins of sociality within *Anelosimus* because social species not only share many unique derived characters associated with sociality, but they are also geographically close. Within the genus *Anelosimus*, there are six independent origins of sociality (Agnarsson 2006) and in *Stegodyphus* there are three (Johannesen et al. 2007). My cladogram shows that diverse clades in the Old World have given rise to subsocial species (e.g. *S. pallida* and *Scytodes* sp. 1 from Asia; *S. rubra*, *S. gooldi* and *S. socialis* from Africa) (Fig. 6-1). Multiple origins may imply that sociality can be advantageous over the short term (Agnarsson et al. 2006). The subsocial species are nested well within the phylogenetic tree, while solitary behaviour optimizes to the base of the scytodid phylogeny (i.e. is primitively present), suggesting that subsocial behaviour is derived. At present sociality occurs only within the genus *Scytodes*. Within a single genus, sociality could range between two extremes: the first being ‘a single origin followed by diversification, in which case social species form a single clade’, and the second being ‘multiple origins, in which case sociality is polyphyletic (Agnarsson 2006)’. With at least four independent origins of sociality in *Scytodes*, the latter is likely to be true.

Where would the subsocial *Scytodes intricata* observed from Panama (i.e. New World) fit inside the cladogram? Is it truly a neotropical species or did it originate from the Old World? If the former is true then this would imply that sub(social) scytodids are present in the New World as well. At this point, I am unable to address these questions because so little is known about the social behaviour, and even the taxonomy of scytodids in the New World. Is sociality in the family Scytodidae rare, or has it been underestimated due to so little attention being paid to the social behaviour of these spiders? Only with more research into this group will the questions be answered.

The first scytodids may have been troglobitic (e.g. *Scytodes magna*). In such dark surroundings it would not have been possible to rely on vision to capture prey and escape predators. The six-eyed condition (as opposed to eight eyes in most other spiders) and (mucilaginous) glue-spitting (since it can be performed from a distance of up to 6 cm (Millot 1930; Dabelow 1958; McAlister 1960)) as a means of capturing prey may have evolved from the ancestral cavernicolous scytodids. Based on the relative characteristics of the three habitats (i.e. cave, ground and aerial vegetation) in which scytodids can be found, I identify those factors which may be promoting the evolution of sociality in this group of spiders. The simplified scheme employed in this study is not necessarily exclusive because certain species such as *S. fusca* is widespread and can be found in forests (Bowden 1991), caves (this study) and even in houses (personal observation). However, it does serve to categorize individual species in the broad sense and will provide a first indication of the presence and extent of correlated changes between habitat type and social behavioural traits.

The ancestral conditions for scytodids may be solitary, and the derived condition – social - that arose with the availability and renewability of prey. It has long been postulated that environmental factors such as predation risk and high prey abundance can function as selective pressures promoting the evolution of sociality in spiders (Alexander 1974; Henschel 1998). The results from previous studies that have succeeded in prolonging the group-living phase of subsocial species by supplementing the colonies with food (Krafft et al. 1986; Ruttan 1990; Gundermann et al. 1993; Schneider 1995; see also Rypstra 1986) suggest that the level of resources available in the environment may facilitate a delay in the timing of dispersal in subsocial scytodids which then leads to group cohesion. Cave scytodids are solitary possibly due to the scarcity of food in this harsh environment (i.e. reduction or absence of light, constant (usually very high) humidity, high carbon dioxide and low oxygen content, and very limited energy (food) input (Howarth 1983, 1993; Parzefall 1985; Culver and Sket 2000; Culver et al. 2003))., whereas scytodids that live under rocks, in dead logs and aerial vegetation have access to abundant arthropod prey.

I have shown that all the subsocial scytodids in this study demonstrate the following key traits: extended maternal care in the form of food provisioning that involves prey-capture and transport of wrapped prey back to the natal web, joint prey-capture and communal feeding among siblings, absence of aggression from the mother and low cannibalism among siblings. The subsocial pathway to sociality proposed by Kullmann (1972) suggests that sociality should arise in clades with extended maternal care through the retention of young in the maternal web. It is premature to confirm Kullmann's proposal, because I do not yet understand the social systems of *S. quarta*, Yunnan *Scytodes* sp. 7 and Otjoz *Scytodes* sp. 19; however, I have shown that with the exception of *S. socialis* the subsocial species occur as sister-taxa in which extended maternal care has been reported. In general, prey-abundant habitats and maternal care may be the basic conditions that underpin scytodid (sub)social structure. Future work could thus involve detailed studies of the social behaviour of more species of scytodids especially those from the Neotropics and caves, thus helping to resolve the number of origins of subsociality/sociality in this family. With more taxa, more genes and perhaps the inclusion of morphological data, I am confident that the current phylogeny will be made more robust such that it can facilitate the study of many other traits such as the evolution of spitting.

The evolution of social behavioural traits

Egg-guarding behaviour

Egg-guarding may be a plesiomorphic (ancestral) character in scytodids. *Scytodes magna* and Guangxi *Scyloxes* sp. 1 did not carry the egg-sac in their chelicerae though females were always within close proximity of their eggs. When disturbed (e.g. moving the cage) the females immediately moved closer to their egg-sacs. As in the case of *Loxosceles*, although the female *S. magna* and Guangxi *Scyloxes* sp. 1 do not carry their egg sac, they relate to it in various ways; she repairs it, adding lines to the whole structure and sometimes repositions it. A few days before the emergence of *Loxosceles* spiderlings the mother will make an aperture in the egg-sac and begin to knock persistently at the cover plate, hitting at it with the tip of her forelegs, as if signaling to the spiderlings the appropriate moment to emerge (Japyassú et al. 2003). However, carrying egg-sac in the chelicerae *per se* renders this family polymorphic and it is uncertain whether egg-sac carrying is plesiomorphic or apomorphic (derived) because all pholcids display this behaviour but it is not known whether all drymusid females carry their egg-sacs in the chelicerae or if there is a similar trend in scytodids (i.e. a few such as the cave-dwelling species do not carry the egg sac, but just stays within close proximity to it). The question of whether it is a case of all troglobitic scytodids not carrying their egg-sacs or all "basal" scytodids such as *Scyloxes* not practicing egg sac-carrying arises. The latter is more likely the case because Rheims and Brescovit (2001) reported that *Scytodes eleonorae*, a species found deep inside caves, carry their egg-sacs. That said, only a behavioural study of *Scyloxes* or an "older" scytodid living outside of caves can reveal the answer.

Valerio (1974) observed *Drymusa dinora* carrying egg-sac, but Labarque and Ramírez (2007) were not able to document *D. serrana*, a caverniculous species, carrying and transporting the egg-sac with the chelicerae, although all the egg-sacs that they collected were attached to the webs. It is possible that *D. serrana* females do not carry the egg-sacs but just stay in close, persistent contact with it, like *S. magna* and Guangxi *Scyloxes* sp. 1. Carrying egg-sac could be an apomorphic character that evolved in *Scytodes* and *Dictis*, and is absent in scytodids such as *S. magna* and Guangxi *Scyloxes* sp. 1 (Fig. 6-2 and Fig. 6-4). Alternatively, it could have been a plesiomorphic character that was lost in *S. magna* and Guangxi *Scyloxes* sp. 1 (Fig. 6-4).

This study is the first to show that Guangxi *Scyloxes* sp. 1 and Guangxi *Stedocys* sp. 1 females do not carry their egg-sacs. In addition, I found that the eggs of *S. magna* (a nominal *Scytodes*) and Guangxi *Scyloxes* sp. 1 were covered in a dense layer of silk whereas those of the other egg-carrying scytodids were wrapped in relatively thinner silk egg case. It may be a case in which as scytodids produce less silk (since it is an expensive protein) to cover the eggs, a different form of egg-guarding arose, that is from staying within close proximity of the egg-sac to carrying it in the chelicerae. By carrying the egg-sac, the females practise extremely close egg attendance (Li et al. 1999) such that when faced with predators (whether it is preying on the spider itself or the eggs), the females are able to escape without having to lose their progeny. In the Los Baños *Scytodes* sp., attended egg-sacs more often survive in the field than unattended ones (Li et al. 1999). By carrying the egg-sac, the female is also capable of foraging without having to leave the egg-sac too far away, because the habitats where prey are rich are also likely to be rich in egg predators. All *Scytodes* and *Dictis* (documented and from this study) females use their chelicerae to hold their eggs up till the point of hatching. Baylis and Haplin (1982) proposed that parental care is the most common antecedent behaviour from which sociality has evolved, and the behavioural antecedent to parental care, or maternal care in the case of spiders, is the maternal-offspring association. For all scytodids that carry their egg-sacs in their chelicerae until hatching and then proceed to assist the spiderlings in the emergence from the egg-sac, the potential for maternal-offspring association exists. Therefore, egg-sac carrying may be the antecedent condition from which any form of sociality in the scytodid can evolve.

Foraging during egg-sac guarding

Foraging during egg-sac guarding may be plesiomorphic, and different selection pressures have acted on this particular trait. These selection pressures are likely to be microhabitats and predation risk or prey, and they possibly have not acted on this trait alone. The costs and benefits of egg-sac tending may also be a selective force in shaping the evolutionary pattern of this trait. Among the 21 taxa studied, subsocial species and a few solitary species rarely or never forage during egg-guarding. If *Scytodes magna* and Guangxi *Scyloxes* sp. 1 are indeed the "basal" scytodids, then one would expect the two species to display the plesiomorphic behavioural trait of foraging during egg-sac guarding. However, this is not the case. I attribute it to the fact that caves also prove to be very harsh environments with the existence of roaches, crickets, centipedes and large huntsmen – arthropods that may readily prey on egg-sacs. Predation may be the driving force behind the rarity of foraging during egg-sac guarding in these two species of scytodids. On the other hand, *S. fusca, S. cavernarum* and the Philippines *Scytodes* sp. 2 that are also cave-dwelling species, were observed to forage frequently (Chapter 2: Fig. 6-5). These three species that construct their nests in tiny holes on the cave walls have the advantage of leaving behind their egg-sacs in the deep tunnels that may serve as a natural protection against egg-sac predators.

Two species of solitary *Scytodes* and six *Dictis* species (all solitary) living in aerial vegetation were observed to forage during egg-sac guarding. One possibility is that females may produce anti-predatory chemicals onto the silken threads forming the sac, or perhaps these spiders can produce a second or third clutch as a strategy to have a higher lifetime reproductive success. Egg-carrying scytodids have fewer foraging opportunities than non-carrying individuals. By not providing care, a female can reallocate time and energy into increasing its survival and growth, and perhaps into finding a new mate sooner. The female in order to recover the energy invested in egg laying will leave the egg-sac aside to forage so as to off-set some of the costs (including decrease in lifetime egg production) of maternal care. Therefore, the trade-off is that in order to produce more eggs, the females would have to forage to gain body weight resulting in egg-sacs being left unguarded for a period of time. However, I postulate that the females do not forage too far from their egg-sacs because in the laboratory when the cages were moved or tapped on, these females immediately returned to their egg-sacs and picked them up in their chelicerae.

Both subsocial *S. rubra* and *S. gooldi* did not forage during the egg-sac attendance (Chapter 3). Since *S. rubra* females produced only one egg-sac per year, the maternal investment is expected to be very heavy, and extremely close egg-sac attendance would thus be expected. For *S. gooldi*, other arthropods such as centipedes, cockroaches and large spiders in microhabitats such as fallen logs may be potential egg-sac predators, thus carrying their egg-sacs at all times may be an important measure in the prevention of egg-sac loss.

The *Loxosceles gaucho* (Sicariidae) female maintains foraging activities throughout the entire egg-guarding phase (Japyassú et al. 2003). In Drymusidae, a sister taxon to the Scytodidae (Forster 1995), *Drymusa dinora* and *D. spectata* females build white, spherical, wrinkled egg-sacs, which are occasionally hung in the web, close to them. Like *Scytodes* (Valerio 1974; Alayón García 1981), *Drymusa* females also carry and transport their egg-sacs. However, whilst attacking prey or when their web is perturbed, they sometimes leave their egg-sacs (Valerio 1974). Lubin and her colleagues (1978) reported that females of an unidentified *Miagrammopes* species hung their egg sacs from threads at night and resumed prey capture actively until dawn at which time they again tended to their egg-sacs. Fink (1986) demonstrated that *Peucetia viridians* (Oxyopidae) egg-guarding females lost 9% of their mean weight after oviposition, and feed only one-tenth as often as non-guarding females. The females guard, rather than desert, their egg-sacs during foraging; this behaviour is likely to defend against generalist predators that might eat the eggs - such predation can be very severe.

Foraging during the egg guarding period may be plesiomorphic because I have observed examples of the outgroup species (i.e. *Loxosceles* and *Drymusa*) as well as “younger” spider groups such as the uloborids performing this behaviour. Within the scytodids, however, it is difficult to reach a conclusion because foraging during the egg-sac guarding phase occurs in solitary as well as subsocial species, albeit at a low frequency. Its multiple occurrences and lack of association with any unique behaviour suggest an independent adaptation that may be influenced by environmental factors such as predation pressure, or even by the individual’s decision when weighing the costs and benefits, and not shared ancestry.

Food provisioning

The early stages of maternal care in the subsocial scytodids are closely similar to the solitary species: they both practice extremely close egg attendance by either carrying in the chelicerae or maintaining body contact with the egg-sac. (Sub)sociality then must have arisen through the temporal extension of maternal care leading to spiderlings spending a longer duration in the maternal web. This is the hypothesis of the “maternal care pathway” to sociality (Burgess 1978;

Vollrath 1982; Uetz 1983; Smith 1986, 1987; Avilés 1986, 1997, 1999, 2000; Avilés and Gelsey 1998; Agnarsson 2002). My results partially support this hypothesis. Females of the five subsocial species/populations – *Scytodes* sp. 1, *S. rubra*, *S. gooldi*, Malaysia *S. pallida* and Singapore *S. pallida* demonstrated extended maternal care in the form of food provisioning through prey capture and transport of prey back to the natal nest, showing that extended maternal care precedes sociality. Mother-offspring and sibling associations, with suppression of antagonistic interactions are thus derived traits that lead to the evolution of sociality in scytodids.

The three optimized trees produced by mapping of the four characters, 'food provisioning by maternal female', 'dragging prey back to natal nest', 'mother feeding alongside brood', and 'aggression from mother towards spiderlings during feeding', show that all four characters are absent (or present in the case of the character aggression from mother during feeding) in the ancestral states (i.e. not known in the "older" families as well as sister families of Scytodidae such as Drymusidae and Sicariidae). It is known, however, that spiderlings of the mygalomorph spider, *Ischnothele caudata* (Dipluridae) feed together on a prey item provided by the mother. This is almost certainly a derived character for *I. caudata* although one cannot rule out the fact that whilst sociality has been widely studied in the araneomorph spiders, it has remained relatively unexamined in the mygalomorphs (Avilés 1997). Another case in point is that in an 11-week study conducted by Reichling and Gutzke (cited in Varrecchia et al 2004), spiderlings of *H. crassipes* Pocock 1897 (Theraphosidae) swarmed together on a prey item subdued by the mother, which was then completely devoured, suggesting that sociality in *Hysterocrates* siblings can extend well beyond the first instar.

Within Scytodidae, the traits evolved three times: once in *Scytodes* sp. 1, once in Malaysia *S. pallida*-Singapore *S. pallida* clade, and once in the *S. rubra*-*S. gooldi* clade (Fig. 6-9A). Alternatively, the traits might have been initially present in the ancestors of the species ranging from *Scytodes* sp. 1 to *S. gooldi* (in a downward sequence) were lost at the node leading to the (Cat Tien *Scytodes* sp. 6 + *Dictis*) and *S. pallida* clades, but then regained in *S. pallida* - Singapore *S. pallida* clade (Fig. 6-9B). From Fig. 6-9C I conjectured that the traits were again initially present in all the species ranging from *Scytodes* sp. 1 to *S. gooldi* (in a downward sequence) but this time lost at the node leading to the (Cat Tien *Scytodes* sp. 6 + *Dictis*) clade and lost in Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4.

Food provisioning by the maternal female such as regurgitation and matriphagy, dragging prey back to natal nest regardless of whether the prey is wrapped, mother feeding alongside brood, and lack of aggression from mother towards spiderlings during feeding all constitute forms of extended maternal care. It is unlikely that all these traits were present initially across the 15 taxa (from *Scytodes* sp. 1 to *S. gooldi* in a downward sequence) since most of these traits are absent in the majority of the araneomorph spiders. Avilés (1997) indicated 16 families showing some form of maternal care of offspring beyond the egg stage. The underlying mechanisms that promote and maintain these traits would appear to be complex, but several factors may possibly be involved. Abundance in prey, large prey or high predatory pressures in the microhabitats of the four species: *Scytodes* sp. 1, *S. pallida* (both Singapore and Malaysia populations), *S. rubra* and *S. gooldi* may have promoted the evolution of extended maternal care in these species. I will discuss the evolutionary correlates that occur between habitat type and sociality in the next section. Nevertheless, I am in favour of Fig. 6-9A which may represent three independent origins of subsociality.

Emergent phase

Loxosceles spiderlings emerge as second instars (Fischer and Vasconcellos-Neto 2005). *Holocnemus pluchei* spiderlings (Pholcidae) emerge as first instars and the first molt occurs 2 days after eclosion, while the spiders are clinging to the egg cocoon (Maughan 1978). As for *Stegodyphus lineatus* (Eresidae), after 30 days of development the female opens the egg-sac and releases the young that are in a premature stage (Schneider 1996). The first instars are tiny and the female will feed them immediately. In my study I found that five species of scytodids, which happen to be cave-dwelling spiders (with the exception of *S. fusca* that can be found in different types of habitats), have spiderlings that emerge from the egg-sacs as first instars (Fig. 6-6). It is possible that predatory pressure in the hypogyean environments is exceptionally high and in order to shorten the guarding period – the duration in which the female is restricted in her movements- the female manipulates the sac to release the spiderlings whilst in the first instars.

I am unable to conclude whether scytodid spiderlings emerging from the egg-sacs as first instars is an ancestral or derived state since the outgroup species, *Loxosceles*, have spiderlings that emerge as second instars and there is no information about how and when drymusid spiderlings emerge. However, since cave scytodids may be ancestral, it is possible that spiderlings emerging as first instars is plesiomorphic and hence there are two independent origins of evolution into the ‘emergence as second instars’ state: one in Yunnan *Scytodes* sp. 3 and the second in clades in which the scytodids have moved from the ancestral habitat of caves to the ground stratum and aerial vegetation.

Behaviour of emergents

Spiderlings of_*Ischnothele caudata* (Dipluridae) hatch over several hours and spread out over the web within a few days of hatching (Jantsche and Nentwig 2001). *Coelotes terrestris* exhibit a clumping tendency even in the mother's absence (Horel, Leborgne and Roland 1982). Maughan (1978) reported that *Pholcus muralicola* spiderlings flex and wave their legs about as they cling to the empty cocoon after hatching. After 3 days of close association to the cocoon the spiderlings disperse throughout the web. To the best of my knowledge, congregation on the maternal female's body is only known in *Pediana regina* (Sparassidae) (Rowell and Avilés 1995), *Xysticus kochii* (Thomisidae) and lycosids (*Trochosa* and *Lycosa*) (Foelix 1996). The broods of the burrowing wolf spider (Lycosidae) are known to mount the female's body after emergence (Miller 1989). Wolf spider females show maternal care and carry their egg-sac attached to their spinnerets; after hatching, the spiderlings climb on their mother's back, despite their ability to run and feed independently and are transported for a certain period. Once spiderlings leave the female's abdomen, they do not climb back on to it (Bonte et al. 2007). Currently, the advantages of this maternal care are not known.

In my study, I found that the emergents of at least six species of *Scytodes* (see Fig. 6-7) mount the female's body (both cephalothorax and abdomen) immediately after eclosing from the egg-sac. Newly-emerged spiderlings attaching to the broken sac seems to be a plesiomorphy and Fig. 6-7A shows that another form of attachment, which is to the mother's body instead, evolved three times: in Yunnan *Scytodes* sp. 3, *Scytodes* sp. 1 and the (Khao Chong *Scytodes* sp. 5 + Vietnam *Scytodes* sp. 4) + (Malaysia *S. pallida* + Singapore *S. pallida*) clade. Fig. 6-7B, however, shows that the hatchlings of the (*pallida* + *Dictis*) clade, *Scytodes* sp. 1 and Cat Tien *Scytodes* sp. 6 all at once attached themselves to the mother's body upon emergence but it was subsequently lost in the *Dictis* clade and Cat Tien *Scytodes* sp. 6. Clustering of young on the body of females is very common in plesiomorphic arachnid orders, Scorpiones and Amblygi (sister group to Araneae), and could imply that this behaviour is a result of parallel evolution. It is also possible that mounting the adult female's body immediately on eclosion is a secondarily derived trait in scytodids (Fig. 6-7A). Fig. 6-7B is not parsimonious because it represents a gain, and then loss again of this trait in *Dictis* and Cat Tien Scytodes sp. 6. The reason why the emergents mount their mother's body is unknown, but it is possible that the hatchlings behave as such so as to either suppress the predatory instincts in the adult female or to gain further protection such that in the event the nest is attacked by a predator, the female is able to escape to a safer location bringing the spiderlings along with her. Another possible reason is that the female immediately upon the emergence of her spiderlings will migrate to another site, although this is unlikely because females in the field are known to remain in their webs with the spiderlings dispersing instead, or if the females do abandon the nest, it is once the offspring have moulted to a certain instar.

In wolf spider, *Pardosa monticola*, the spiderlings dismounted the maternal abdomen within 150 - 185 h of hatching (Bonte et al. 2007), but in *Scytodes* the spiderlings left their mother's body within 24 – 36 h, and then congregate in the natal web. The reasons for aggregating on the mother's back are at present unknown, but I postulate that it is a form of maternal defense against predators. Why then do a few spiders exhibit this trait but not others? This could be attributed to the different ecological pressures acting in different microhabitat type. Although I did not observe the other *Scytodes* females carrying around the broken egg-sac with the newly emerged spiderlings still attached to it, as in the case of pholcids (Huber 2009), such a behaviour may be performed in the natural habitat. If so, this is also a way in which the mother can protect her offspring from potential predators. In the laboratory observations, the emergents of most species were seen to aggregate in a tight cluster in the natal web after detaching themselves from the broken sac.

Scytodes magna and Guangxi *Scyloxes* sp. 1 spiderlings do not aggregate in the maternal web after emergence possibly due to the fact that there is no extensive maternal web. I found two Guangxi *Scyloxes* sp. 1 females on the walls of the caves next to their egg-sacs which were suspended by threads, and although I did not collect any *S. magna* females with egg-sacs, I observed their webs to be non-extensive and sparse in nature. Although I scored this trait as being 'absent' for the six species (*S. magna*, Guangxi *Scyloxes* sp. 1, Singapore *Dictis* sp. 3, Hainan *Dictis* sp. 2, Singapore *Dictis* sp. 2 and Singapore *Dictis* sp. 4)(i.e. they did not form a tight cluster in the maternal nest), most of the hatchlings were spread out within 6-10 cm of the mother. I am unsure if this is a reflection of what occurs in their natural settings in which there is no space constraint.

Regardless of the duration, aggregating in the maternal web is characteristic of almost all known species of spiders. The mapping of this character onto the molecular phylogeny yielded six optimizations, for which I opined two were very possible. Fig. 6-8A shows that this trait was lost three times: once in *S. magna*, once in Guangxi *Scyloxes* sp. 1 and once at the *Dictis* clade, with secondary gains in Thailand *Dictis* sp. 1 and Hanoi *Dictis* sp. 5. In Fig. 6-8B, there are four losses: in *S. magna*, Guangxi *Scyloxes* sp. 1, Singapore *Dictis* sp. 3 + (Hainan *Dictis* sp. 2 +

Singapore *Dictis* sp. 2) clade and in Singapore *Dictis* sp. 4. However, it is difficult to decipher an evolutionary pattern of this character. It may be that there is no “loss” nor “gain” in this trait, simply that forming a tight aggregation is not indication of a social behaviour, that is, spiderlings upon eclosing from the egg-sac will stay in the natal nest for some time, independent of the distance they maintain from each other.

Cooperative prey-capture and food sharing among siblings

Cooperative prey-capture is a key character of social spiders (reviewed in Avilés 1997; Kullman 1972; Lubin and Bilde 2007; Whitehouse and Lubin 2005). All cooperative spiders that evolved via the subsocial route have a phylogenetic history of feeding alongside siblings in the maternal web (Whitehouse and Lubin 2005). Group hunting and communal feeding among adults in these societies are extensions of behaviours already present in the juvenile stages (Burgess 1976; Buskirk 1981; Uetz 1988). Colonial spiders are formed in part by aggregations around a resource and therefore individuals are not closely related (Whitehouse and Lubin 2005). The five subsocial species/populations in my study, Malaysia *S. pallida*, Singapore *S. pallida*, *Scytodes* sp. 1, *S. rubra* and *S. gooldi*, originated via the subsocial route, which rooted from maternal care. To date, there is no information on *Loxosceles*, *Sicarius* and *Drymusa* maternal females providing food to their progeny, and, since cooperative prey capture and communal feeding are absent in most of the scytodids, these two traits must be derived characters in which group living, regardless of the duration, enhances individual foraging success. How? In the presence of a large prey a single individual might not be able to subdue it without help from siblings. Although there are predation costs, the benefits from cooperative prey hunting outweigh the costs.

When spiders cooperate, large prey are attacked and usually fed upon by more than one individual (Fowler and Diehl 1978; Pasquet and Krafft 1992). All spiders that are candidates for the subsocial route show communal feeding (Schneider 1996; Lubin and Bilde 2007). The evolution of communal feeding in group-living spiders may depend on the average size of prey caught, the cost of sharing prey, the ease of monopolizing prey and the risk associated with uneaten prey, as well as phylogenetic constraints (Whitehouse and Lubin 2005). Mapping of the characters cooperative prey-capture and prey sharing by captors yielded the same three optimization trees as the characters: food provisioning by maternal female; dragging prey back to natal nest; mother feeding alongside brood; and (lack of) aggression from mother towards spiderlings during feeding). Therefore, the following discussion is still based on Figs. 6-9A to 6-

9C. Cooperative prey capture and food sharing were observed in five of the *Scytodes* broods used in this study.

Such cooperation is commonly observed in the most advanced social spiders (e.g., *Agelena consociata*; Krafft 1969), but is not a universal characteristic of subsocial or social systems. Indeed active defence of prey items by individual spiderlings has been observed in several social species (e.g. *A. eximius*: Christenson 1984; *A. consociata*: Riechert 1985) and in the subsocial *Scytodes intricata* (Eberhard 1986) as was also observed in *Scytodes* sp. 1 when a fruit fly was captured by a single individual (Yap and Li 2009). However, if the prey was larger (e.g. a house fly or cricket), then there was sharing of prey observed not only in *Scytodes* sp. 1, but also in the two *S. pallida* species, *S. rubra* and *S. gooldi.* This is in agreement with Riechert et al (1986): a large variety of prey are small enough to be handled by a solitary spider, and individual food intake decreases as the size of the cooperative group increases. This suggests that cooperative feeding behaviours may not have evolved as an antecedent of sociality but rather as a consequence of it. Packer and Ruttan (1988) suggested that there will be a temptation to cheat, in this case to join in feeding without participating in the actual capture if the prey is large but not if it is small enough to be monopolized by a single individual, and I cannot rule out this possibility: the observed prey sharing is not voluntary; juveniles are unable or it takes too much effort to defend their prey against rivals, as was hypothesized by Vanacker et al (2004).

Nonetheless, cooperative foraging and prey-sharing are important traits necessary for the evolution of sociality in Scytodidae and other spiders. A number of spider species live in groups, but few of them satisfy the criterion of (quasi)social behaviour: co-operation in prey-capture and brood care (see chapter 1). Miller (2006) observed several instances of cooperative prey capture in the subsocial *Scytodes* - *S. socialis*- whereby two individuals cooperated in the capture of a relatively large prey item (moth or fly). In all instances, additional colony members eventually joined in the feeding and no aggressive interactions among colony members were ever observed. As I discussed in the earlier sections (see maternal care: food provisioning), I am also in favour of Fig. 6-9A when it comes to the mapping of the characters: cooperative prey capture amongst siblings and food sharing by captors.

Cannibalism

The benefits of living in groups may be outweighed by costs such as cannibalism by larger group members (Whitehouse and Lubin 2005), thus neotenic retention of juvenile tolerance has been assumed to be the first step towards communal living (Kullmann 1968; Buskirk 1981). Juvenile cannibalism is well known in spiders. Because cannibalism or the lack of intraspecific tolerance among nest-mates is rampant in most spider species, including my outgroups, *Loxosceles*, *Sicarius* and *Drymusa*, tolerance among siblings in the scytodids is likely to be a derived behaviour.

Mapping of the character, stage when cannibalism first occurs resulted in five optimizations (Fig. 6-10A–E). The tree in Fig. 6-10B represents three separate origins, and may be the most parsimonious because congruent with the "maternal care pathway" to sociality hypothesis, the extension of the mother-offspring phase has led to increased tolerance among nest-mates in *S. rubra* as a result late occurrence of cannibalism is observed. Fig. 6-10A which represents four separate origins of intraspecific tolerance is also a possible scenario, but it would mean a jump from early cannibalism (i.e. second instars) to late cannibalism (i.e. fourth instars) among the ontogeny of *S. rubra*. This is inconsistent with the usual understanding that evolution usually occurs gradually.The scenario in Fig. 6-10C is improbable because a reversal to a less social state in *S. gooldi* is unlikely. Moreover, *S. gooldi* juveniles start to cannibalize each other in the late 3rd instars, nearing the 4th instars, thus it is more likely a case of *S. gooldi* developing a higher tolerance within the brood. Similarly, Figs. 6-10D and 10E are also improbable because delayed cannibalism is most likely to be an apomorphic trait that appeared recently in the five aforementioned species.

A major aspect of sociality is tolerance, so it is not surprising that cannibalism (other than matriphagy) in spider social groups is rare and, when it does occur, appears to be related to food scarcity (D'Andrea 1987). Hunger level affects the propensity to cannibalize most clearly in solitary spiders. In subsocial spiders hunger can affect rates of cannibalism (Bilde and Lubin 2001), but not always (Miller 1989), and in some social spiders cannibalism is absent even if the spiders suffer extreme hunger (D'Andrea 1987). Out of the 21 species of scytodids from this study, 16 display cannibalism from the second instars. Spiderlings of *Scytodes* sp. 1, Malaysia *S. pallida*, and Singapore *S. pallida* start cannibalism from early third instars, *S. gooldi* from late third instars, and in *S. rubra* the tolerance for nest-mates starts to break down from the fourth instars (Table 3-7). A slight overestimation of the extent of juvenile cannibalism is possible, because spiderlings in nature may be more able to avoid being eaten by each other; however, the set-ups in which the experiments were carried provided sufficient space for the spiderlings/juveniles to evade one another. Since it is evident that there exists some degree of tolerance among these five species, why then does cannibalism still occur among them? Perhaps compared to the fruit flies, conspecifics maybe high-quality prey in terms of nutrient content, and only upon dispersal will they eventually diversify their diets with heterospecific prey. Alternatively, it could be a built-in mechanism for maintaining population stability (Wise 2006) since in the case of for example the Philippines *Scytodes* sp. 2, the female produces numerous clutches of eggs (3.33 clutches/female) throughout the reproductive phase. Through cannibalism, competition for resources such as food, space and mates among individuals may be reduced hence playing a significant role in the regulation of population densities (Elgar and Crespi 1992; Wagner and Wise 1996).

Natal dispersal

The transition to quasisociality in spiders is thought to have involved the suppression of the dispersal phase characteristic of ancestral subsocial species (Avilés and Gelsey 1998). The subsocial *Scytodes* sp. 1, Malaysia *S. pallida*, Singapore *S. pallida*, *S. rubra* and *S. gooldi* all form mother-offspring and sibling associations that break down before the third or fourth instars. As the juveniles stop relying on their mother to provide food and begin to capture prey on their own, dispersal starts in the absence of a group foraging function. Dispersal at a later instar or delayed dispersal may be an ancestral state in scytodids. Early dispersal may have arisen in Yunnan *Scytodes* sp. 3, Vietnam *Scytodes* sp. 4, Khao Chong *Scytodes* sp. 5, Thailand *Dictis* sp. 1, Singapore *Dictis* sp. 2, Singapore *Dictis* sp. 3, Singapore *Dictis* sp. 4 and *Dictis venusta* perhaps due to lack of further protection offered by the mother, insufficient space and more importantly, high costs (e.g. mutual aggressiveness) of remaining in groups.

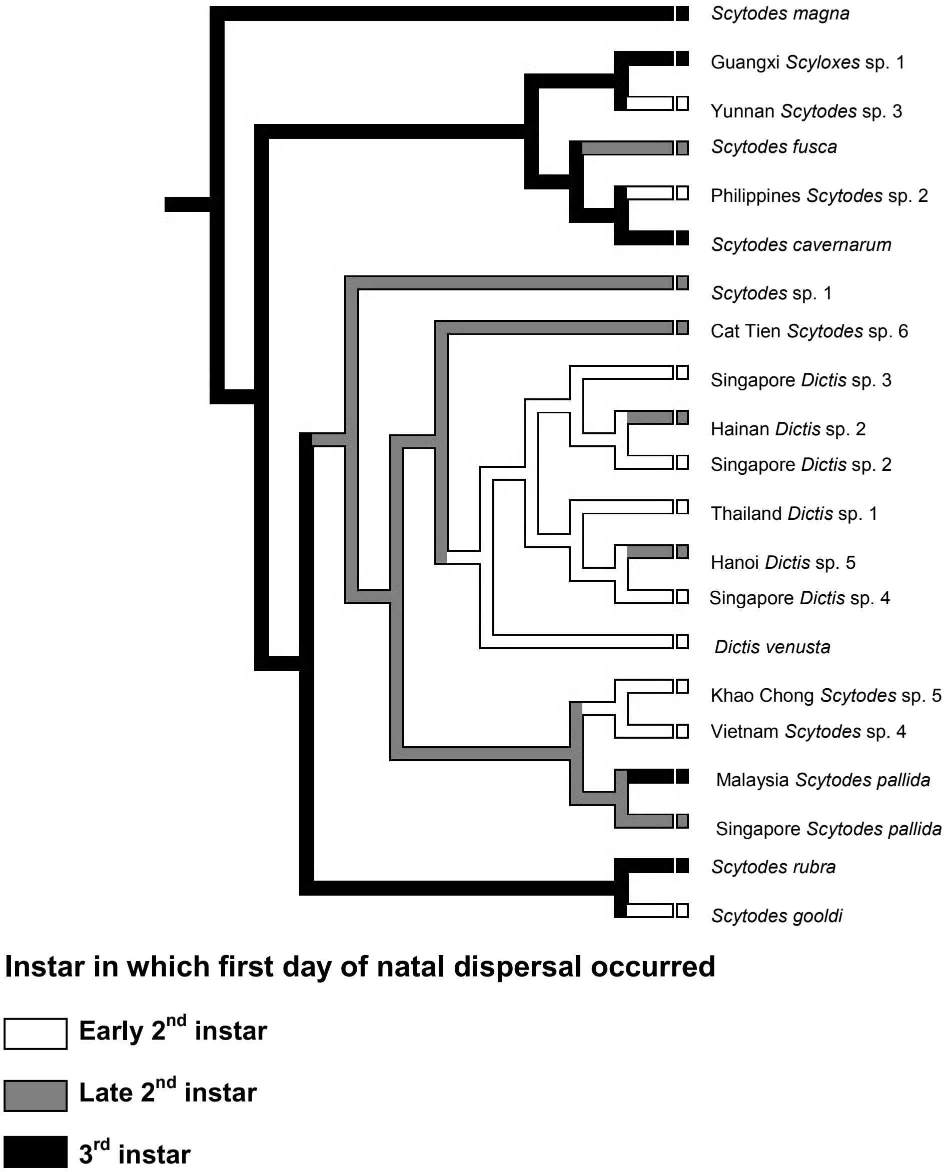
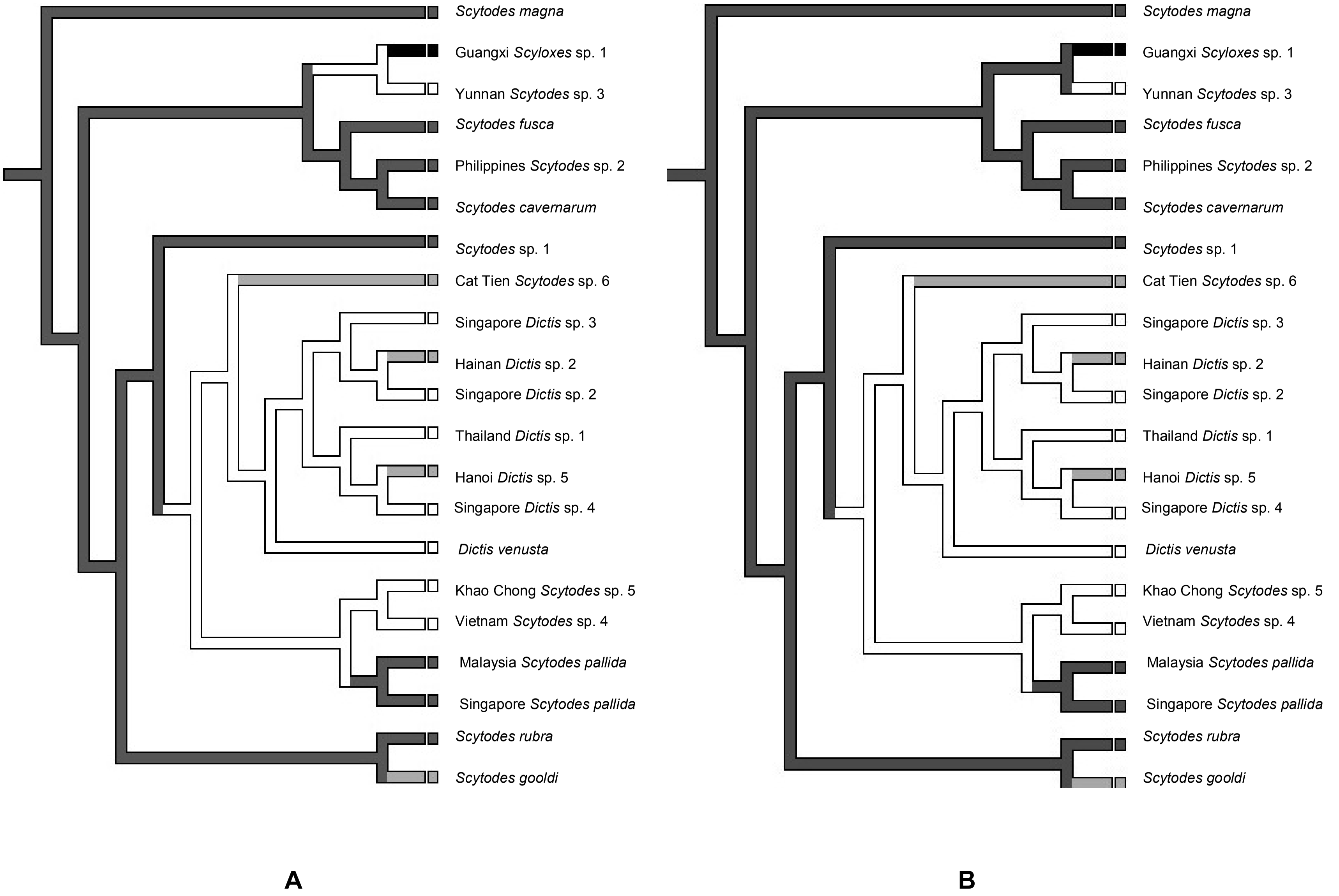


Fig. 6-11. Optimized trees of the character: instar in which the first day of natal dispersal occurred; mapped onto the MPT = gaps as 5th character state for 21 taxa

Extended maternal care provides sufficient benefits, such as improved survival and faster growth, for siblings to remain in the natal nest and delay dispersal (Agnarsson et al 2006; Avilés 1997). The delay of juvenile dispersal appears to be a gradual phenomenon and may show plasticity both among and within species. Young that disperse at a later stage tend to have larger body size and thus will have an advantage in survival and growth over smaller dispersers (Aviram 2000). Mapping of the character, instar of the spiderlings in which the first day of natal dispersal occurs, yielded only one optimized tree (Fig. 6-11). However, the result seems to contradict our earlier discussion. Later dispersal (i.e. at the third instars) seems to be an ancestral state and it spreads unevenly across the 21 taxa – three occurrences in the solitary cave species *S. magna*, Guangxi *Scyloxes* sp. 1 and *S. cavernarum*; and the other two in the subsocial species Malaysia *S. pallida* and *S. rubra*. To date, there is no literature on the natal dispersal patterns in the sicariids or drymusids to validate this. For spiderlings to remain longer than usual in the maternal web, they must be receiving some form of benefits. However, as discussed above, the solitary *Scytodes* receive no maternal care from females other than protection during the egg stage. Siblings do not participate in group foraging or prey sharing and on top of that, cannibalism commences at the second instars, *albeit* the later second instars when the yolk has been used up (as evidence from their distended abdomens deflating). A possible reason for this perplexing situation could be that under laboratory conditions in which prey is offered *ad lib,* the spiderlings of the solitary species make the decision not to disperse despite the threat of being eaten by their siblings because the benefits of receiving more nutrition far outweighs the costs of being cannibalized. For these small-sized spiderlings the chances of being preyed upon as opposed to finding prey are very much higher in harsh cave environments. Evading just their siblings they stand a higher chance of survival than having to evade a variety of predators, such as crickets and other larger spiders. Another unexpected scenario is that, other than Malaysia *S. pallida* and *S. rubra*, the other subsocial species do not display late natal dispersal. We should expect that, in the subsocial species, natal dispersal would only commence at the third instars, or later. Perhaps this is not a true reflection of what occurs in nature although the first occurrence of natal dispersal took place when the spiderling was in the late second instar. Most of the dispersal might be actually during the third instar or later. Many studies showing natal dispersal patterns in spiders describe a sigmoid graph in which dispersal commences gradually, accelerates and then plateaus (e.g. Kim 2000). Indeed when I extended our investigation of natal dispersal pattern into the 6th week (from day of emergence) I found that in all the subsocial species, except for *S. gooldi*, 50% of the spiderlings dispersed in the third instar (see Chapter 3).

The 12 optimizations (Figs 6-12A – 6-12L) attained from mapping the character ‘instar at which 50% of spiderlings disperse from the natal nest’ again showed that delayed dispersal is the plesiomorphic state. Figs 6-12A, 6-12D, 6-12G and 6-12J are not likely because it shows that at the Guangxi *Scyloxes* 1 + Yunnan *Scytodes* sp. 3 node there is a loss (i.e. a transformation to early dispersal at the early second instars), and then a reversion to a much delayed dispersal (at the fourth instars) in Guangxi *Scyloxes* sp. 1. This is unparsimonious. Figs. 6-12F, 6-12H, 6-12I, 6-12K and 6-12L are also impossible because within the ‘*pallida*’ clade the change from third-instar dispersal to early second-instar dispersal at the (Khao chong *Scytodes* sp. 5 + Vietnam *Scytodes* sp. 4) branch is too abrupt. Figs. 6-12B and 6-12C represent a loss of this behaviour in the *Dictis* and *pallida* clades + Cat Tien *Scytodes* sp. 6 node, and then a secondary gain (i.e. revert back to later dispersal) in Cat Tien *Scytodes* sp. 6, Hainan *Dictis* sp. 2, Hanoi *Dictis* sp. 5 and the two *S. pallida* populations. However, the reversion from early second-instar to third-instar dispersal is once again too sudden. Fig. 6-12E implies three losses: one at the (Cat Tien *Scytodes* sp. 6 + *Dictis*) node and the other at the (Khao Chong *Scytodes* sp. 5 + Vietnam *Scytodes* sp. 4) node, followed by three separate reversions to later dispersal habit in Cat Tien *Scytodes* sp. 6, Hainan *Dictis* sp. 2 and Hanoi *Dictis* sp. 5. Therefore, it is my opinion that Fig. 6-12E is the most parsimonious. One could argue that the change from late dispersal to very early dispersal in Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 is also very abrupt, however, “losing” the inter-attraction among brood-mates may be easier than “gaining” it. If the latter is true then why are there so few social spiders.



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Scytodes sp. 1
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Malaysia *Scytodes pallida*
Singapore *Scytodes pallida*
Scytodes rubra
Scytodes gooldi
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Scytodes sp. 1
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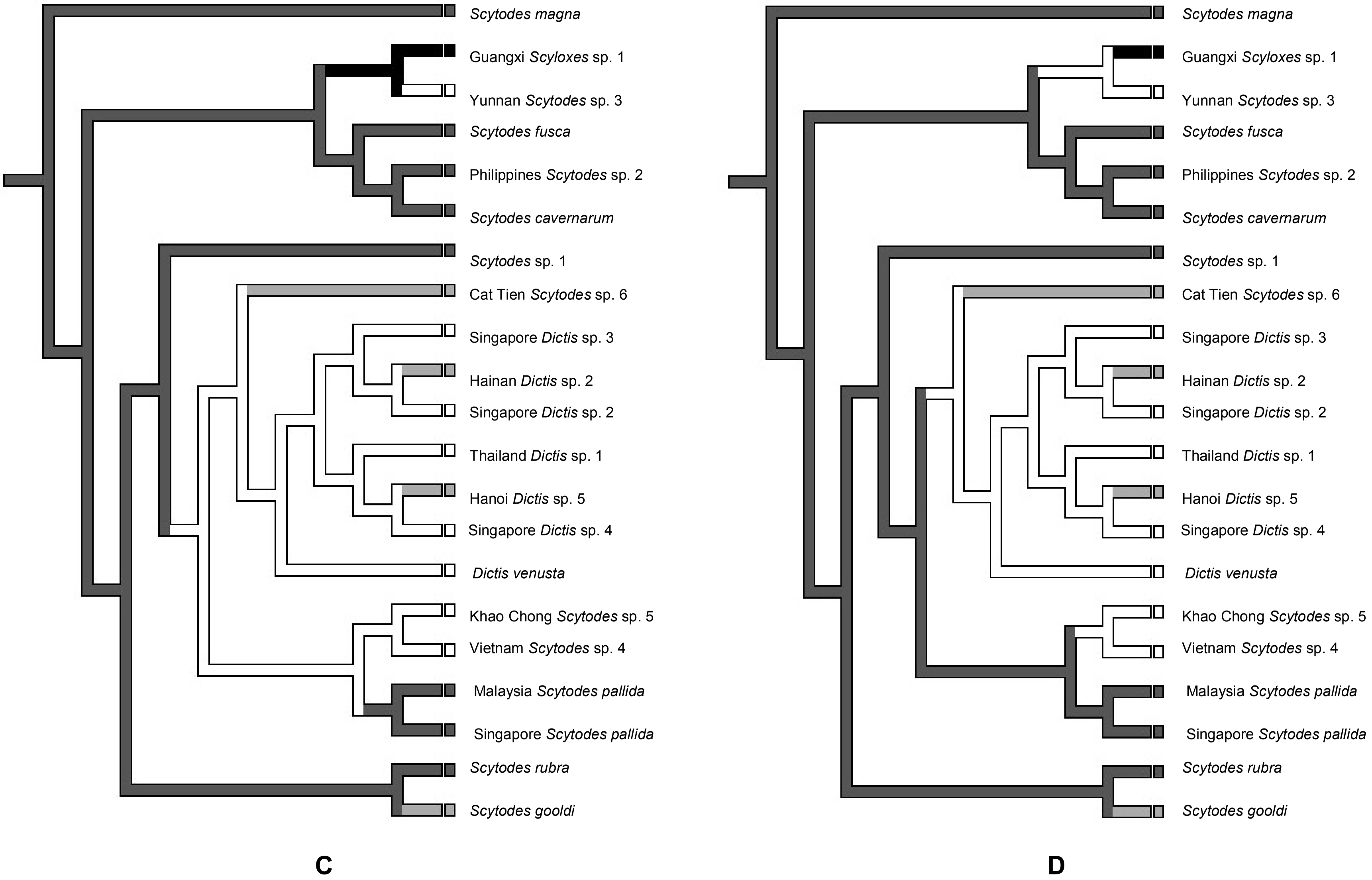
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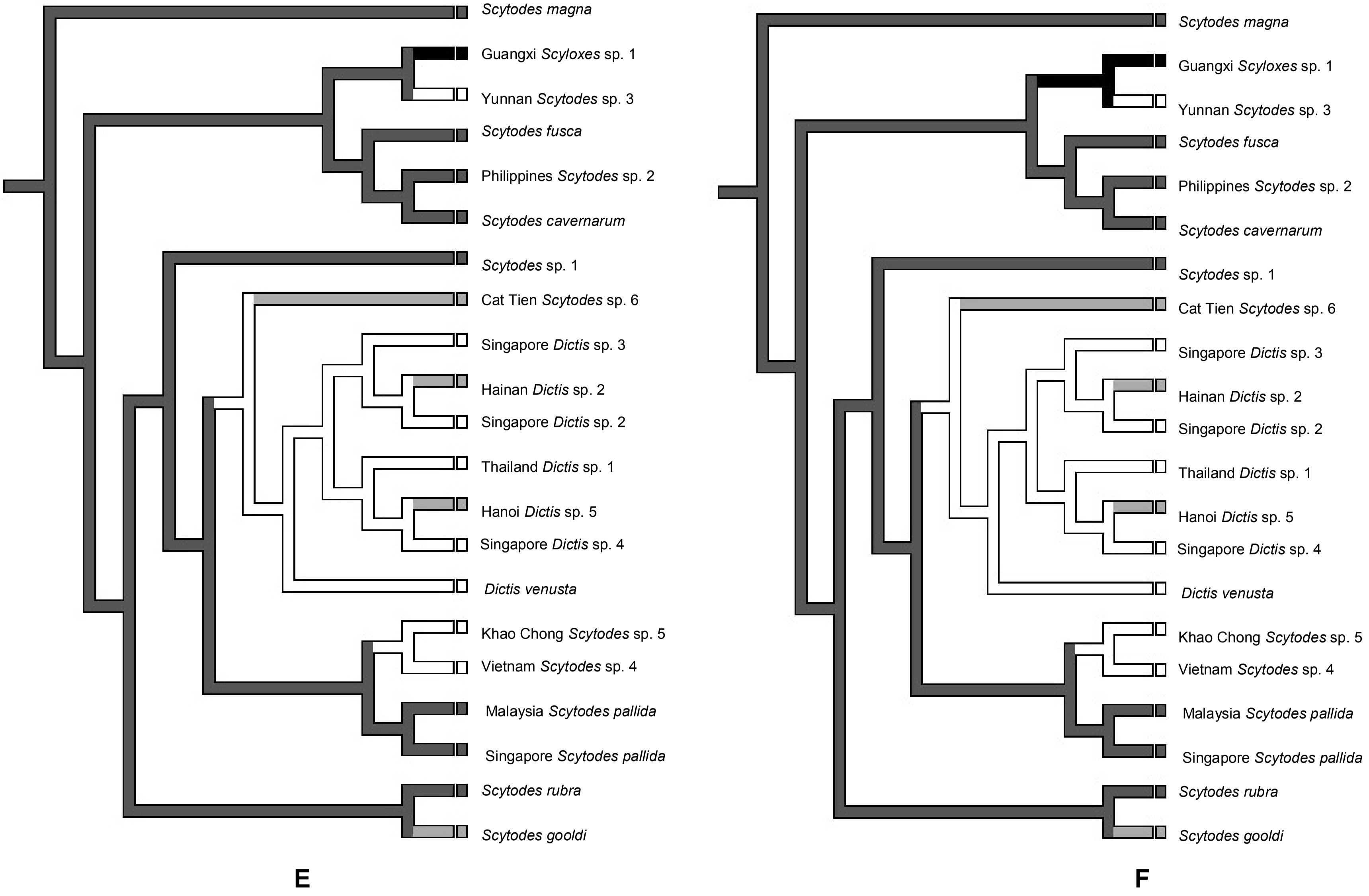
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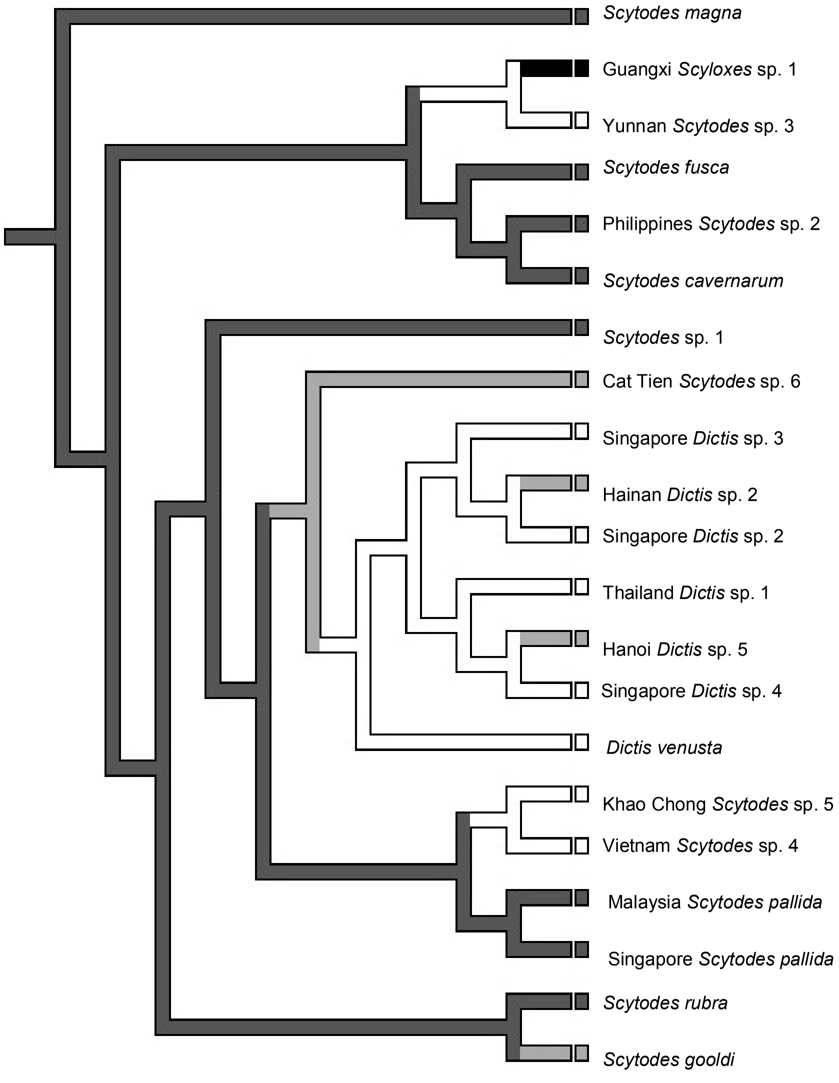
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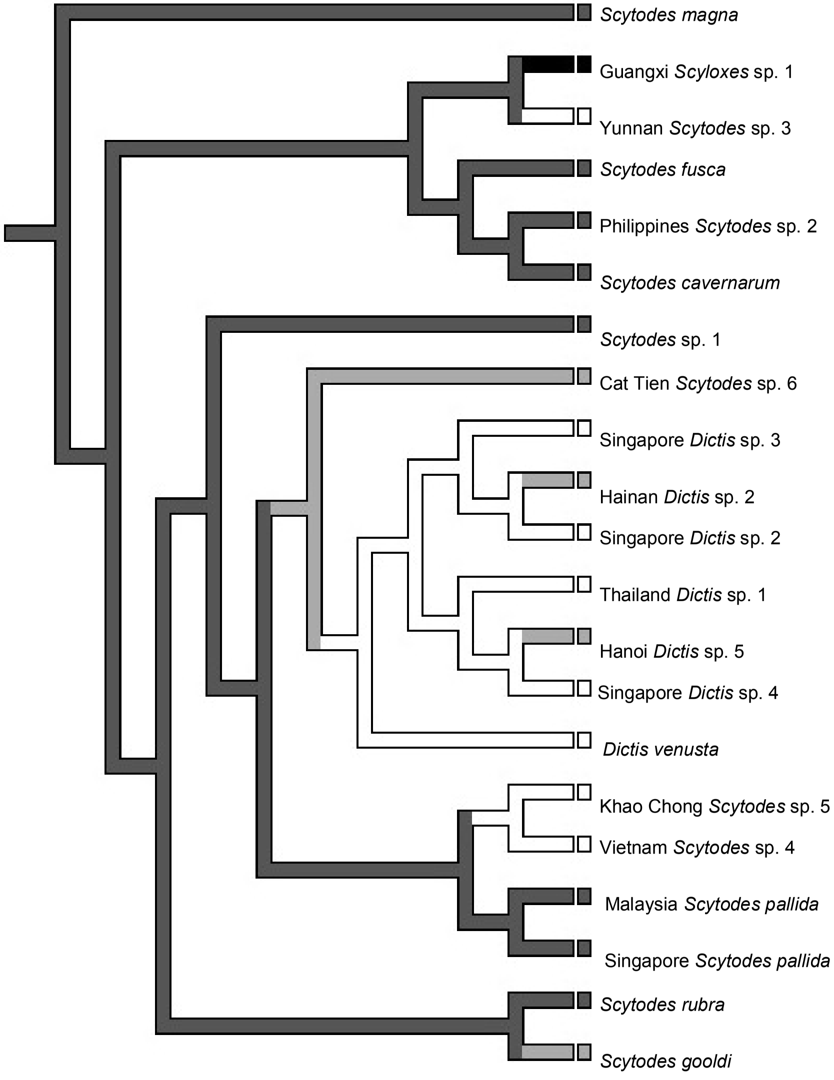
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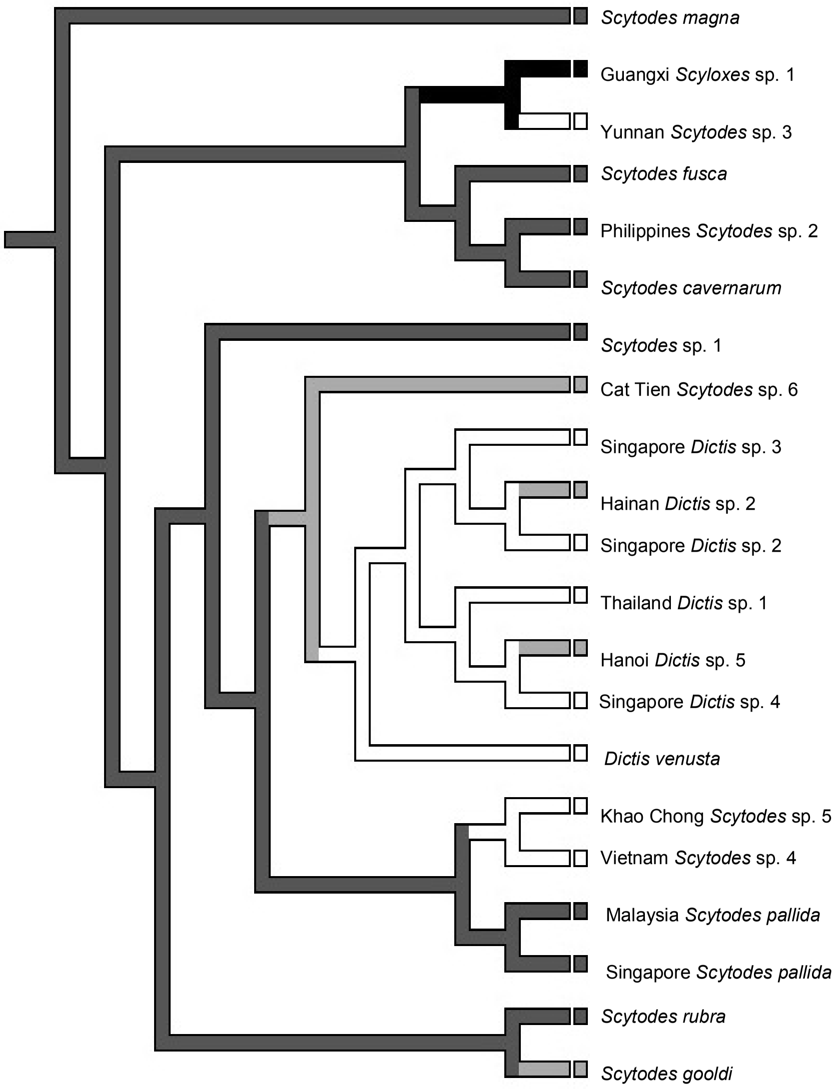
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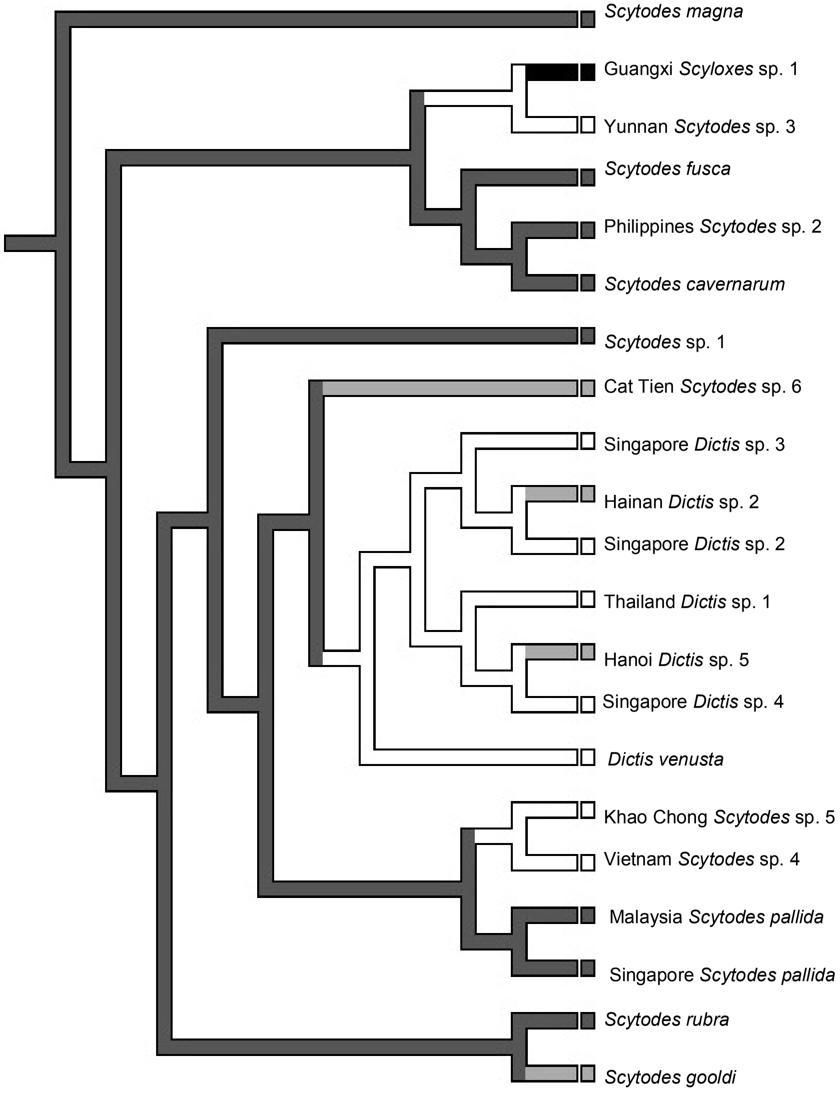
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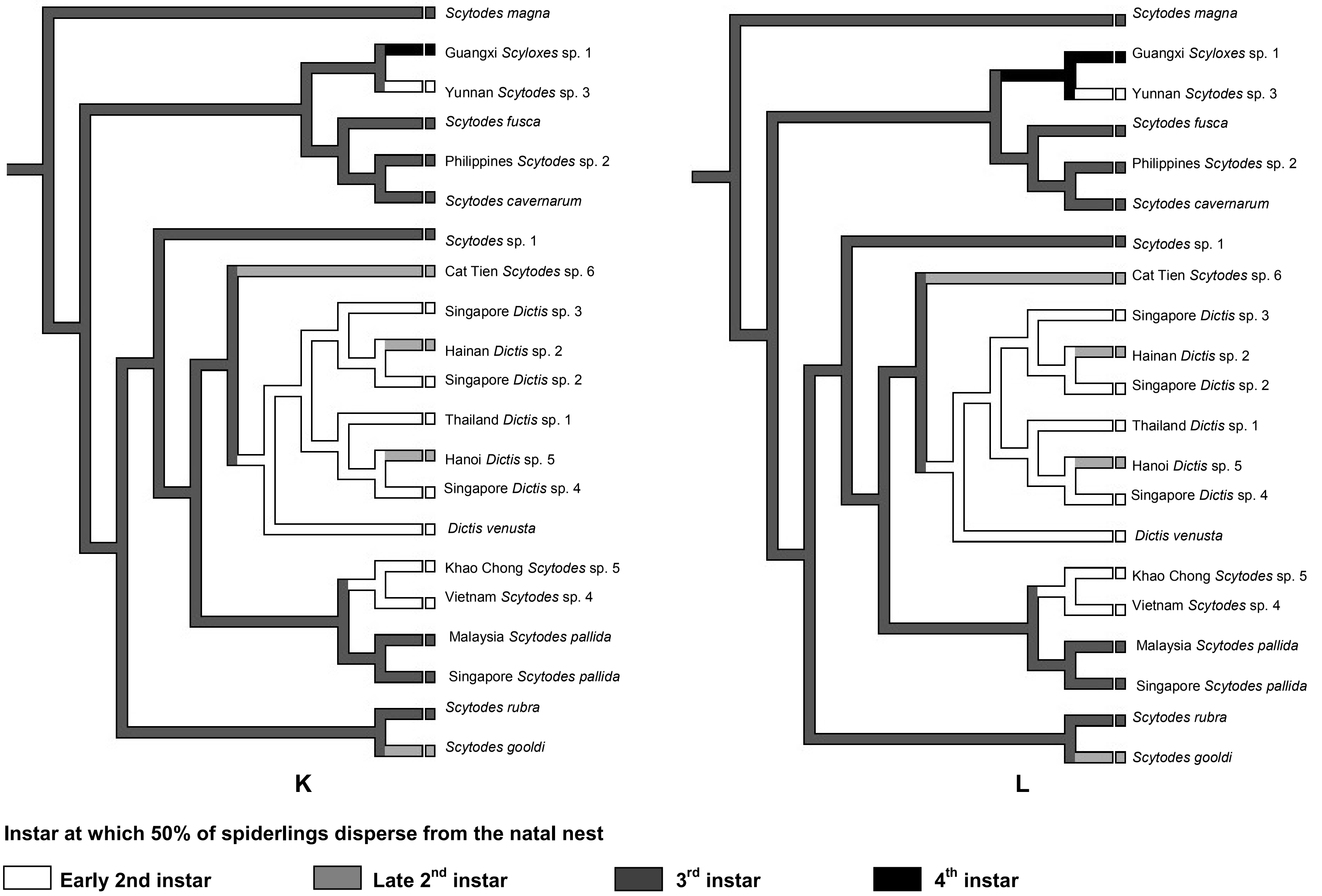


Fig. 6-12. Twelve optimized trees (A – L) of the character: instar at which 50% of spiderlings disperse from the natal nest; mapped onto the MPT = gaps as 5th character state for 21 taxa.

Dispersal is sensitive to environmental factors such as resource availability, population density or predation risk (Cahan et al. 2002). Such factors appear to have promoted delayed dispersal in desert isopods and beetles, as well as in African cichlids (Shachak and Newton 1985; Taborsky 1994; Rasa 1997), in polygynous ant species, low dispersal success has caused permanent failure to disperse from the parental colony (Creel and Waser 1994; Keller 1995). The dispersal decision is also strongly influenced by social interactions among individuals occupying the same natal nest. Social dominants, often the parents, can either promote or discourage offspring dispersal through the level of aggression directed toward subordinates. At the extremes, this can result in forced eviction (Taborsky 1994). This was not investigated in my study, but it is possible that the maternal females of the solitary species produce chemical cues to “chase” the spiderlings off her web. In sum, a combination of different factors may be acting on different species making it difficult to delineate specific factors that are acting on the overall scytodids. However, from the experimental data, it seems likely that cooperative foraging plays a significant role in the evolutionary maintenance of delayed offspring dispersal in scytodids. There is evidence of plasticity in the timing of dispersal and is influenced by prey availability in the nest (Morse 1980; Krafft et al 1986; Ruttan 1990). Permanent social organization could evolve in the subsocial *Scytodes* if the young opt out of dispersal and remain in the maternal nest regardless of the costs.

Reproduction

Clutch size in spiders is correlated with foraging patterns (Enders 1976), parental care (Buskirk 1981), and sociality (Kullmann 1972; Smith 1982). Several authors (e.g. Kullman 1972) have noted an inverse relationship between levels of social development and fecundity in spiders. In general, social spiders are expected to lay fewer eggs than spiders of the same genus showing a lower level of sociality (Krafft 1982). Kullmann (1972) was the first to observe that social *Stegodyphus* produced smaller clutches than their less social relatives, and suggested that this decrease in clutch size is a result of the increased maternal care invested by the more social species. When compared between species of sheet-web spiders having similar body size, Buskirk (1981) found that species of spiders exhibiting greater maternal care produced smaller clutches than those that displayed less maternal care. This led me to hypothesize that the more social scytodids would produce fewer spiderlings than their solitary relatives. However, this trend was not recovered in the scytodids, that is, my results showed that *S. cavernarum*, Hanoi *Dictis* sp. 2 and Yunnan *Scytodes* sp. 3, all three solitary scytodids, produced fewer offspring throughout their reproductive cycle compared to subsocial *Scytodes* sp. 1 and *S. gooldi*. Several parameters may attribute to the unexpected results: female body size, food intake, duration and number of copulation.

Within some groups it certainly appears to be true that more social spiders tend to be less fecund, but Smith (1982) found a different trend in clutch size that clutch size increases with increasing level of sociality. A complication is introduced into this analysis by the fact that clutch size and body size are in general positively correlated in spiders (Eberhard 1979). In her study Smith (1982) showed that for orb-spinning spiders such as *Cyrtophora* (Araneidae) and *Philoponella* (Uloboridae) the solitary species tend to have smaller clutches than communal species. Females of larger body size produce larger clutches than females of smaller body size. Interspecific variations have been recorded for *Loxosceles laeta* and *L. intermedia* (Andrade et al. 2000) with the former species laying a greater number of eggs because of its greater size and mass. As discussed previously, Yunnan *Scytodes* sp. 3 and *S. cavernarum* are small in size; Yunnan *Scytodes* sp. 3 being the smallest scytodid in this study, tended to lay fewer eggs. In Linyphiidae, the relationship between the duration of mating and egg viability was related to the time required for the transfer of sufficient sperm for the construction of egg-sacs (Suter and Parkhill 1990). Conditions in the laboratory may not be conducive for some scytodid species to mate and may result in low quantities of sperm being transferred into the female. In my study, all laboratory-raised virgin females were mated once with virgin males. It may be possible that for some scytodid species, multiple matings are required to ensure reproductive success resulting in larger number of spiderlings. According to Horner and Stewart (1967), female *Loxosceles reclusa* that mated repeatedly were more fertile since additional mating protected against the gradual loss of sperm viability and inadequate storage capabilities. The amount of food a female takes in affects its physiological state. A lowered food intake will result in low body mass and so the female produces fewer eggs (Fischer and Vasconcellos-Neto 2005). All scytodids reared in the laboratory were fed fruit flies and house flies, following the protocol of other studies, however, these prey may not be suitable for all scytodid species.

In addition, I hypothesize that the more social scytodids would have longer egg-guarding duration and longer time interval between consecutive ovipositions than the solitary species, due to higher investment in their progeny (i.e. by tending to the egg-sac for a longer period, the females can ensure that the spiderlings emerge only when they are physiologically ready to do so). However, I again found my results inconsistent with my prediction. Other factors may influence the length of incubation time. For example, the length of incubation in *L. intermedia* appeared to be related to the air temperature and relative humidity, and these factors appeared to influence how long the spiderlings remained within the egg-sac (Fischer and Vasconcellos-Neto 2005). The time interval between successive egg-sacs may also be influenced by the spiders' nutritional state, sperm storage and stress (Fischer and Vasconcellos-Neto 2005).

It thus seems that all reproductive traits are not good characters for defining the level of sociality of scytodids (see Appendices A).

Sociality within the family Scytodidae

In addition to *Scytodes* sp. 1, Malaysia *S. pallida*, Singapore *S.pallida*, *S. rubra* and *S. gooldi,* extended maternal care has also been documented in *S. socialis* (Miller 2006) and *S. intricata* (Eberhard 1986). The fact that in this genus (within Scytodidae) the females remain in close proximity to their egg-sacs and tolerate the presence of and physical contact with their hatchlings for two to three weeks clearly indicates a degree of intraspecific tolerance beyond that found in other solitary spiders. The more advanced social organization present in *Scytodes* sp. 1, Malaysia *S. pallida*, Singapore *S. pallida* , *S. rubra*, *S. gooldi*, *S. intricata* and *S. socialis* may have evolved, at least in part, from such mother-offspring associations.

It is evident that Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 are "transition species" exhibiting both solitary and subsocial traits. What is unclear, however, is the polarity of the social state. That is, it is unclear if there is a reversal from the subsocial to solitary state in Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 or if they are transiting into the subsocial state. My findings suggest that detailed studies of Yunnan *Scytodes* sp. 7 might be particularly valuable in solving the above question since this species is placed between Khao Chong *Scytodes* sp. 5 + Vietnam *Scytodes* sp. 4 clade and the *S. pallida* clade. However, because there is no evidence or hypothesis to suggest that loss of sociality in spiders is more likely than gains, I favour the idea that Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4 are "quasisolitary" (a term coined by Agnarsson (2006) to include species that exhibit maternal care, but the young disperse from the maternal web at an earlier stage than in their subsocial counterparts), transiting into the subsocial state.

The genus *Scytodes* contains solitary, subsocial, and "transition to social" species, thus offering an independent opportunity to examine the consequences of reversal to solitary behaviour on other behavioural traits. However, to date no phylogenetic hypothesis for *Scytodes* species exists, and while existing evidence suggests several independent origins of sociality, it remains to be seen if sociality has ever been lost within the genus. Agnarsson and colleagues (2006) provided the first evidence for a reversal in behaviour from social to solitary in *Anelosimus* lineage. Linked with this is a reversal in other traits, such as web type, use of sticky silk, and the ability of juveniles to emerge from the egg-sacs, and build and maintain webs without help from the mother. To date, there is no evidence for a reversal from subsocial to solitary living in spiders.

I term *S. rubra* a "transition into the social" species based on certain traits that it portrays: cooperative prey capture both in the presence and absence of the maternal female, late natal dispersal and cannibalism occurring only from the fourth instars. It is evident that members of this species are more tolerant of each other. Also from the colony composition drawn out during the field sample survey, subadult males and females (range: two to four) were found regularly in the same web under rocks. I also observed a similar trend in the laboratory in which cooperative prey-capture also occurred, but I did not observe siblings attempting to mate with each other. Furthermore, I found from my laboratory observations that *S. rubra* females generally produce only one egg-sac throughout their reproductive phase. This conclusion, however, requires further confirmation because I cannot be certain if this phenomenon is partially due to unsuitable breeding conditions in the laboratory or prior to being caught and transported back to the laboratory, the females had already undergone one reproductive cycle. Miller (2006) observed 12 *S. socialis* individuals comprising of juveniles, females and males occupying the same web in two separate occasions, but the degree of relatedness among nest mates has not yet been established although given the unbiased sex ratio in this species; it would appear that dispersal is common.

Inbreeding requires that members of both sexes remain in the natal nest, while sociality does not, as is clear from the fact that most social organisms are, in fact, not inbred. In spiders, the existence of at least two outbred social species, *Tapinillus* sp. (Avilés 1994) and *Delena cancerides* (Rowell and Avilés 1995), suggest that even in spiders inbreeding is neither a requirement for sociality nor a necessary consequence of it. Outbreeding in *Tapinillus* sp. is accomplished by dispersing males so colonies consist mostly of siblings plus a few immigrant males. More detailed observations from across the phenological cycle are necessary to determine whether sociality in *S. rubra* and *S. socialis* conform to the *Tapinillus* model of sociality, although results from my colony survey has shown thus far that *S. rubra* males do not cohabit with females implying that the males must be leaving the natal nest before they reach sexual maturity. A lack of female-biased sex ratio also indicates outbreeding in *S. rubra* (personal observations).

Although the natural history of *S. quarta* and Otjoz *Scytodes* sp. 19 in the '*thoracica*' group are unknown, their behaviour can be predicted based on their phylogenetic position. They are nestled amongst the subsocial/non-territorial multiple-adult species and can thus be predicted to be subsocial, unless sociality has been secondarily lost. A similar phylogenetic prediction recently led to the discovery of numerous subsocial *Anelosimus* species in Madagascar (Agnarsson and Kuntner 2005). Recent studies have shown that quasisociality evolves from intermediate subsociality (Avilés 1997; Bukowski and Avilés 2002; Powers and Avilés 2003; Agnarsson 2002, 2004). Understanding the evolution of quasisociality thus requires the study of subsocial (or even secondarily solitary) species, and the '*thoracica'* group is a promising source of case studies. Any behavioural study of the least known nonsocial species would help us to understand the factors that could favour the evolution of the sociality in scytodids and even in other spider groups.

Although sociality is often discussed in terms of discrete classes, within this family there appears to be a continuum of levels of subsociality from dispersal of young at the end of maternal care at a very early stage (e.g. Hanoi *Dictis* sp. 5), through dispersal at later instars (e.g. *S. pallida*) to groups of subadults remaining together and sharing a nest (e.g. *S. socialis*) (Miller 2006). Even in *S. intricata* in which food provisioning by the adult female and prey sharing are lacking, Eberhard (1986) still considered this species to be subsocial on the basis that colonies generally consisted of mother-offspring associations. Ecological factors are likely to be playing a dominant role in determining the length of the group-living stage. The resource dispersion hypothesis (see Carr and Macdonald 1986; Macdonald 1983) states that the quality and dispersion of resources influence the social structure of population in a given habitat. Environment and abundance of prey in open habitats of the tropics may have favoured the occurrence of sociality. Abundant food resource may have facilitated group formation in *S. fusca* in the Queensland community. Other than food conditions, the stage at which dispersal occurs may also be dictated by intranest competition (Aviram 2000).

Some authors have suggested that paedomorphosis might be the mechanism by which adult individuals have retained the tolerant phase (Kraus and Kraus 1988), pointing to the trend towards smaller body size that characterizes some of the quasisocial species relative to their subsocial or solitary relatives. I found this to be an interesting point because species of the genera *Stedocys* and *Scyloxes* are of a large body size, and the species of these genera are solitary.

Phylogenetics and the multiple origins of scytodid subsociality

My study, the first intensive behavioural study of scytodids, was performed on 21 taxa across possibly ("possibly" because I am at present unsure if *S. magna* should be placed in a separate genus of its own) four genera – *Scyloxes*, *Scytodes*, *Dictis* and '*Magnodes*'. Other than detailed studies performed on two genera – *Stegodyphus* and *Anelosimus* – no other studies carried out on such a scale have been performed.

All the studies of social spider so far have shown that multiple independent origins of sociality exist in all the known social spider clades and the number of origins vary with clades. The genus *Stedogyphus* contains three independently derived permanent-social (quasisocial) species that fall into three species groups (Kraus and Kraus 1988). Despite their ancestral antiquity, the social *Stegodyphus* have not diversified and evolved cladogenetically (Johannesen et al. 2007). Low diversification applies also to clades of subsocial *Stegodyphus*. Without a complete phylogeny of the genus, it remains unresolved whether lack of diversification is characteristic of all *Stegodyphus* or of social spiders in general. The cobweb spider genus *Anelosimus* (Theridiidae) is well known for containing numerous social species. Many of these have been intensively studied behaviourally (see Kullmann 1972; Avilés 1997 for review), and the genus has been used frequently as a model system to advance our understanding of the evolution of spider sociality (e.g. Avilés 1993, 1997; Agnarsson 2002, 2004, 2006; Agnarsson and Kuntner 2005; Bukowski and Avilés 2002; Powers and Avilés 2003). Agnarsson et al (2006, 2007) produced a detailed phylogeny of the social *Anelosimus* which led to three conclusions: (1) there are 5 or 6 independent origins of sociality; (2) several pairs of social-subsocial sister species can be recognized; and (3) there seem to be no instances of diversification within the social clades, a pattern also seen in the social *Stegodyphus.*

Avilés (1997) inferred at least 12 derivations of sociality in spiders, counting at least three times in theridiids (*Anelosimus*, *Theridion*, *Achaearanea*); three times in *Stegodyphus* (Eresidae); and at least once each in *Agelena* (Agelenidae*), Aebutina* (Dictynidae), *Mallos* (Dictynidae), *Tapinillus* (Oxyopidae), *Delena* (Sparassidae), and *Diaea* (Thomisidae). With the addition of newly confirmed (*A. dubiosus*) or discovered (*A. guacamayos*, *A. oritoyacu* and *A.puravida*) social taxa in the Theridiidae and recently described sociality in the Nesticidae, there are now at least 18 independent derivations of sociality in spiders (Agnarsson et al. 2006). The social spiders originate from subsocial ancestors, through prolongation of intraspecific tolerance and cooperation to adulthood (Kraus and Kraus 1988; Agnarsson 2006). Cooperation in social species include cooperative brood care performed by multiple females in the nest and web construction by several individuals simultaneously (Lubin and Bilde 2007). Regular inbreeding which is another key characteristic of quasisocial spiders (Lubin and Bilde 2007) arises from the suppression of pre-mating dispersal phase (Kullman 1972; Avilés 1997).

Interestingly, sociality has a "spindly" phylogenetic distribution in spiders (Agnarsson et al. 2006). Although sociality has evolved repeatedly, in no cases did a diverse social clade arise: each origin has yielded at most a small clade of one or two species. Thus, inbred sociality may be an example of an evolutionary dead-end, in which further evolution of social clades ceases. Although perhaps initially successful within species, inbred sociality may be unusually vulnerable over the long term. The "spindly" phylogenetic pattern may be explained by extinction rapid enough that a nonsocial sister group does not have time to diversify while the social lineage remains extant (Agnarsson et al. 2006). In my study, although no quasisocial species has been identified, the subsocial spiders portray a "spindly" distribution, albeit being confined to the Old World clade. Will the subsocial scytodids evolve into social species resulting in a "dead-end" or revert to the solitary lifestyle? Perhaps there will be no "dead-end" in scytodids because if they conform to the *Tapinillus* sp. model of sociality, then there will be no potential for inbreeding depression.

Subsociality is rare but phylogenetically scattered in spiders with about 30-40 species distributed in Amaurobiidae (Horel et al. 1982), Eresidae, Lycosidae, Pisauridae, Salticidae, Scytodidae, Thomisidae, and Theridiidae (Avilés 1997; Whitehouse and Lubin 2005; Agnarsson et al. 2006; Lubin and Bilde 2007;). On the preferred phylogeny, the subsocial species of scytodids are also scattered, implying at least four independent origins of sociality (Fig. 6-1). Diverse clades in the Old World have given rise to sub-social species, e.g. *S. pallida* and *Scytodes* sp. 1 from Asia; *S. rubra*, *S. gooldi* and *S. socialis* from Africa. The subsocial species are nested well within the phylogenetic tree, while solitary behaviour optimizes to the base of the scytodid phylogeny (i.e. is primitively present). Hence, subsocial behaviour is derived. Future work could involve resolving the number of origins of subsociality/sociality. Within the araneomorph spiders, cooperative or communal behavior occurs more often within families whose lifestyle depends on the use of a web (irregular or orbicular) (D'Andrea 1987). A web is important because it provides a means of communication among individuals and may promote cohesiveness to the group (Krafft 1979). Thus, this could mean that *Scytodes*, having irregular webs, may be pre-disposed to being social. However, there are thousands of species with irregular webs, out of which only 16 are known to be cooperative and quasisocial. What then are the other pre-requisites? Krafft (1979) noted that a predisposition to sociality might be present in all spiders given that they spend their first instar after eclosion confined within the common sac. This initial phase of tolerance among siblings may have served as the basis from which longer lasting associations could have arisen (Kullman 1972; Krafft 1979). If this initial period of tolerance can be extended by maternal care, coupled with a suppression of the competitive instincts, a permanent-social species could arise.

I have shown that Scytodidae offers another independent opportunity within spiders to explore evolutionary processes and to address other important questions. Do social scytodids exist in temperate regions or the neotropics? Can solitary species transit into subsocial species with high prey availability, abundance of large prey and large spaces? Does kin recognition exist in the subsocial scytodid species, and if so would individuals cannibalize intruders first before eating their siblings? What are the life history advantages of sociality- for example body mass, growth rates, and fecundity? With the addition of more taxa and further social behavioural traits uncovered, more evolutionary hypotheses within Scytodidae and even in other spider groups can be generated.

The *Scytodes* genus contains mostly solitary species, and solitary life is almost certainly the primitive condition in scytodids since no social species are known from the sister families Sicariidae and Drymusidae. The question that should be asked is: why is sociality in scytodids so rare? Many authors have argued that the first most immediate consequence of the evolution of cooperation was probably a switch from an outbred to inbred breeding system, so to avoid the negative repercussions (i.e. loss of fitness) caused by inbreeding depression, these spiders remain in colonies. However, I have discussed (see above) that inbreeding is neither a requirement for sociality nor a necessary consequence of it as exemplified by the *Tapinillus* sp, and even in the Australian subsocial huntsman spider, *Delena cancerides* (Rowell and Avilés 1995)

Did “modern” scytodids evolve from cave ancestors?

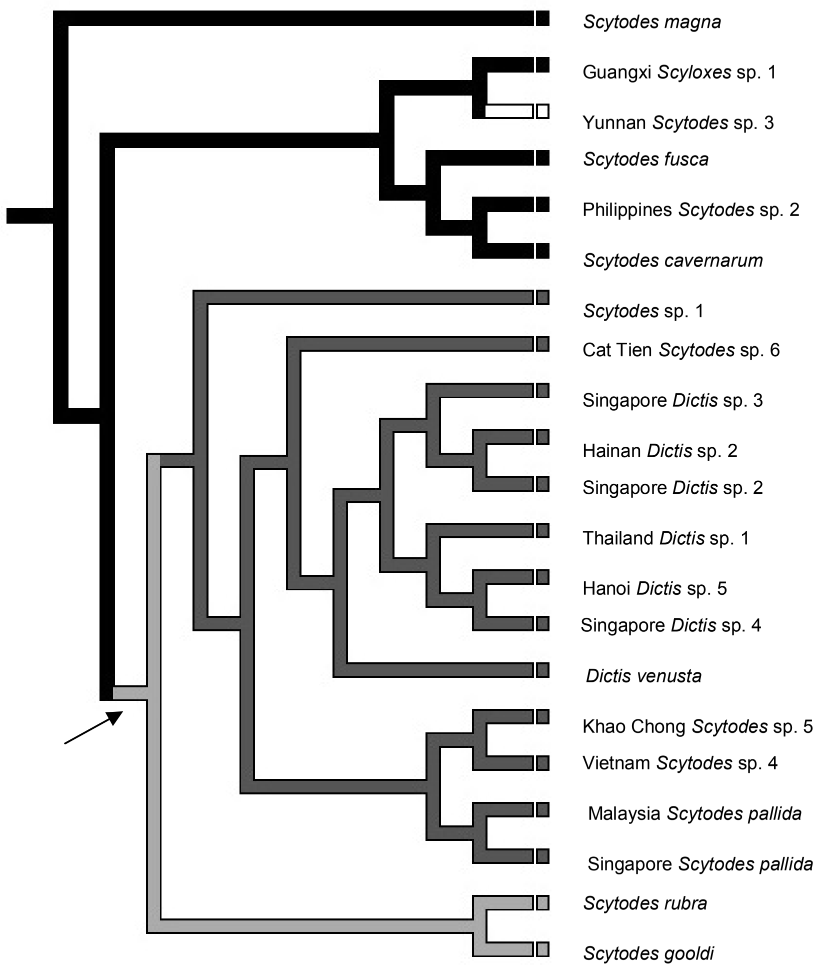
The evolution of troglobites is often considered a unidirectional, irreversible, evolutionary dead-end (cited in Predini et al. 2010). Reversal, the recolonization of endogean or epigean habitats by hypogean taxa is assumed to be prevented through competitive exclusion by species already occupying, and better adapted to, those habitats (Conway Morris 1995). Endogean or epigean generalists are thought to be more adaptable to changing environmental pressures than hypogean specialists that are used to environmental stasis, due to possession of a wider range of features.

The phylogeny of Typhlochactidae (Scorpiones) challenges the conventional wisdom that ecological specialization (stenotopy) is unidirectional and irreversible, falsifying Cope's Law of the unspecialized and Dollo's Law of evolutionary irreversibility. A recent study has shown that troglobitism is not an evolutionary dead-end: endogean scorpions evolved from hypogean ancestors on more than one occasion (Prendini et al. 2010). My results suggest that the troglobitic condition may be ancestral in Scytodidae (*Scytodes magna*), evolved to a trogloxitic condition (as in *S. univittata*, a species considered occasional visitors to cavernicolous habitats), and then reverted back to the triglobitic/hypogean condition (Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2). The endogean condition then evolved once from the hypogean condition: in Yunnan *Scytodes* sp. 3.

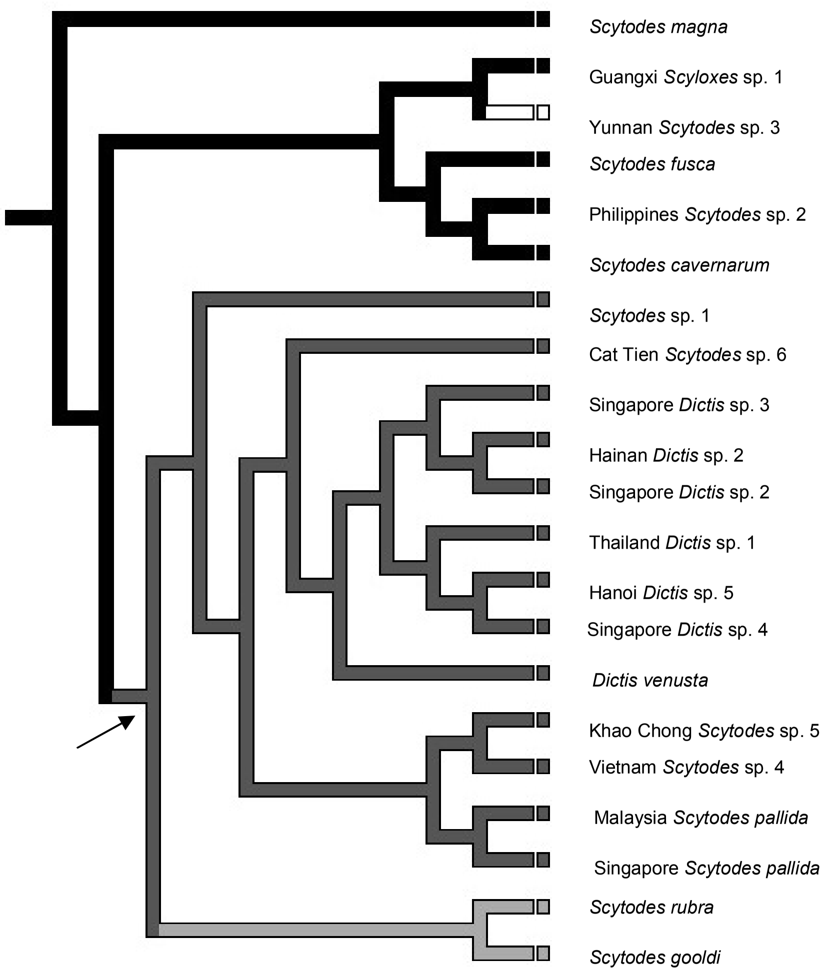
It is evident that the non-hypogean scytodids (i.e. the ground-dwelling and aerial vegetation scytodids) evolved from the hypogean ancestors (Fig 6-13). When looked into the natural history of Drymusidae, the sister family of Scytodidae, I found that webs of *Drymusa serrana* were found in the inner half of the cave, which was wetter and darker than the outer half, and attached to large supporting objects such as the crevices in the cave's walls or under rocks. *D. rengan* are usually found between rocks and roots well covered by earth and leaf litter, on a hill slope. Other *Drymusa* species such as *D. dinora* can be found on forest floor, e.g. underside of fallen logs. *Loxosceles laeta*, another species from Sicariidae that was included as an outgroup in my molecular phylogeny, is usually found in microhabitats such as indoors in wooden houses, spaces behind picture frames and furniture, and outdoors in rock cavities, beneath rocks, and beneath loose bark, in hollow trees, in crevices of decaying logs, under leaf litter, in caves and grottos (Fischer and Vasconcellos-Neto 2005). *Loxosceles* are also noted to be ubiquitous in the karstic environments (Gonclaves-de-Andrade et al. 2007). *Sicarius*, like *Loxosceles*, live in shallow caves and in crevices made between natural debris and the ground, but they are often in highest abundance in patches of fine sand, such as at the base of cliffs or under rocky overhangs (cited in Binford et al. 2008).

My study appears to support Prendini et al's (2010) hypotheses that the evolution of troglobites may not necessarily be unidirectional (i.e. cave colonization must evolve from non-hypogean ancestors), and it is not an evolutionary dead-end. My study is of course still in its infancy stages, and due to lack of information, I am unable to make a firm conclusion on which mapped tree is the most parsimonious. The difference in all three optimizations is the node marked with an arrow. In Fig. 6-13A, it suggests that the evolution of hypogean ancestors to an epigean state and subsequently to aerial habitats is a progressive one. Fig. 6-13C suggests that the divergence from a hypogean ancestor into the epigean and aerial condition occurred simultaneously. Fig. 6-13B seems to be the least parsimonious in that from the hypogean condition, there is a shift to the aerial habitats but the (*S. rubra* + *S. gooldi*) clade reverted back to a condition that is more ground-level – they became epigeal.

Herein lies one possible future for a study investigating the evolution of habitat types and morphological and social traits in Scytodidae. In recent years, Rheims and Brescovit (2000, 2001, 2004) have made numerous discoveries and described many new species of *Scytodes* from Central and South America that are found in a wide variety of habitat types, some of which include caves, under rocks and leaf litter. By incorporating these species into the molecular, morphological, and behavioural data we will be able to gain better insights as to whether our study supports or rejects Predini et al's hypotheses.


Scytodes magna
Guangxi Scyloxes sp. 1
Yunnan Scytodes sp. 3
Scytodes fusca
Philippines Scytodes sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien Scytodes sp. 6
Singapore Dictis sp. 3
Hainan Dictis sp. 2
Singapore Dictis sp. 2
Thailand Dictis sp. 1
Hanoi Dictis sp. 5
Singapore Dictis sp. 4
Dictis venusta
Khao Chong Scytodes sp. 5
Vietnam Scytodes sp. 4
Malaysia Scytodes pallida
Singapore Scytodes pallida
Scytodes rubra
Scytodes gooldi

A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Yunnan Scytodes sp. 3
Scytodes fusca
Philippines Scytodes sp. 2
Scytodes cavernarum
Scytodes sp. 1
Cat Tien Scytodes sp. 6
Singapore Dictis sp. 3
Hainan Dictis sp. 2
Singapore Dictis sp. 2
Thailand Dictis sp. 1
Hanoi Dictis sp. 5
Singapore Dictis sp. 4
Dictis venusta
Khao Chong Scytodes sp. 5
Vietnam Scytodes sp. 4
Malaysia Scytodes pallida
Singapore Scytodes pallida
Scytodes rubra
Scytodes gooldi

B

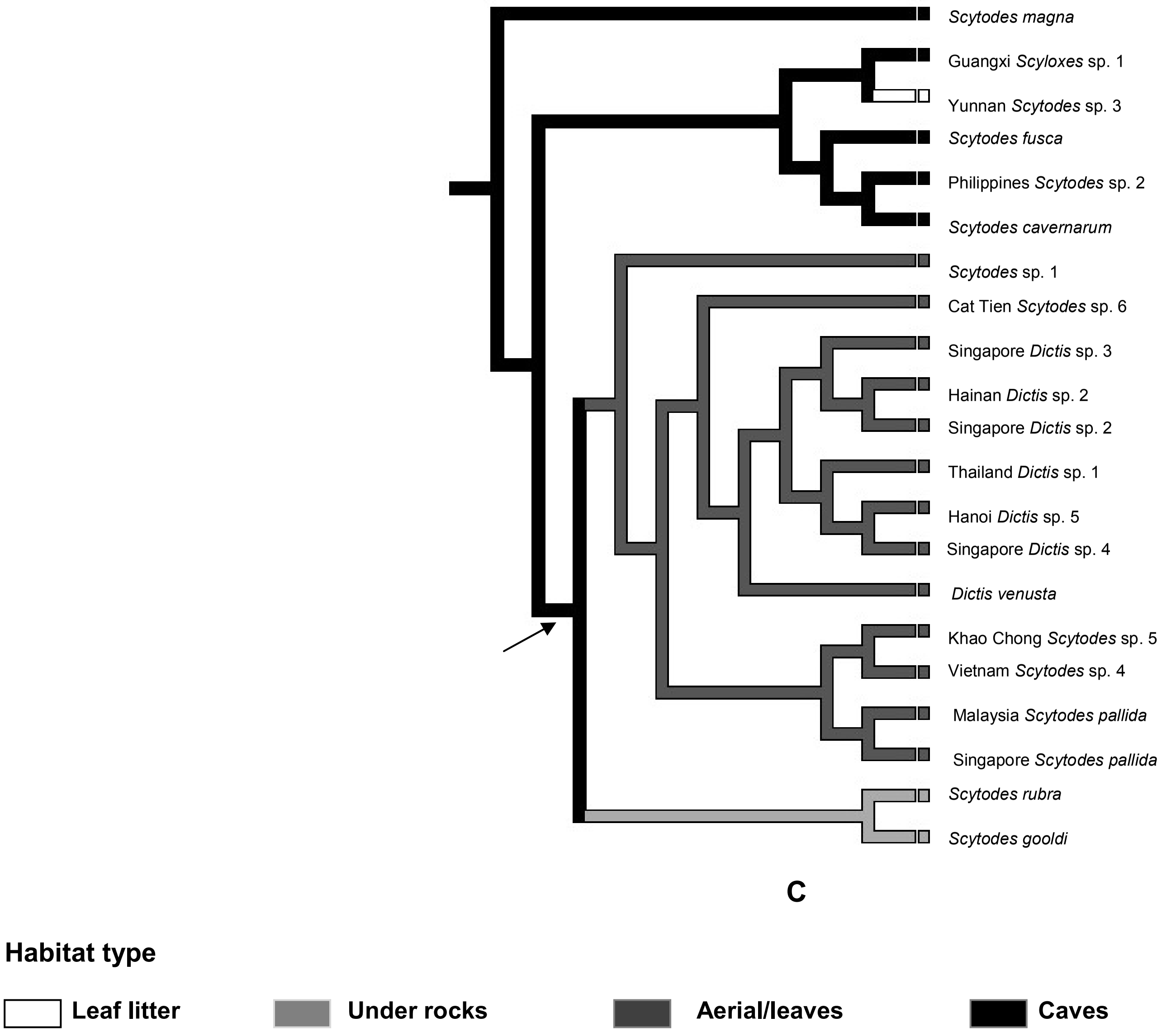
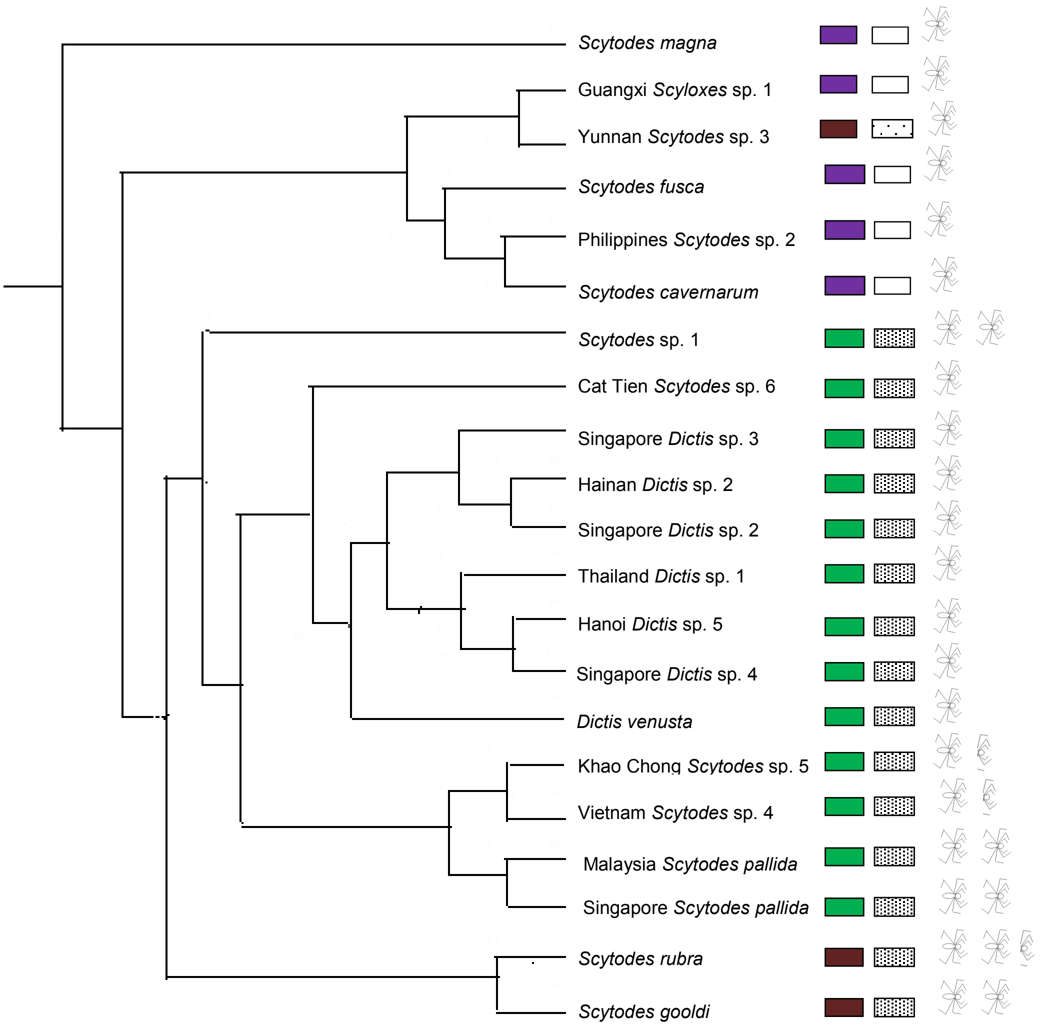


Fig. 6-13. Three optimized trees (A - C) of the character: habitat type; mapped onto the MPT = gaps as 5th character state for 21 taxa.

The costs and benefits associated with the decisions of dispersal and individual reproduction are largely determined by ecological factors. As with multicellularity (i.e. cells forming groups to become larger in size), sociality is also thought to allow the colonization of ecological niches not accessible to solitary individuals (Avilés 1999). Naked mole rats, for instance, are capable of inhabiting the extremely arid deserts of southern Africa by cooperatively searching for new food patches after heavy and unpredictable rains have softened the soil enough for digging (Jarvis et al. 1994). Among cooperative foragers, tree-killing bark beetles and social carnivores are capable of obtaining resources—live trees or large animals, respectively—that solitary individuals are unable to access (Baird and Dill 1995; Caraco and Wolf 1975; Nudds 1978; Raffa and Berryman 1987). Social spiders are notable among cooperative foragers for their ability to capture prey that are many times larger than the spiders themselves (Nentwig 1985; Rypstra 1990; Powers and Avilés 2007), thus gaining access to a rich supply of insects not available to most solitary spiders (Nentwig 1985; Avilés 1997).

A relationship between habitat type and the level of sociality inferred from Figs 6-13 shows the species that live in caves, regardless of obligatory or facultative, and leaf litter lead a solitary lifestyle. As I have argued in the earlier chapters, perhaps living in caves and leaf-litter may have precluded these scytodids from evolving into more social species because of: (1) low prey abundance; (2) abundance of predators; and (3) avoidance of being too conspicuous. A similar trend has been observed in cockroaches from the forests in Brazil. In performing a phylogenetic analysis of the social behaviour evolution of these cockroaches, Pellens and colleagues (2007) analysed the relationships between habitat and their social behavior, and found that reversals toward a solitary way of life occurred with an ancestral habitat, the ground litter, which could be part of an anti-predator strategy in the particular context of mass predation by army ants (Grandcolas and Deleporte 1994). The four subsocial species of scytodids (Singapore *Scytodes* sp. 1, *S. pallida* (Singapore and Malaysia populations), *S. rubra* and *S. gooldi*) and two possible "transition to subsocial" species (Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4) are found under rocks or on green leaves of shrubs or plants. The solitary species of the *Dictis* clade and Cat Tien *Scytodes* sp. 6 also stay in leaves, but a point to note is that most of these leaves are brown or shriveled up. *Dictis venusta*, for example, was sampled from dried and brown pitcher cups of *Nepenthes ampullaria* and *N. gracilis*.



Habitat:
cave vs. ground vs. leaf
🟪 🟫 🟩

Prey availability:
abundant vs. moderate vs.scarce
▩ ⬚ □

Level of sociality:
Solitary vs. subsocial vs. social
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Fig. 6-14. Pictogram depicting the relationships between habitat type, prey abundance and the level of sociality attained in the 21 taxa

A pattern of correlation between the habitat type, prey availability and level of sociality cannot be made (Fig. 6-14). Although there is no doubt that prey abundance is an important factor influencing the level of sociality, it does not necessarily mean that spiders inhabitating prey-rich habitats develop higher levels of sociality. None of *Dictis* spiders has been found to be subsocial/social, although they dwell in the aerial vegetative habitats where prey are abundant. One possible reason is that since *Dictis* is likely to be a highly derived genus (i.e. "younger" spiders), sociality within this genus may develop over time. Due to prey scarcity, predator abundance and the need to be inconspicuous, cave scytodids have been genetically programmed to lead solitary lifestyles. Levels of prey may be in the moderate range for leaf-litter dwelling scytodids, however, as these species are especially small in size, they may be easy targets for predators as such Yunnan *Scytodes* sp. 3 makes the decision to be solitary. Having said that, I did observe certain behavioural traits that can be classified as being social. For example, newly emerged spiderlings mount the female's body and the female rarely feed during the egg-sac guarding phase. Khao Chong *Scytodes* sp. 5 and Vietnam *Scytodes* sp. 4, which are found in a similar microhabitat as that of their subsocial counterparts (Singapore *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida*), are categorized as "transition into subsocial" or "quasi-solitary" species. There is a possibility that Cat Tien *Scytodes* sp. 6 may also evolve into a more social species given its similar microhabitat as that of the abovementioned species. *S. rubra* and *S. gooldi*, both ground-dwelling, are subsocial species. This could be attributed to prey abundance, prey richness or presence of numerous large-sized prey. As I have discussed above, dead logs teem with a variety of arthropods that can serve as potential prey for *S. gooldi*. Ants and spiders, together with other notably widespread groups of invertebrates such as Acarina, Collembola, Blattodea and Coleoptera, are abundant under rocks (Goldsbrough et al. 2003). As I mentioned before, *S. rubra* were sampled from under layers of rocks/stones. It has been shown that associations with rock-on-rock could be structurally driven for spiders that require bare rock for depositing egg-sacs (Goldsbrough et al. 2003). High prey availability coupled with safe habouring sites may be the factors responsible for *S. rubra* evolving into a "transition to social" species.

By comparing solitary and subsocial species which share the same habitat (i.e. aerial/leaf), I obtained results on several traits of these spiders that can be integrated into a more general comparison of these two ways of life. There appears to be a slight advantage when living in temporary groups. When I housed the spiderlings that had dispersed, individually, in small (cylindrical) cages and fed them with fruit flies, more than 80% of the spiderlings that had dispersed as second instars died within two weeks due to reasons such as inability to capture the prey (and therefore starved) or that they were physiologically weak. Early dispersers tend to have less mobility because these instars have shorter legs relative to their bodies than later-instar spiderlings. Those that dispersed as late second or third instars fared much better with about 75% moulting to fourth instars. These spiderlings belonging mostly to the subsocial species dispersed at a larger body size, appeared to be fitter with more coordinated movements and hence were more capable of catching the fruit flies. The solitary females produced more eggs.

Social spiders and hymenopteran insects are two groups that seem particularly able to adapt, or facultatively adjust, their level of sociality to the environment. Although sociality is rare among spiders, the non-orb-web building families are ‘pre-adapted’ for sociality in that social individuals mainly need to evolve conspecific tolerance, not novel behaviours (Krafft 1979). The rapid diversification of *Scytodes* together with their ecology and high sociality potential makes this genus a promising candidate for future studies of the evolution of sociality. The phylogeny of these spiders coupled with detailed behavioural studies provides an ideal background for comparative studies of the relative influence of various ecological factors on the evolution of sociality.

CONCLUSION

My findings show that in Scytodidae, subsociality is derived. My study also supports the long-held view that extended maternal care pre-adapts spiders for sociality. Subsociality and quasisociality can gradually evolve by extension of maternal care to older and older offspring. As seen in the model systems of *Stegodyphus* and *Anelosimus*, the evolution of sociality in spiders was accompanied by changes in the dispersal pattern, degree of cooperation and breeding structure. Moreover, the subsociality in Scytodidae have been gained and lost frequently throughout its evolutionary history. My study was designed to focus on social traits that can be found in subsocial species that may have facilitated the transition to sociality and I have discussed some ecological parameters that may have influenced this transition. Ecological factors such as prey availability are likely to play a key role in determining the length of the group-living phase. Subsocial species in possessing traits of both solitary and social spiders make excellent study subjects because they provide a window into the ways these changes might have come about as well as provide a good model for the study of factors that may influence the evolution of social behavior. The current phylogeny offers a robust hypothesis of Scytodidae interrelationships that serves as a backbone to future, more detailed phylogenetic studies. A point to note, however, is that such hypotheses are always only as good as the phylogenies upon which they are based; any changes in the cladistic structure will entail changes in the evolutionary hypotheses (Ryan 1995). Nonetheless, the scytodid system of sociality as presented here does provide new information bearing on the puzzle of whether or not ‘permanent sociality is truly an evolutionary dead-end’.

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CHAPTER 7

GENERAL DISCUSSION

Sociality of scytodid spiders

One of my primary objectives is to determine the form of sociality for the scytodid species available as well as to check for the possibility of further, as yet unrecorded forms of sociality. In examining 21 species and populations, my study of social behaviour in scytodids in this thesis has revealed four more subsocial species in Scytodidae. *Scytodes gooldi*, *S. rubra*, *Scytodes* sp. 1, Malaysia *S. pallida* and Singapore *S. pallida* exhibit the suite of traits associated with subsocial spiders. However, within these four subsocial scytodids, there exist variation in certain traits such as length of tolerant phase among siblings, time in which cannibalism first occurred and fecundity rates. Subsociality has been used as a collective term to lump all associations from transient parent-offspring associations to much more complex social groups that include long-lasting mother-offspring-sibling associations extending until or even beyond sexual maturity of the offspring and the death of the mother (Rayor and Taylor 2006).

Over the last decade, there has been an increasing recognition that sociality occurs along a behavioural continuum, rather than in discrete categories, and that definitions of sociality need to be broadened to more accurately reflect the diversity of social dynamics in a broad range of animals (e.g., Sherman et al. 1995; Costa and Fitzgerald 1996; Choe and Crespi 1997; Crespi and Choe 1997; Costa and Fitzgerald 2005; Whitehouse and Lubin 2005). The designation of species into the subsocial category can be somewhat tricky. Some species do not fit comfortably in a single category. For example, Eberhard (1986) termed *S. intricata* as subsocial despite the fact that agonistic interactions within groups of siblings seem to be especially common. Furthermore, maternal female is not known to provide food for her young. At the other end of the subsocial spectrum, there is *S. socialis,* a species of web-building scytodid in which webs may contain multiple males, females and juveniles. Multiple nest-mates participate in cooperative prey-capture of large prey items, and both participants and non-participants are allowed to feed on the captured prey (Miller 2006).

Rayor and Taylor (2006) proposed that in describing and assessing social dynamics in subsocial arachnids, both the duration and patterns of association among mothers, offspring, and siblings should be taken into account. Key variables are the age of dispersal from the natal nest, degree of cooperative behaviour, and patterns of tolerance or social interactions within the group. It is through incremental changes in these variables along the social continuum that higher sociality evolves. In agreement with Rayor and Taylor (2006), I used the term "transition into social species" to describe *S. rubra* which shows extended mother-offspring-sibling associations and "quasi-solitary species" (i.e. transiting into the subsocial state) to describe Vietnam *Scytodes* sp. 4 and Khao Chong *Scytodes* sp. 5 that exhibit both solitary and subsocial traits such as females rarely foraging during egg-sac guarding and emergents mounting the mother's body. In future, "prolonged subsocial" and "transient subsocial" (Rayor and Taylor 2006) may be used.

This study found two subsocial *Scytodes* that live in leaves of shrubs, adding to the growing pool of known subsocial scytodid species from the aerial vegetation zones. The other two subsocial scytodids are found in the ground substratum. All five cave scytodids were found leading solitary lifestyles. I propose that this pattern may be correlated to the level of prey and prey diversity, as well as extent of predation pressure found in the habitat. Prey scarcity and presence of numerous predators may account for non-social spiders in caves whereas abundant food supply and presence of large-sized prey may be the reasons why most of the social scytodids and social spiders as a whole reside in the aerial vegetative zones. Within the scytodids, I have shown that the duration of mother-offspring-sibling association is a continuum that may vary with ecological factors. In a habitat where there are many large prey such as roaches and moths, sibling groups that participate in collective prey capture stand to gain as compared to solitary individuals which would be unable to access large prey items, hence favouring group-living. With an extension of the tolerance phase over time, juveniles will remain longer and longer in the natal nest, eventually forming long-lived colonies (Fig. 7-1).

By comparaing behaviour of *S. fusca* that live in aerial vegetation (Bowden and Jackson 1988) with *S. fusca* that live in caves (this study), I found the cave species to lead solitary lifestyle whereas those found in web complexes made on the trunks of large trees are communal territorial. There appears to be considerable flexibility in the social life histories of *S. fusca*. In *Anelosimus studiosus*, communal or solitary breeding is found to be an alternative reproductive strategy depending on environmental conditions (Jones and Riechert 2008). Social groups can form when individuals aggregate because of inherent advantages of group living, and in this case the vegetative zone in which *S. fusca* naturally occurs could be rich in large prey and so in building large web complexes whereby single units are linked together by interstitial silk the chances of capturing large prey are higher. Conversely, it is possible that foraging-related forces other than prey availability have driven group living in *S. fusca*. For example, joining webs into larger capture sheets may allow individuals to exploit niches unavailable to solitary individuals (e.g. open areas; Whitehouse and Lubin 2005), particularly in sites where prey are scarce. Such a trend has been observed in populations of the orb-weaving spider *Parawixia bistriata* that exhibits a greater tendency to forage in habitats offering lower resource (Campón 2008). Here is an example of sociality arising via the parasocial pathway: *S. fusca* groups, if they do exist, are formed parasocially by aggregations of individuals around a prey-related resource such as abundant food supply or large prey item, and their composition is variable (Lubin 1980).

Is quasi-sociality attainable in the subsocial scytodids? It is evident from field observations of *S. socialis* that mature males and females, and juveniles all participate in prey capture of large prey items such as moths and roaches (Miller 2006). The survey of sixty-one colonies has shown *S. socialis* to be a non-territorial multiple-adult species. Inbreeding is not known in this species but as I have discussed elsewhere in this thesis, inbreeding is neither a requirement for sociality nor a necessary consequence of it (also see Whitehouse and Lubin 2005; Lubin and Bilde 2007). The group might then comprise of siblings and immigrant males. The breeding females can then make a choice to remain in the same nest and cooperate in raising the brood. There is a possibility that these populations are currently in flux and evolving towards higher levels of sociality. *S. socialis* – a "transition to social" species – may be a hint that non-territorial permanent sociality (read: quasisociality) may, if not already present, evolve within the scytodids one day. In the case of *S. rubra*, perhaps they need only to extend the period of group cohesion and mutual tolerance into adulthood to become permanently social. In this regard, the timing of juvenile dispersal in *S. socialis* and *S. rubra* could be under selective control by ecological conditions (see Fig. 7-1).

Phylogeny of Scytodidae

Other than investigating and describing detailed social behavioural traits exhibited by each of the 21 taxa, my second primary goal is to reconstruct a phylogeny for the family Scytodidae. This study provides the very first molecular phylogenetic tree for Scytodidae. It includes 44 representatives from four different genera, and shows strong support of the monophyly of Scytodidae. Research, however, disclosed an order of magnitude more genera and species than expected at the outset. The family Scytodidae has traditionally consisted of five genera: *Scytodes*, *Dictis*, *Scyloxes*, *Soeuria* and *Stedocys*. Although tentatively placed in *Stedocys* and *Scyloxes* in this study, I found that Guangxi *Stedocys* sp. 1, and Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2 do not fit well into their respective genera. In fact these taxa, including *Stedocys leopoldi*, are placed within the genus *Scytodes* rendering it paraphyletic. While the present analysis provides only a weak support for the phylogenetic positions of the aforementioned taxa, it strongly underlines the need for further revisionary work, and may hint at the need to divide *Scytodes* into several clades. I support Lehtinen's (1986, 2010) proposal of the establishment of more genera to accommodate peculiar scytodid species with primitive palpal morphology, unique serrular formation and different carapace shapes. The *Dictis* genus is re-validated with this group of spiders recognized on the basis of female genital morphology and having two tarsal claws (as opposed to three in the other scytodids).

Evolution of scytodid sociality

Prey abundance has been implicated in the evolution of sociality in spiders in many cases. Aggregations of the orb-weaver, *Nephila clavipes* form around areas of high prey abundance in Peru (Rypstra 1985). Some species that build big web conglomerates do so to take advantage of high insect densities (Uetz et al. 1982). These examples, including *S. fusca*, indicate that high prey abundance is important in the evolution of sociality via the parasocial route (sensu Wilson 1971). Perhaps under such conditions, the solitary scytodids such as Cat Tien *Scytodes* sp. 6 or *Dictis venusta* may evolve into colonial spiders (Fig. 7-1). Alternatively, like *Goniosoma* that aggregate for defensive purposes, some scytodid species may also take this route to gregariousness. *Goniosoma* aggregations may strengthen the repulsive signal of the scent gland secretions to predators (Machado et al. 2000). Other than used in capturing prey, the spitting behaviour of scytodids can also be for defense, thus it is possible that under high predation pressures otherwise solitary scytodid individuals may form groups for the purpose of strengthening the defense mechanism (Fig. 7-1).

The other route which is from a subsocial precursor, is where group-living evolved from extended maternal care. Parental care, including the defense and feeding of offspring in a nest or other protected cavity have been crucial for the evolution of eusociality (Andersson 1984).

Baylis and Haplin (1982) proposed that parental care is the most common antecedent behaviour from which sociality has evolved, while Buskirk (1981) suggested that in spiders more elaborate sociality could evolve from parent-neonate association by extension of parental care and delayed dispersal of young. All scytodids documented so far are known to practice close egg attendance either by carrying the egg-sac in the chelicerae or maintaining close body contact with it. Furthermore, in most species the females will assist in the emergence of the spiderlings by tearing open the silk sac. Extended maternal care that involves dragging prey back to the spiderlings in the nest and delayed dispersal of the young are characteristics of subsocial scytodids Los Baños *Scytodes* sp. (Li et al. 1999), *S. socialis* (Miller 2006), *Scytodes* sp. from Singapore (Yap and Li 2009), *S. gooldi* and *S. rubra* (chapter 3, this study), and *Scytodes* sp. 1 and *S. pallida* (chapter 4, this study).

Through food provisioning there is suppression of cannibalism among young leading to a prolongation of tolerance among the siblings. With the extension of relations between siblings, mutualistic cooperation may arise in which large prey can be jointly subdued. Hence high prey availability especially the presence of large prey items such as moths, grasshoppers and roaches, may be one of the pivotal ecological factors to drive sociality in scytodids. It has been suggested that an ability to avoid cannibalizing kin will favour the evolution of cooperation and group-living in spiders (Bilde and Lubin 2001), therefore, over time the potential benefits of kin-biased behaviour such as nepotism (e.g. related spiders cooperate better and are more efficient at foraging and are more motivated to share digestive enzymes with the other spiders, allowing them to consume their prey more quickly) should favour selection for kin recognition (Fellowes 1998) in the scytodids. With a positive feedback mechanism the association among brood-mates gets prolonged thus leading to a permanent non-territorial group-living lifestyle. I propose that in scytodids there is an outbreeding system, maintained through the dispersal of males before they reach sexual maturity to other colonies for mating with non-sister females. If tolerance among the brood-mates break down, and then the competitive instincts for resources such as food and space becomes dominant and with the onset of cannibalism, pre-mating dispersal occurs and adults live in isolation (Fig. 7-1).

Clearly, phylogenetic context and a revised taxonomy are essential to progress in understanding the phylogenetic distribution and evolution of social behaviour. With data on social behaviour of 21 scytodid species/populations and a partial molecular phylogenetic tree available from this study, my third primary goal is to test the hypotheses about the origin and evolution of scytodid sociality. My results reveal that there are at least four independent origins of sociality within Scytodidae, with a subsocial lifestyle being an apomorphic condition. Miller (2006) had suggested the possibility of multiple independent origins of non-territorial multiple-adult sociality in scytodids, hence this result is not unprecedented. It is evident that in *Scytodes*, maternal care precedes delayed dispersal of juveniles from the natal nest, however, I am unable at this point of time to confirm whether all the subsocial/social scytodids form clades which have a phylogenetic history of maternal care because the current cladogram is not properly resolved as more genes and taxa are required. Behavioural studies of more scytodids, especially those of Yunnan *Scytodes* sp. 7, Otjoz *Scytodes* sp. 19 and *S. quarta* are crucial.

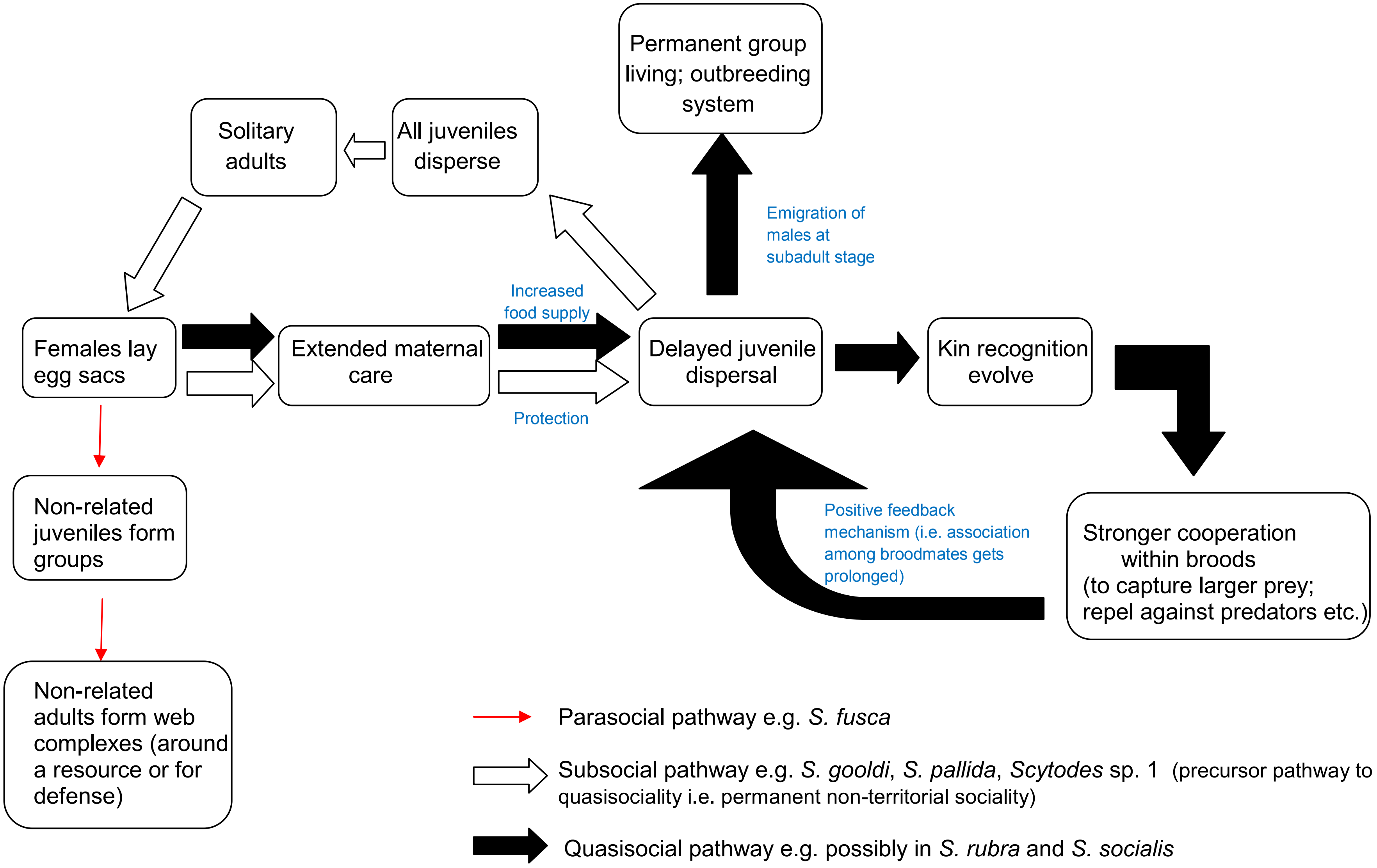

Fig. 7-1. Diagrammatic representation showing possible route to quasisociality in scytodids via the "maternal care" pathway, taking into consideration various ecological factors

A total of 13 social behavioural traits, 6 reproductive traits and one ecological trait were mapped onto the preferred phylogenetic tree. The reproductive traits do not seem to be useful characters for determining the social status of scytodid spiders because the data was erratic with no clear pattern, inconsistent with my prediction of social species producing fewer offspring than their less social relatives. The homology of certain behavioural traits can be difficult to verify (e.g. egg-sac carrying in the chelicerae have been reported in pholcids and drymusids, however, information at this time is insufficient to confirm whether all drymusid females practice egg-sac carrying) and may be influenced by ecology or other derived life history attributes. I am thus left to draw inferences based on suites of characters considered to be primitive, compiled from the outgroup taxa (i.e. *Loxosceles* and *Drymusa*) to yield productive insights regarding both commonalities and differences, and therefore, potentially significant influences favouring the evolution of sociality in the spitting spiders. I hypothesize that the "modern" scytodids (i.e. those found in the endogean and epigean habitats) arose from cave ancestors. Two lines of evidence lead to this hypothesis. First, all other scytodids form a sister group to the nominal '*Scytodes*' *magna* which are found in the dark zones of Batu Caves (see Fig. 5-1). Second, within Lehtinen's (2010) proposed subfamily Scytodinae, the most plesiomorphic of *Scytodes* auct is the *longipes*-group. *Scytodes longipes* are distinct in that these spiders have very long legs, similar to the cave scytodids, which are particularly useful in sensing around dark environments.

Subsocial spiders are important in the study of evolution of sociality because they provide a window into the ways changes such as breeding structure and degree of cooperation in reproduction from subsocial-to-social system came about (Lubin and Bilde 2007). It is evident that the evolution of sociality in scytodids is accompanied by changes in the dispersal patterns. In all scytodid species, juvenile dispersal from the natal nest follows a period of maternal care, in this case close egg attendance either via carrying the egg-sac in the chelicerae or close body contact with the egg-sac. The timing of dispersal is delayed if the duration of maternal care is extended. For the scytodids, this includes food provisioning for the young through transport of captured prey to the natal nest. Extended maternal care thus provides sufficient benefits for siblings to remain in the natal nest and postpone dispersal. I have shown that the origin of group-living in *Scytodes* is maternal care, thus supporting the hypothesized matrifilial route to permanent sociality in spiders (Shear 1970; Wilson 1971; Kullmann 1972). Clearly, the preconditions necessary for the transition to sociality differ from the mechanisms that maintain it. Ecological factors are likely to play a key role in determining the length of the group-living stage in the subsocial *Scytodes*. The timing of dispersal may be dictated by food conditions and intranest competition (Aviram 2000).

Expressions of sociality in Scytodidae are diverse, with considerable variation in the duration of maternal care timing of dispersal. With only four out of 21 taxa found to be subsocial, is sociality rare in this family or has it been under-estimated due to insufficient research carried out on this poorly-studied group of spiders? Only new advances in the study of scytodid natural history, phylogeny and systematics will reveal the answer. The discovery of subsociality in *Scytodes* corroborates predictions derived from the phylogenetic distribution of maternal care (Agnarsson 2002, 2004; Agnarsson and Kuntner 2005), and provides another evolutionary replicate for comparative studies of subsocial behaviour and the evolution of sociality. I am confident that many more subsocial/social scytodid species await discovery. More fieldwork is an urgent priority.

This study can be further extended to include more species from the different genera especially the currently monotypic *Soeuria* and *Scyloxes*, as well as *Stedocys* (e.g. *S. uenorum* and *S. pagodas*). Also, with the availability of a scytodid tree, the origin and evolution of various different interesting traits of this group including the origin of spitting, morphological characteristics and courtship and mating behaviour can be further studied and understood.

Future studies

I hope that this study can pave the way to further in-depth research into the social behaviour of more scytodids from a wide range of habitats. Of special interest would be to gather more behavioural and morphological data on the cave scytodids because only then can the question of whether they are the ancestors of all other scytodids distributed in other habitats including those found under rocks, in the leaf litter and live green leaves, and even those in houses and old buildings be answered.

Furthermore, revision of the family Scytodidae is pertinent. Undescribed species need to be described and intense sampling of Asian scytodids will certainly unravel more new species. Species such as ‘*Scytodes*’ *magna* are in need of re-description and establishment of new genera will be essential for species such as Guangxi *Scyloxes* sp. 1 and Yunnan *Scyloxes* sp. 2, and perhaps other undescribed species that do not fit into the genus *Scytodes*.

Reconstruction of the phylogenetic tree should be based on more genes such as 12S and CYTB, more taxa, and combined with morphological data such as genital structures, spinnerets and legs morphology so that current unresolved clades can be resolved and the phylogeny will be made more robust such that it can address further evolutionary hypotheses related to sociality, as well as facilitate the study of many other traits such as the evolution of spitting. With more behavioural and molecular data, the puzzle of whether scytodid sociality is distributed as few large clades or phylogenetically scattered i.e. “spindly” can be solved.

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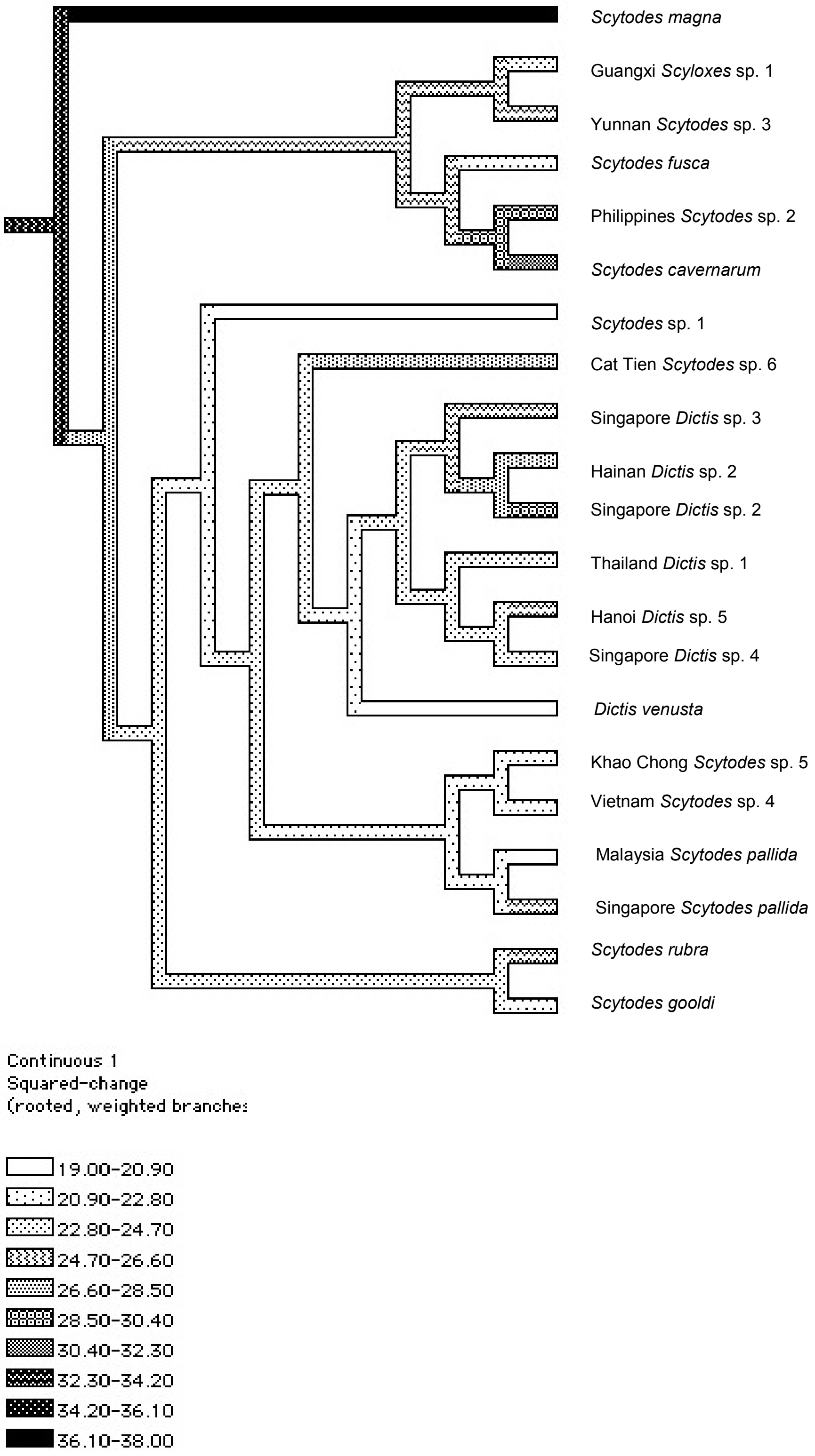
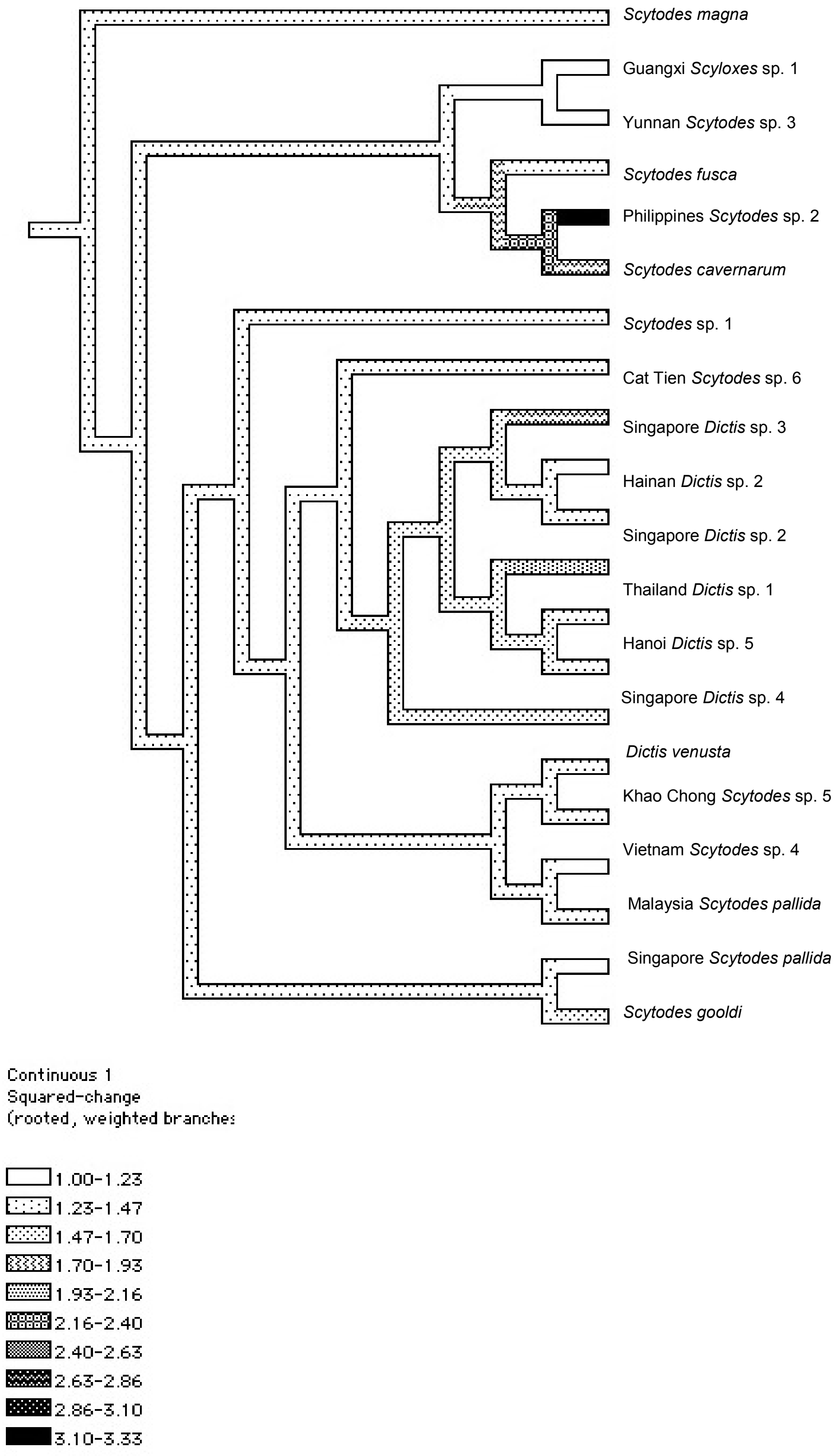
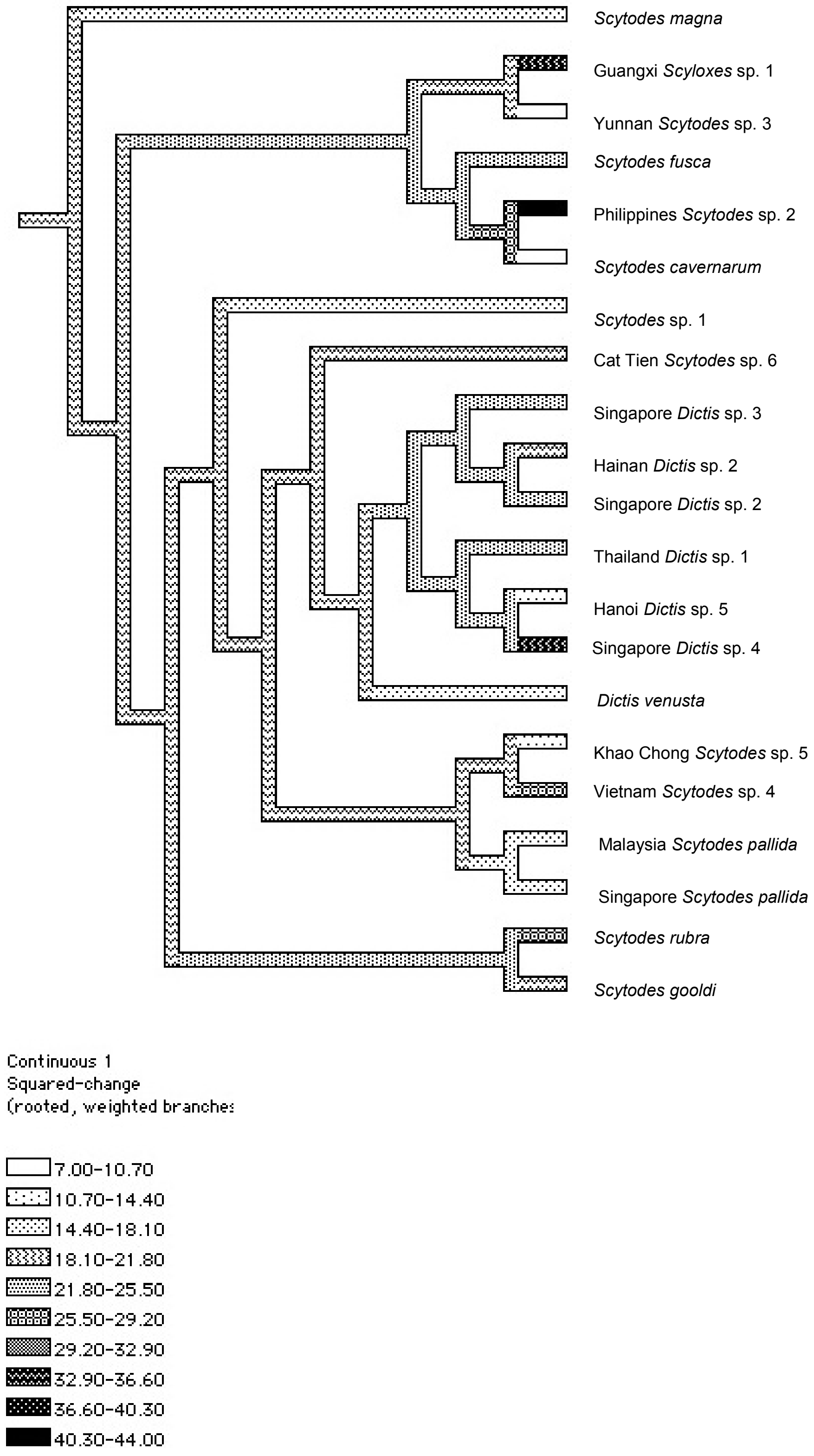
APPENDIX



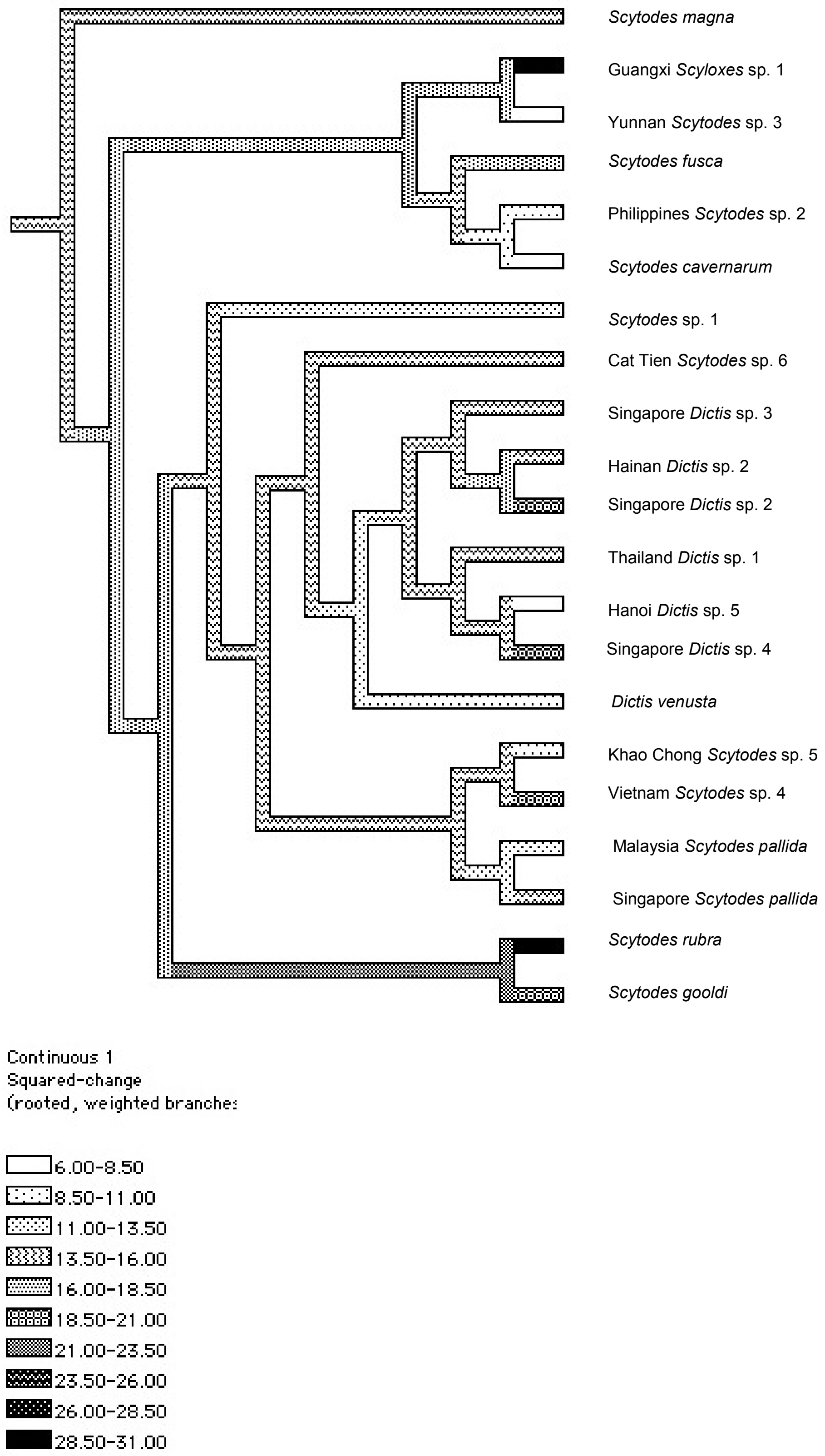
Appendix 1. Character: Mean egg-sac guarding duration mapped as continuous data onto the MPT = gaps as 5th character state for 21 taxa



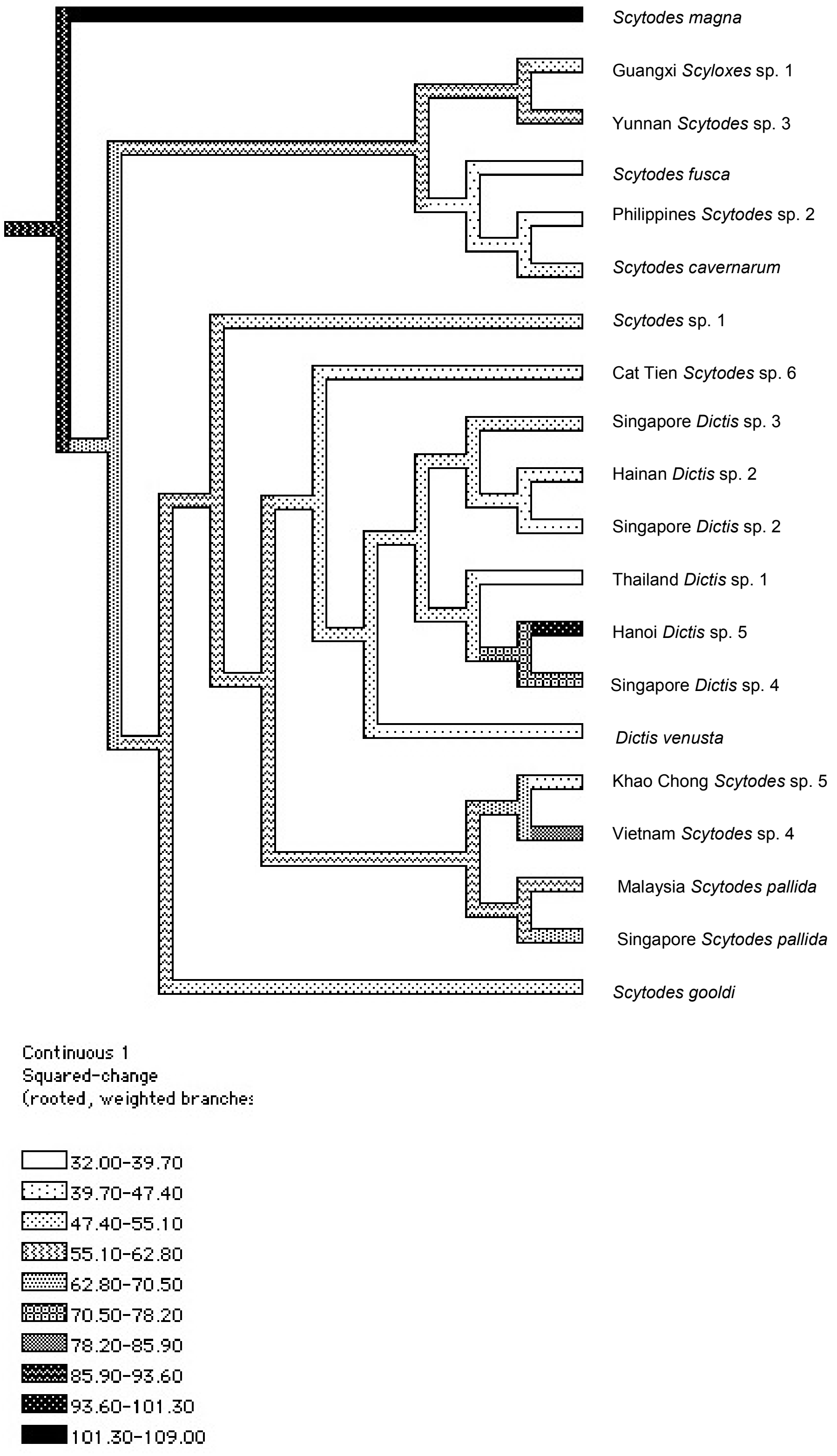
Appendix 2. Character: Mean number of clutches per female mapped as continuous data onto the MPT = gaps as 5th character state for 21 taxa



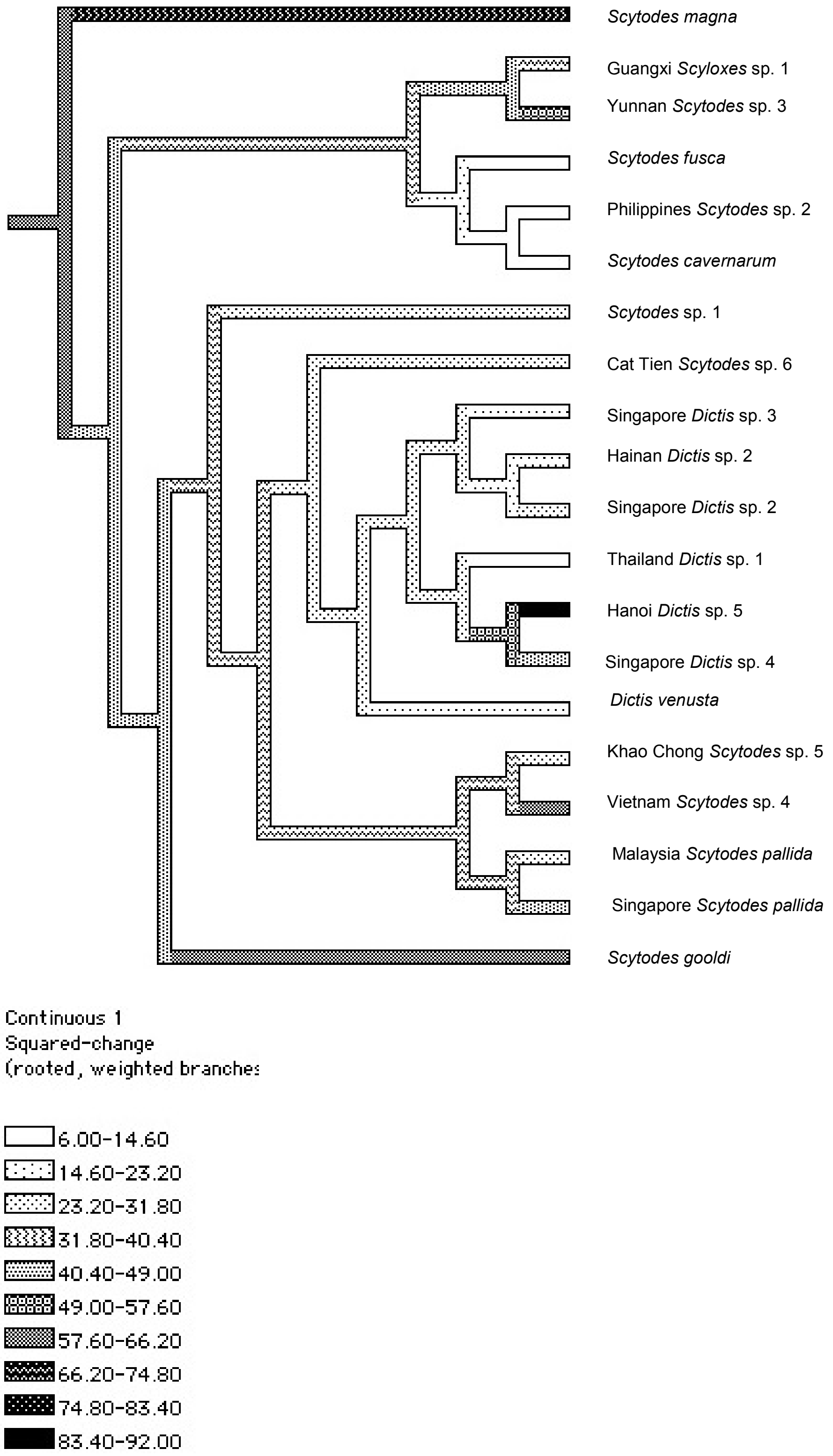
Appendix 3. Character: Mean total number of spiderlings/species mapped as continuous data onto the MPT = gaps as 5th character state for 21 taxa



Appendix 4. Character: Mean number of spiderlings per clutch mapped as continuous data onto the MPT = gaps as 5th character state for 21 taxa



Appendix 5. Character: Interval between successive egg-sacs mapped as continuous data onto the MPT = gaps as 5th character state for 21 taxa



Appendix 6. Character: Interval between hatching and next egg-sac production mapped as continuous data onto the MPT = gaps as 5th character state for 21 taxa.