A gang of thieves — evolution of cooperative kleptoparasitism in the subfamily Argyrodinae (Araneae: Theridiidae).

By

Yong-Chao Su
University of Kansas, 2012

Submitted to the graduate degree program in the Department of Ecology and Evolutionary Biology and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Chairperson : Deborah R. Smith

Member : Rafe M. Brown

Member : Bruce Cutler

Member : Mark T. Holder

Member : Paul A. Selden

Member : Matthew Buechner

Date Defended : May 14th , 2012

The Dissertation Committee for Yong-Chao Su certifies
that this is the approved version of the following dissertation:

A gang of thieves — evolution of cooperative kleptoparasitism in the subfamily Argyrodinae (Araneae: Theridiidae).

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Chairperson : Deborah R. Smith

Date approved: May 16th, 2012

Abstract

This is the first comprehensive study of group-living behavior in kleptoparasitic Argyrodinae, and the first species level molecular phylogenetic analysis of the Argyrodinae (Araneae: Theridiidae). I included four research chapters in this dissertation. In Chapter 2, I showed the first empirical study of cooperative kleptoparasitism in *Argyrodes miniaceus*. The results showed that, at least at the level of foraging, group-living behavior has adaptive function of cooperation. Using a game theory model, the payoff of being cooperator in a group is greater than the payoff of being solitary. In Chapter 3, I concluded that kleptoparasites do not aggregate simply because the webs are large and can support multiple kleptoparasites. Social interactions among group members provide additional benefits that favor individuals remaining in groups. In Chapter 4, I concluded that group members could gain indirect benefit of fitness by cooperating with group members, who are potentially related individuals. This is because in group-living *Argyrodes*, group members are significantly more closely related than the individuals drawn randomly from the population in a small geographic scale. In Chapter 5, the phylogenetic analyses showed several independent origins of group-living behavior in different species groups. The evolutionary sequence of foraging strategies of Argyrodinae is from free-living to araneophagy, then to kleptoparasitism. The comparative analyses showed the specialization to large host is correlated with the evolution of group-living behavior. In addition, the processes of specialization thus becoming group-living may have caused diversification within species groups.

Acknowledgements

Professional support

I thank Dr. Deborah Smith for her support for all my projects. I thank Dr. M. Helen Alexander, Dr. Ford Ballantyne, and Mr. Chao Song for the suggestions on statistics. I thank Dr. Bruce Cutler, Dr. Paul A. Selden, and Steve R. Davis for the suggestions on systematics. I thank Dr. Rafe M. Brown and Dr. Mark T. Holder for the assistance on phylogenetic methods and comparative analyses. I thank Dr. Yael Lubin's valuable comments on my behavioral project.

Field support

I thank the assistance of fieldwork for from Dr. Daiqin Li's lab at National University of Singapore; Dr. I. M. Tso's lab at Tunghai University, Taiwan; Dr. Rafe Brown's lab at the University of Kansas; and Dr. Aimee Barrion Dupo's lab at the University of Philippines, Las Baños. I thank Ms. Seok Ping, Mr. Diego Pitta De Araujo, Mr. Jun Hao Tan, and Mr. Shichang Zhang for the sample collection from China, Singapore, and Malaysia. I thank Mr. Ren-Chung Cheng and Ms. Yin-Ting Lin for the help in sample collection and filed survey in Taiwan, Thailand, and Japan; I thank Dr. Yael Lubin's samples from Israel; I thank Dr. Dan J. Bennett for the specimens from Peru. I thank Ms. Shan-Hui Su for her assistance of fieldwork in Kansas, Taiwan, Philippines, Singapore, and Malaysia.

Lab work

I thank Ms. Shan-Hui Su for the help in laboratory.

Funding

I thank all the funding sources including the grants for graduate research from American Arachnological Society, Entomology Endowment of Entomology Program at the University of Kansas. I thank the grant (grant number 2006353) from the US-Israel Binational Science Foundation to Dr. Yael Lubin and Dr. Deborah R. Smith.

Personal support

I thank my wife Shan-Hui, my daughter Zih-Chiao, and my son Zih-Chi for their love and warm support. I thank my parents and my parents-in-law in Taiwan for their support to my family.

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Chapter 1 Introduction

Spiders in the subfamily Argyrodinae (Araneae: Theridiidae) are known for their associations with other web-spinning spiders (Whitehouse 2011). These associations include entering another spider's web and eating the resident spider ("araneophagy; Wise 1982, Cobbold and Su 2010), eating its eggs and spiderlings (Smith Trail 1980), or eating its silk (Miyashita et al. 2004); in some cases, individuals build their own webs to catch prey (Eberhard 1979). However, most Argyrodinae forage as kleptoparasites in the webs of other host spiders. The kleptoparasites can scavenge small prey that are ignored by the host (Koh and Li 2003), feed on prey that have been wrapped and stored by the host, or feed simultaneously with the host on large prey items (here called "creep-up-and-share") (Vollrath 1979). One species may use multiple foraging strategies, depending on the circumstances (Cobbold and Su 2010; Vollrath 1979; Whitehouse 1988).

About 20 of the 238 named Argyrodinae species show an even more unusual behavior: they are group-living kleptoparasites in the webs of their hosts—literally, "gangs of thieves". These group-living species specialize in invading the webs of larger, orb-weaving spiders, such as *Nephila* (Nephilidae), *Argiope*, and *Cyrtophora* (Araneidae). Multiple individuals forage in one host web and show strong conspecific tolerance, especially when sharing prey items directly with their hosts (Elgar 1993). Group size is usually five or more, and may exceed 40 individuals in a single host web. Groups include adult males and females and juveniles.

Group-living kleptoparasites are found primarily in the argyrodine genera *Argyrodes* and *Faiditus*, in species inhabiting tropical rainforest habitats. These spiders steal food bundles that have been wrapped by the host, glean small insects caught in the host web,

eat the silk of the host web, and feed directly with their host (see review in Whitehouse 2011). Group-living behavior in Argyrodinae was reported as early as 1928 (Wiehle 1928), but surprisingly, interactions among conspecific kleptoparasites sharing a host web have received little attention (though interactions between kleptoparasites and their hosts have been relatively well researched (e.g., Vollrath 1979, Tanaka 1984, Whitehouse 1988). One of the few investigations of group-living behavior in argyrodines is a study of *A. antipodianus* by Whitehouse (1991), who described their behavior from the perspective of intra-specific competition. Hénaut (reported in Whitehouse et al. 2002) was perhaps the first to suggest that group-living argyrodine kleptoparasites might cooperate in the webs of their hosts. He reported "host distracting" behavior by *F. globosus*, in which some members of a group would make a disturbance in the web, attracting the attention of the orb-weaver host, while others used the opportunity to steal prey.

This dissertation is a broad investigation into the group-living Argyrodinae. In it, I address several major aspects of the biology of the group-living behavior. First, I investigate whether group-living in the argyrodine kleptoparasite *A. miniaceus* provides foraging benefits to group members, and can be considered a form of cooperation (Chapter 2). Next, I investigate whether group-size fits the expectation of an ideal free distribution (Fretwell and Lucas 1970) of individuals in resource patches (host webs), and whether groups in a host web are likely to consist of related individuals. Group size is expected to follow an ideal free distribution if individuals are simply aggregating in resource patches (host webs) of different sizes and responding to competition with conspecifics. For this study I used five group-living species: *A. fissifrons*, *A. flavescens*,

A. kumadai, *A. lanyuensis*, and *A. miniaceus* (Chapter 3). In this chapter I also investigated environmental parameters that might predict the presence of kleptoparasites in a host web for the five group-living species and the solitary species *A. fasciatus* and *Neospintharus trigonum*. I measured relatedness among group or "gang" members in the group-living species *A. miniaceus* and *A. kumadai,* and analyzed population structure in *A. miniaceus*, *A. kumadai* and two solitary species, *A. fasciatus* and *N. trigonum* (Chapter 4). If group-members are more closely related than would be expected in random assemblages of individuals, then inclusive fitness benefits may contribute to the maintenance of group-living. Finally, in Chapter 5, I present a phylogenetic analysis of species level relationships in the Argyrodinae, using data from three mitochondrial genes and three nuclear genes. I use the resulting tree to investigate whether group-living behavior in Argyrodinae evolved once or several times, whether there is evidence for speciation in a group-living clade, and whether group-living is associated with specialization on large hosts. My work also provided many observations on natural history of the Argyrodinae, and revealed a wealth of undescribed species.

Taxonomy of Argyrodinae

There are 238 named species and six genera in Argyrodinae (Platnick 2012). The taxonomy of the subfamily Argyrodinae has always been difficult (Exline and Levi 1962, Yoshida 2001, Agnarsson 2004). Currently six genera are recognized: *Argyrodes*, *Ariamnes*, *Faiditus*, *Neospintharus*, *Rhomphaea*, and *Spheropistha* (Platnick 2012). Simon (1893) erected the genus *Argyrodes* in which he included all the taxa currently placed in the subfamily Argyrodinae, excluding the genera *Ariamnes* and *Rhomphaea*.

Exline and Levi (1962) revised the New World species and recognized six species groups: *Argyrodes, Ariamnes, Cancellatus, Cordillera, Trigonum*, and *Rhomphaea*. Tanikawa (1998) added the genus *Spheropistha* in the genus *Argyrodes*. Yoshida (2001) elevated Exline and Levi's one genus to the subfamily level. In this subfamily, Argyrodinae, Yoshida (2001) retained the genus *Argyrodes,* elevated *Ariamnes* and *Rhomphaea*, and *Spheropistha* to genus level. Agnarsson (2004) carried out a revision of Theridiidae using morphological characters. His results strongly supported Yoshida's results; he also combined Exline and Levi's (1962) *Cancellatus* and *Cordillera* species groups in a single genus, *Faiditus,* and he renamed their *Trigonum* species group as genus *Neospintharus*.

Group-living species of Argyrodinae in general are found only in the genera *Argyrodes* and *Faiditus. Ariamnes*, *Rhomphaea*, and *Neospintharus* contain only solitary species. The behavior of *Spheropistha* is largely unknown except for the species I collected. Whitehouse (2011) lists 14 group-living species in *Argyrodes* and three group-living species in *Faiditus*. In addition to these, I observed group-living behavior in *A. lanyuensis*, in three species that are similar to *A. elevatus*, in two species that are similar to *A. fissifrons*, and in two species that are similar to *A. miniaceus.* In addition, *Spheropistha* sp. is group-living (see Chapter 5 for the phylogenetic relationships of these species). However, the taxonomic treatment of the undescribed species listed above has not been concluded; thus to be conservative, there are at least 20 group-living species in Argyrodinae.

Group-living behavior in other spiders

Although group-living behavior has received little attention in the Arygyrodinae, group-living behavior is well known among the spiders. There are two major types of spider social groups: communal (or colonial) societies and cooperative societies. The former constitute aggregations of individuals at resource patches, such as rich food resources or suitable nesting sites. Individuals in communal societies defend individual webs, and do not cooperate in group life (Uetz 1997). The main benefits of group-life in these societies are access to resources and energetic saving in web-maintenance (reviewed in Uetz 1997). Indeed, inidvidual members in a communal group may have greater foraging success if other colony members are removed.

Cooperative behavior is known from approximately 40 of the 40,000 named spider species (Bilde and Lubin 2011, Platnick 2012). These spiders live in groups and cooperate in a variety of colony activities, including web construction, prey capture, feeding and care of the young. In these species, juvenile dispersal is suppressed and mating generally takes place among colony mates; this is accompanied by a female biased sex-ratio. Thus relatedness among colony members is high (Smith and Engel 1994, Smith et al. 2009). These social systems have arisen many times independently, and are believed to have arisen from ancestors with extended maternal care (subocial behavior) (see reviews by Buskirk 1981, D'Andrea 1987 , Aviles 1997, Bilde and Lubin 2011). The high degree of relatedness within colonies and the population dynamics of colony origin and extinction suggest that benefits may accrue to group members through both kin selection and group selection (Smith and Hagen 1996, Aviles 1997).

Size of the host web predicts size of the resident kleptoparasite groups

The variables that predict the size of kleptoparasitic groups have been studied in nine of the ~20 known group-living Argyrodinae species (Cangialosi 1990a, Grostal and Walter 1997, Tso and Severinghaus 2000, Miyashita 2002, Agnarsson 2003, Koh and Li 2003, Hénaut et al. 2005, Kerr 2005, Agnarsson 2010; see Table 2). The major predictor of group size is the size of the host web, which is positively correlated with availability of prey (Elgar 1993). Studies in *A. elevatus* and *F. americanus* (in Agnarsson 2010), *A. flavescens* (in Miyashita 2002), *A. argentatus* (in Kerr and Quenga 2004), *A. globosus* (in Hénaut 2000), and *A. fissifrons* (in Tso and Severinghaus 2000) showed strong correlations between number of kleptoparasites per web and size of the webs of their hosts, *Cyrtophora*, *Argiope* (Araneidae), and *Nephila* (Nephilidae). In *A. antipodianus,* web size is a positive predictor of number of kleptoparasites, although the correlation is weak (Grostal and Walter 1997). For *F. ululans*, which are kleptoparasites of cooperative social spiders such as *Anelosimus eximius* (Theridiidae), larger cooperative host webs hosted more kleptoparasites than smaller webs (Cangialosi 1990b). However, *F. ululans* also prey on their social hosts (araneophagy) as one of their main foraging strategies. Elgar (1993) suggested that the distribution of kleptoparasites per web for group-living species simply follows the expectation of the ideal free distribution (Fretwell and Lucas 1970), which assumes that the interaction among group members is merely competition for resources. In Chapter 3, I investigate whether the size of argyrodine groups in host webs follows the ideal free distribution model. Deviation from ideal free distribution

prediction of group size would indicate that the relations among group members are not strictly competitive, and that group-living may actually provide benefits to group members.

Foraging behavior of group-living argyrodine kleptoparasites

Foraging strategies of a group-living species can be extremely flexible: *A. miniaceus* can use up to four ways to forage in the web of *Nephila* sp. (Nephilidae). In general, species that have similar morphology and similar hosts tend to have similar foraging strategies (Su, personal observation). For example, *A. kumadai* and *A. fissifrons* are among the largest kleptoparasites (body length ~8 mm) in Argyrodinae, and specialize on hosts with three-dimensional webs. Both these species creep up to feed from a large prey item only in the juvenile stage, when the larger host is feeding on it (Su lab and field observations). The adults mainly scavenge small prey items ignored by the host (Baba et al. 2007). They can also conduct araneophagy, preying on the juveniles of their hosts (Tanaka 1984) or on other guest spiders in the host web (Elgar 1993). Group size in these two species is about six to 10 individuals (Tso and Severinghaus 2000), and sometimes as large as 25 individuals (Su field observation).

Other group-living species, such as *A. miniaceus* and *A. flavescens*, are specialists in Nephilidae webs. Their body is ~5 mm in length and usually orange in color. Their primary mode of foraging is the creep-up-and-share strategy, which they use throughout their life, both as juveniles and adults (Su field and lab observations). They also occasionally engage in silk-eating, insect-gleaning, and food bundle stealing (Koh and Li 2003). The spiderlings of *A. miniaceus* begin to utilize the web of *Nephila pilipes* right

after they emerge from their egg-sac, which is deposited in a host web by the mother a week before hatching (Su field observation in Miaoli County, Taiwan, in 2007). My lab rearing experience showed that the life-cycle of this species can be as short as 15 days, and that the host web in the field can last longer than 20 days. This indicates that *A. miniaceus* can possibly complete more than one life cycle in a host web.

Group-living kleptoparasite species with smaller body size (1-3 mm in length) and silver abdomen, such as *A. elevatus*, *A. antipodianus*, *A. argentatus*, and *A. nephilae*, mainly utilize orb-weaving spiders as hosts. They use diverse foraging strategies, including creep-up-and-share, insect-gleaning, and food bundle stealing. Vollrath (1979) observed food stealing trials in the New World species *A. elevatus.* He reported about 50% chance of using food bundle stealing strategy, and only about 10% of chance using creep-up-and-share strategy in total observed feeding trials. This species also preys on its host (Cobbold and Su 2010).

Whitehouse (1986) found that *A. antipodianus* can use strategies of silk-stealing, insect-gleaning, stealing food bundle, araneophagy and the creep-up-and-share strategy, but that they gained the most nutrition when conducting the creep-up-and-share strategy (Whitehouse 1997) and showed reduced aggression toward conspecifics when using this strategy. *Argyrodes argentatus* glean insects, steal food bundles, creep-up-and-share, and occasionally kill their hosts (Kerr 2005). In my lab observations, *A. nephilae* only glean small insects and do not use the creep-up-and-share strategy, at least when *N. clavipes* is the host.

Faiditus caudatus and *F. ululans* do not use the creep-up-and-share strategy; instead they often glean small insects in the host web (Vollrath 1984, Cangialosi 1990a). *Faiditus*

globosus was found to use the creep-up-and-share strategy, and group members performed host-distracting behavior when conducting this foraging strategy (see Henaut's observation in Whitehouse et al. 2002).

In Chapter 2, I investigate whether individuals in groups of *A. miniaceus* have greater foraging success than solitary individuals when using the creep-up-and-share strategy in the webs of *Nephila* hosts.

Foraging behavior of solitary Argyrodinae

The natural history of solitary species in *Argyrodes* and *Faiditus* is largely unknown. From my observations, *A. fasciatus* is a specialist of *Psechrus* sp. on Pulau Ubin Island, Singapore. They creep-up-and-share prey with the host and glean small insects from the host web. The Philippines *A. tripunctatus* mainly uses hosts with three-dimensional webs. However, I did not see any kleptoparasite foraging behavior in any of the 18 webs I observed. *Faiditus xiphias*, the only *Faiditus* species in Asia (observations were made in Singapore and Malaysia), was observed to be a generalist of orb-weaving spiders and performed insect gleaning strategy. *Argyrodes lanyuensis* was described as a group-living species endemic to Orchid Island, Taiwan (Yoshida et al. 1998); in the Orchid Island population typical group size is ~20. However, I have also observed what appears (based on morphology) to be *A. lanyuensis* on the Islands of Palawan, Mindanao, Mindoro, Negros, Negros, and Luzon in the Philippines. In these populations, I have observed solitary behavior; foraging strategies of *A. lanyuensis* include silk-eating, creep-up-and-share, and insect-gleaning (Tso and Severinghaus 1998).

Araneophagy is also practiced by many solitary species. *Rhomphaea* and *Ariamnes*

throw silk to catch other spiders as their prey (Eberhard 1979). Species in *Neospintharus* bite their spider prey directly, and occasionally conduct kleptoparasitism (Cangialosi 1997), or eat the egg-sacs of their host (Smith Trail 1980).

Do kleptoparasite gangs consist of relatives?

The level of social interaction and cooperation shown by group-living Argyrodinae are very poorly known, and the relatedness among group members has never been investigated directly. Some inferences can be drawn from observations of the movement of kleptoparasites among host webs. Whitehouse and Jackson (1993) found that the distances traveled by male *A. antipodianus* were longer than females'. The adults travel by "bridging," connecting an airborne silk thread to a distant object, then climbing along the silk to move from one place to another. Elgar (1989) found *A. antipodianus* also use ballooning; thus group members in a web could be a random assemblage of individuals that arrived in the host web independently. Elgar (1989) also found after the removal of *A. antipodianus* in their host webs, the webs were recolonized in two days. The same pattern was found in *A. flavescens*. Miyashita (2001) did a removal experiment of kleptoparasites and found the kleptoparasite density in the webs of *Nephila* recovered in three days. From my observations, when *A. miniaceus* were removed from webs of *N. pilipes*, it took about three days to recover to the same group sizes in most webs. However, I did the same removal experiment in *A. fissifrons* and the group size recovered slowly and did not recover to the original group size in 7 days when I stopped my observations. These observations suggested for many *Argyrodes* species, there are many floaters searching for host webs in the forests. However, the group sizes of these

observed species in natural populations are very stable and the group members did not change rapidly (Whitehouse and Jackson 1993, Tso and Severinghaus 2000, Miyashita 2001, Kao 2008). These observations suggested there is strong interference competition between floaters and the individuals that are already in the web.

In Chapter 4, I assess the relatedness and population structure of solitary and group-living argyrodines, using DNA fingerprints (specifically, Three-Endonuclease Amplified Fragment Length Polymorphisms, or TE-AFLPs; (van der Wurff et al. 2000) as a source of data. I used spatial autocorrelation to compare population structure (pairwise phenotypic similarity as a function of pairwise spatial distance) of the group-living *A. miniaceus* and *A. kumadai*, and the solitary species *A. fasciatus* and *N. trigonum.*

Working together to the same end?

The emergence of cooperative behavior from groups of competing units is of long standing interest in evolutionary biology. Cooperation is arguably the third fundamental principle in evolutionary biology in additional to mutation and natural selection (Nowak 2006). Cooperation can be the evolutionary pacemaker that leads to specialization, and so promotes biological diversification (Wilson 1975). Traditionally, research on the evolution of cooperation fall into two categories: one based on kin selection theories (Hamilton 1964) and the other based on game theories, or reciprocity (Trivers 1971). West et al. (2007) categorized theoretical models of the evolution of cooperation into indirect benefit of fitness (kin selection based, cooperator contribute to the fitness of relatives) models and direct benefit of fitness (game theory based, cooperators enhance their own fitness) models. The research on sociality of spiders is similar to this

dichotomy (Bilde and Lubin 2011). Cooperative social spiders are believed to have evolved from subsocial species with extended maternal care, which causes juvenile dispersal to be delayed and often only over short distances from the mother's web, leading to local populations composed of related individuals (e.g., Johannesen et al. 2007). Permanent cooperative societies arise when juvenile dispersal is completely suppressed. Colony members can gain direct fitness benefits from, for example, more reliable access to prey (insects captured and shared by colony members) and better protection of their offspring (if a female dies, her young will be cared for by colony mates); and indirect fitness benefits by helping related colony mates (see Kullmann 1972, Aviles 1997, Bilde et al. 2005).

For communal group-living spiders, their collective behavior, such as building communal webs, directly enhances individual fitness, such as higher per capita foraging efficiency or more efficient web maintenance. The sociality of these species is rooted in individual fitness and reciprocity (see reviews in Uetz and Hieber 1997, Bilde and Lubin 2011).

In this dissertation, I investigate whether the group-living behavior in Argyrodinae is merely a form of aggregation around a resource patch, or is a form of cooperative behavior. If it is a form of cooperation, are both kin selection and reciprocity involved in this system? Lastly, what is the evolutionary history of this group-living behavior? Is group-living associated with specialization on large hosts? What is the order of the origin of group-living behavior, kleptoparasitism and araneophagy?

Four approaches were made to comprehensively understand the group-living behavior in Argyrodinae: (1) I used game theory based model, i.e., group foraging model

(Packer and Ruttan 1988) to test if cooperative behavior would be preferred in a group-living species, *A. miniaceus*. (2) I used a group size predicting model, i.e., ideal free distribution model (Fretwell and Lucas 1970), to test whether the group sizes in the webs of natural populations are larger than the prediction of this model, which assumes exploitation competition is the only interaction among group members. (3) I used DNA fingerprinting analyses to test the genetic relatedness across multiple group-living species and solitary species to determine whether kin selection component could possibly exist in this system, and (4) I used comparative analyses to test whether the focal behavioral trait, group-living behavior, is associated with the ecological processes of specialization on large hosts. The four approaches used in this this study are presented in four research chapters.

Chapter 2

Living in groups results in higher foraging success for a kleptoparasitic spider

Abstract

Kleptoparasitic spiders primarily conduct foraging in the webs of larger spiders; about 20 out of 238 species in Argyrodinae are group-living in the webs of their hosts. In these species, multiple individuals forage in one host web, and show strong conspecific tolerance, especially when they share prey items directly with hosts (termed creep-up-and-share strategy). The function of this group-living behavior has never been investigated; I aimed to test whether the group-living behavior of *Argyrodes miniaceus* increases their per capita foraging benefit. *Argyrodes miniaceus* and their host, *Nephila pilipes,* were housed in 60 × 60 × 60 cm cages in the lab. For each experimental trial, a ~0.3 g domestic cricket (*Acheta* sp.) was provided to the host spider. I used time to reach the prey item, per capita time spent feeding, and per capita feeding rate as indicators of foraging benefits and tested group sizes from one to seven. Results of analyses of variance tests showed that solitary kleptoparasites took significantly longer to reach the prey than the first kleptoparasite in a group needed, but there was no effect of group size on the time needed for the first kleptoparasite to reach the prey item for groups of two to seven. However, per capita feeding rate was highest when two or three individuals were in a group. Correspondence analysis showed that individuals in a group size of two or three were more likely to reach the prey and feed. A time series analysis showed that when a group size was larger than four kleptoparasites, *A. miniaceus* individuals took turns feeding and kept two to three kleptoparasites searching around the host for the duration of each experimental feeding trial. I concluded that group-living behavior in *A. miniaceus* is not merely a form of aggregation; instead, it has the function of social foraging.

Introduction

Social spiders are excellent systems in which to study the evolutionary transitions from solitary to social life styles. Because of their cannibalistic nature, a group-living life style, which is the intermediate life style between solitary behavior and sociality in most animals, cannot easily exist (Bilde and Lubin 2001). Therefore, the evolutionary mechanism of avoiding cannibalism then proceeding to sociality by developing social behaviors in spiders has been attracting substantial research effort (see review in Aviles 1997; Bilde and Lubin 2011; Uetz and Hieber 1997).

Like most spiders, the majority of spiders in the subfamily Argyrodinae are solitary; but some species in this subfamily exhibit a form of sociality. These Argyrodinae live and forage in host webs along with multiple conspecific individuals.

Spiders in the subfamily Argyrodinae (Araneae: Theridiidae) are known for their associations with other web-spinning spiders (Whitehouse 2011). These associations include entering another spider's web and eating the resident spider (Wise 1982) (Cobbold and Su 2010), eating its eggs and spiderlings (Smith Trail 1980), or eating its silk (Miyashita et al. 2004); in some cases, they even build their own webs to catch prey (Eberhard 1979). However, most Argyrodinae forage as kleptoparasites in the webs of other host spiders. Here too, there is an array of feeding strategies. They can scavenge the small prey that are ignored by the host (Koh and Li 2003), feed on prey that have been wrapped and stored by the host, or feed simultaneously with the host on large prey items (here called "creep-up-and-share") (Vollrath 1979). One species may use multiple foraging strategies, depending on the circumstances (Cobbold and Su 2010; Vollrath 1979; Whitehouse 1988).

About 20 of the 238 named Argyrodinae species show an even more unusual behavior: they are group-living kleptoparasites in the webs of their hosts — literally, "gangs of thieves". These group-living species specialize in invading the webs of larger, orb-weaving spiders, such as *Nephila*, *Argiope*, *Araneus*, and *Cyrtophora*. Multiple individuals forage in one host web and show strong conspecific tolerance, especially when sharing prey items directly with their hosts (Elgar, 1993). In some cases, group size may exceed 40 individuals in a single host web.

There is a substantial literature on foraging strategies of Argyrodinae. Most focuses were on interactions between the host and its kleptoparasites (Baba et al. 2007; Cangialosi 1990b; Grostal and Walter 1997; Miyashita et al. 2004) or competition among the kleptoparasites co-inhabiting a host web (Whitehouse 1988; Whitehouse 1991; Whitehouse 1997). Even though group-living is a rare and interesting phenomenon among spiders, the interactions among group-living Argyrodinae remain essentially unstudied (see Whitehouse et al. 2002, Whitehouse and Jackson 1998).

Whitehouse (2011) applied a widely used game theory model of cooperative foraging (Packer and Ruttan 1988) to group-living Argyrodinae kleptoparasites that share large prey items directly with their hosts (the "creep-up-and-share" strategy). She proposed that by exhibiting mutual tolerance, kleptoparasites save energy by avoiding competition, and also reduce the risk of being detected by the host. However, she did not address whether mutual tolerance increases the per capita food intake of a group member, which is Packer and Ruttan's first criterion for cooperative foraging. Here we address the function of group-living behavior in kleptoparasitic Argyrodinae from the perspective of social foraging. Moreover, although this model is relevant to the group foraging behavior

of group-living Argyrodinae, there are fundamental differences between the predator-prey systems that Packer and Ruttan (1988) modeled and the host-kleptoparasite systems of Argyrodinae.

Game theory based group foraging model – Packer and Ruttan's (1988) group hunting model includes five parameters: the value of the prey (*V*), probability of hunting success (*H*), cost of handling prey (*C*), cost of searching for prey (*E*), and prey encounter rate (*L*). In cases where *n* group members hunt a single prey, the payoff to each cooperator is:

$$L_n(H_n(V/n - C_n) - E_n) \qquad \text{Equation 1}$$

The payoff to a solitary hunter (group size = 1) that does not share the prey is:

$$L_1(H_1(V - C_1) - E_1) \qquad \text{Equation 2}$$

The payoffs to cheaters and scavengers in Packer and Ruttan's (1988) model do not involve any costs and are simply the product of solo prey encounter rate (L_1), solo hunting success rate (H_1), and shared prey value (V/n).

This model applies to predator-prey situations, but the Argyrodinae system involves a third role, the host. Thus, to apply this model to the Argyrodinae kleptoparasites, the cost of prey handling, C, can be eliminated since the host captures and wraps the prey, but an additional term, the cost of interacting with the host, must be added.

I studied *Argyrodes miniaceus* (Theridiidae), a group-living kleptoparasite in the orb-webs of *Nephila* species (Araneae: Nephilidae). *Argyrodes miniaceus* can use four foraging strategies: creep-up-and-share, silk stealing, scavenging insects, and stealing food bundles (Su, personal observations). I focused here on the creep-up-and-share

strategy of *A. miniaceus* because this is the strategy that requires the most conspecific interactions among these kleptoparasites. Group foraging behavior in *A. miniaceus*, if it exhibits a cooperative component, falls into the *Group hunts a single prey* model in Packer and Ruttan (1988). In this model (1) solo hunting success (H_1) is low; (2) group hunting success (H_n) can easily exceed solo hunting success; (3) when group size becomes too large, the group hunting benefit decreases and approximates that of the solo hunting of small single prey. This model predicts that group hunting in an optimally sized group is an evolutionary stable strategy (ESS) that has better foraging success rate compared to solitary foraging.

Natural history of group foraging behavior in *A. miniaceus* – Field observations show that *A. miniaceus* complete their life cycles in the webs of *Nephila* or *Nephilingys*, with up to 40 conspecific individuals in the same web. Females lay their egg sacs next to their hosts' webs. Newly emerged spiderlings immediately invade the adjacent host web. The host webs can last up to 3 weeks in the field. Even when the host does relocate its web, it usually moves to a nearby location. There is some movement of individuals among host webs: one study that measured turnover of marked individuals in the web found that after three days, ~20% of adult kleptoparasites in the web were unmarked immigrants (Kao 2008). Thus *A. miniaceus* could interact with the same group members multiple times within the course of a web tenure period.

Both male-male and female-female competitive encounters occur. Male-male competition is primarily for food bundles and mating opportunities. Female-female competition was observed in the periphery of host webs, where they may battle over small insects or small prey wrapped by the host. Mutual tolerance among females and

between females and males was observed when they engaged in creep-up-and-share behavior. Other foraging tactics, e.g., stealing small insects ignored by the host, eating silk etc., occur but provide much less nutrition than creep-up-and-share strategy. This is because kleptoparasites not only benefit from access to larger prey, but also by utilizing the digestive enzymes regurgitated by the host (Whitehouse 1997).

The creep-up-and-share behavior is triggered in *A. miniaceus* by host prey-wrapping behavior. When the host returns to the hub of the web and begins feeding, the kleptoparasites creep up to the prey and feed simultaneously with the host at the web hub. To test whether group-living *A. miniaceus* cooperate with conspecific individuals in the same web, I measured the foraging benefits (probability of reaching prey and feeding upon it, per capita search time and feeding time) of the creep-up-and-share behavior. Specifically, if the group foraging observed in *A. miniaceus* is a form of cooperative foraging, *sensu* Packer and Ruttan (1988), I predict: (1) probability of reaching the prey and feeding will be greater when group size is greater than one; (2) per capita search time will be shorter when foraging group size is greater than one; (3) per capita time spent feeding will be higher when foraging group size is greater than one; and (4) per capita feeding rate will be higher when foraging group size is greater than one, particularly if groups consist of females; (5) because the prey is a limited resource, there will be an optimal group size, larger than one, showing highest per capita feeding rate when prey size is controlled; (6) there will be no dominant individuals in a group such that the value of the prey will be shared equally among foraging group members.

Material and Methods

Sample collection – The group-living species, *A. miniaceus*, is a specialist kleptoparasite of *N. pilipes*. I collected live specimens from forests in Huoyenshan, Taiwan (N24°21'51.3", E120°44'20.3"), where large populations of *A. miniaceus* and their hosts can be found, from June 11 to July 15, 2010. I collected one subadult individual (see below) *A. mineaceus* from each of ~ 40 webs in the same forest on each of five collecting trips for a total of approximately 200 spiders. These individuals were used to form artificial groups in the lab. According to my DNA fingerprinting tests (see Chapter 4 for details), this avoided kinship among individuals in groups under my lab settings.

Experimental design – Eight 60×60×60 cm mesh cages were used to house adult female *N. pilipes*. Adult crickets (*Acheta* sp.) with body weights of about 0.3 g were used as prey items, and only one cricket was provided in each trial. I recorded foraging behavior of adult female *A. miniaceus* in groups of one to seven individuals. The female-only groups prevented disturbance due to mating behavior, which might affect foraging efficiency. To control the condition of these females, I collected and kept subadult females in the lab until they molted into adults. These newly molted females then were used for my experimental trails and I did not reuse the individuals. I treated each individual as one replication no matter in what group size. The number of replications (individual spiders experiencing groups of a particular size) ranged from 20 to 48 (see Figure 1 for the sample size of each group size treatment). After the hosts produced orb webs, one *Argyrodes* that had been starved for two days was released in each cage. I allowed *Argyrodes* to acclimate overnight before I started the experiment. After the first set of observations on a group size of one, I removed the previous *Argyrodes*, put a fresh pair of

two-day starved *Argyrodes* in each cage and repeated the same observations. I repeated the procedure with a larger group of *Argyrodes* each time until group a size of seven was reached. The hosts were starved for at least one day before their next experimental trial. The *Argyrodes*' search time (approach to the wrapped prey) and feeding were recorded using digital video-recorders, SONY Cyber 1 (Japan).

Measurements and analyses

From the videotaped foraging sessions, I recorded (a) the success or failure of each kleptoparasite to reach the prey, (b) total duration of the feeding bout, from the time the first kleptoparasite began feeding to the time the last kleptoparasite stopped feeding, (c) per capita search time (i.e., the time each individual required to reach the prey), (d) per capita time spent feeding and per capita feeding rate, and (e) the number and identity of feeding individuals for evidence of dominance or egalitarianism.

(a) Feeding success: I divided the foraging attempts into three categories: (1) *feeding*, in which the *Argyrodes* contacted the prey with its mouthparts (presumably feeding); (2) *reaching*, in which the *Argyrodes* reached the prey and touched it with its legs, but did not contact it with the mouthparts (and thus presumably did not feed); and (3) *no contact*, in which the *Argyrodes* did not reach the prey item at all.

I analyzed these data with correspondence analysis (CA), a multivariate ordination analysis using categorical data, which is similar to Factor Analysis in Principle Component Analyses. I use this analysis to test if the proportion of feeding, reaching, and no contact foraging attempts differed among kleptoparasites in groups of different sizes. I formed a two-way contingency table of three categories of foraging attempts (columns) × seven group sizes (rows). The counts of *feeding*, *reaching*, and *no contact* by

individuals in each group size treatment were entered in the cells of the contingency table, and plotted as points in a multidimensional Euclidean space. The distances between pairs of points were weighted by their "masses" or the percentage contributions of each column or row category (that is, the frequency of each particular distance category). Then, the weighted chi-square distances of these points to the centroid (or "inertia") was calculated in Minitab 1.4, USA.

The dispersions of row points and column points according to chi-square distances to centroids were plotted onto a two-dimensional space with two principle component axes. These two axes delineate four quadrants. The proximity of column points (group sizes), or row points (outcome of foraging attempts), and their occurrence in the same or different quadrants show the degree of association between these points. The interpretation of the association between a group size and the feeding success of individuals in that group is based on where they fall on the two-dimensional plot with respect to the quadrants. If a group size data point and a feeding attempt data point fall in the same quadrant, it shows a high association between them. The numerical outputs of the ordination axes showed the percentages of the total inertia that were explained by that component. See Greenacre (2007) for literature on this method.

(b) Duration of feeding trial and feeding bouts: I measured the *length of the feeding trial* beginning when the host began wrapping the prey, and ending when the host discarded the prey remains. I measured the *length of kleptoparasite feeding* from the time the first kleptoparasite began feeding until the last kleptoparasite stopped feeding. The endpoint of the kleptoparasite feeding bout is determined primarily by the host: when the host has finished consuming the prey, she discards it out of the web and the

kleptoparasites are forced to stop feeding. I used one-way ANOVA (Minitab 1.4, USA) to test the effects of group size on the mean durations of feeding trials, search time of the first *Argyrodes* that reached the prey item in each trial, and duration of kleptoparasite feeding bouts following Fisher's method for multiple comparisons.

(c) Search duration: Individual or per capita search time represents the cost of searching (*E*) in Parker and Ruttan's model. An individual's search time was defined as the period beginning when the host started wrapping the prey item (which signals the kleptoparasites to begin searching for the prey item), to the time when the kleptoparasite reaches the prey. In estimating average per capita search time I included the kleptoparasites that successfully contacted the prey with their legs or mouthparts (*reaching* and *feeding* categories). I did not include individuals that searched but did not reach prey (as shown in Figure 1, nearly every kleptoparasite reaches the prey). I used one-way ANOVA (Minitab 1.4, USA) to test the effects of group size on the individual search time following Fisher's method for multiple comparisons.

(d) Time spent feeding and per capita feeding rate: I measured the time (in seconds) each *Argyrodes* in a group remained feeding on the wrapped prey item and calculated mean time spent feeding by individuals in each group size. Because the duration of feeding trials differed (see results) I also calculated a per capita feeding rate by dividing the time each *Argyrodes* spent feeding on the prey item by the total length of the feeding trial, from start of prey wrapping to the time the host discarded the prey, to standardize among different hosts and prey items. One-way ANOVA (Minitab 1.4, USA) was used to test the effect of group size on the per capita feeding rate and time spent feeding following Fisher's method for multiple comparisons.

(e) Dominance or egalitarianism: To determine if there were dominant individuals in a group, I recorded the number of *Argyrodes* per minute searching and feeding (hereafter termed foraging *Argyrodes*) near the prey in the web's hub during each experimental trial. The number of foraging *Argyrodes* was recorded every minute of each experimental trial until the end of that trial. I tracked the identity of each individual and its feeding duration. I plotted the number of *Argyrodes* foraging at the hub/min along a time series. For each group size treatment I tested for significant differences among individuals in time spent feeding.

Time series analyses of the number of foraging *Argyrodes* at the hub: I used the moving average process (Minitab 1.4, USA) to detect if there is a limit on the number of foraging *Argyrodes* at hub, given the size of the prey provided (~0.3 g). For each group size, the number of *Argyrodes* foraging at the hub per minute was plotted along a time axis. A five-minute moving average window was used to remove the random noise in the data. If there is a limit to the number of *Argyrodes* that can forage simultaneously at the hub, and if group size is larger than that limit, then the number of *Argyrodes* foraging simultaneously at hub should be smaller than the total group size. This could be achieved in two ways: (1) some individuals might dominate the foraging opportunity and exclude others from the prey, or (2) individuals might take turns to forage. I used ANOVA to test if the times spent feeding by some individuals in a foraging trial were significantly higher than others, i.e., some were dominant foragers.

Results

I recorded over 100 hours of videos from the experimental trials in seven group sizes. The time required for the first spider to reach the prey was significantly longer for solitary kleptoparasites (group size of one) than for groups of two to seven. There was no difference among group sizes of two to seven in the time it took the first kleptoparasites to reach the prey. However, averaging over all members in a group, there was no significant difference between solitary and group-living spiders in mean search duration. Kleptoparasites in groups of two to three individuals have a higher probability of feeding and spent more time feeding on average, than individuals in groups of other sizes. In trials with groups of four to seven kleptoparasites, individuals appeared to take turns feeding, so that usually only two to three individuals were feeding at any one time. Larger groups of foraging kleptoparasites distracted the host more, so that it took longer for her to consume the prey and the duration of feeding bouts lasted longer.

Success or failure of each kleptoparasite to reach the prey: Figure 1 presents the proportion of individuals whose foraging attempts were categorized as *feeding*, *reaching*, and *no contact* in groups of one to seven kleptoparasites. In the correspondence analysis (Figure 2) the x^2 value of the two-way (3×7) contingency table was 18.55 (*DF*=6, *p*=0.005), which indicates feeding success is not independent of group size. The results showed the first component (CP1, the horizontal axis) explained 59.38 % of inertia, and the second component (CP2, the vertical axis) explained 46.62 % of inertia. The dispersion of the data points for group sizes two and three were close to each other, and thus had similar results. Because these two data points fell in the same quadrant as the *feeding* category for both CP1 and CP2, this indicates the individuals in these two group

sizes were more likely to feed successfully than individuals in the other group sizes. In contrast, none of the data points for group sizes four though seven fell in the same quadrant as *no contact*. This shows there was no group size in my experiments that would cause all individuals to fail to feed. According to the CA results, individuals in groups of two or three had the highest chances of feeding successfully.

Duration of the feeding trials and feeding bouts: I compared the durations of feeding trials among different group size treatments. The ANOVA result showed that the mean durations of the feeding trials were significantly different among group sizes (F=5.31, p=0.0001, Figure 3). There was no significant difference in mean duration of feeding trials for groups of size one to four, and the mean duration of feeding trial is highest when group size is five or six. Duration of feeding trials in groups of size seven were intermediate, and were not significantly different from groups of five and six on one hand, or from groups of one through four on the other. The overall trend is smaller groups have shorter experimental trials, which means the host finished the prey items faster. With larger groups the host takes longer to finish feeding on the prey and the kleptoparasites have a longer time in which to feed.

The comparison of the length of time from the moment the host started wrapping the prey item to the moment the first *Argyrodes* touched the prey item showed that solitary individuals (group size one) took significantly longer than *Argyrodes* in other group sizes (F=6.16, p=0.001, see the filled bars for Fisher's grouping in Figure 3).

The mean feeding bouts of group size five, six, and seven are significantly longer than the feeding bouts of smaller group sizes (F=6.32, p=0.001). The results of Fisher's grouping showed the mean feeding bout of group size one, which grouped with group

size two to four, is shortest. Group size two, three, four and seven formed a group with longer mean feeding bouts. The lager groups, i.e., group size five, six, and seven, formed a group with longest mean feeding bouts. In general, when group size gets larger the feeding bouts get longer (hollow bars in Figure 3).

Search duration: Because my aim was to compare the search efforts of those individuals that successfully reached and/or fed on the prey item, I did not include individuals that failed to reach the prey (*no contact*). However over all the trials, only a very few kleptoparasites failed to reach the prey (Figure 1). I did not detect any difference in per capita search time among individuals in groups of different sizes (ANOVA: $F = 1.32$, $p = 0.252$).

Time spent feeding and per capita feeding rate: Kleptoparasites in groups of two, three and six fed significantly longer than individuals in groups of other sizes (ANOVA: F=4.95, $p<0.001$). According to Fisher's grouping method, the kleptoparasites in groups of two and three can feed longest (grouped in "a", Figure 4a). However, the mean time spent feeding by individuals in groups of size one and six were not significantly different from the mean time spent feeding by individuals in groups of three. For other group sizes, the mean time spent feeding was lower than that of group size two and three.

The average proportion of per capita feeding rates in groups consisting of two and three kleptoparasites (adjusted for total length of the feeding trial) were significantly higher than the averages of other group sizes (ANOVA, F=9.08 p=0.001). When group size was larger than three, the average per capita feeding rates from group size four to seven were not significantly different from the average per capita feeding rate of group size one (Fisher's multiple comparison test; Figure 4b). The results showed the average

per capita feeding rates of group size two and three ($Mean G_2 \pm SD = 671.18 \pm 587.12$ sec/*Argyrodes*/hour and $Mean G_3 \pm SD = 566.33 \pm 463.02$ sec/*Argyrodes*/hour) were about two times higher than those of other group sizes.

Dominance or egalitarianism: The results of time series analyses showed no dominant individuals in the larger groups. I only conducted time series analyses on group sizes four to seven. This was because the results of per capita feeding rate had shown the optimal foraging group size was two or three, and my goal was to examine the behaviors of *Argyrodes* at the web hub when the group size was larger than the optimal size. The fit lines of time series analyses (Fig 5) showed that the number of *Argyrodes* foraging at the hub at any one time remains at or near two to three individuals even in test groups of 4 or more individuals. The feeding durations of individuals in groups of four are marginally significantly different from each other (p=0.04). From group size five to seven, the feeding durations of the individuals in a group were not significantly different from each other (p-values of ANOVA range from 0.06 to 0.24). These results showed that individuals in a larger group take turns to feed on the prey.

Discussion

The results showed group-living behavior in *A. miniaceus* has the function of social foraging as defined in social foraging theory (Giraldeau and Caraco 2000), i.e., the payoffs or penalties to individuals that forage in groups are economically interdependent with group size. The per capita feeding rate and time spent feeding indicate that individual kleptoparasites gain the most benefits in groups of two or three individuals; individuals in groups can reach the prey sooner than solitary kleptoparasites, and feed

longer than single individuals or individuals in groups larger than four. This energy gained from the creep-up-and-share strategy presumably readily converts into the energy for reproduction. In the field, male and female *A. miniaceus* mate right after feeding with the host. The female then produces an egg sac at the periphery of or next to the host web. The finding of higher foraging rate and the observation of the mating behavior of *A. miniaceus* showed the group foraging with conspecific individuals can increase the chance of reproduction and thus increase their fitness.

An individual's search cost not significantly different when group size is different. The interactions among individuals in different group sizes did not increase or decrease the effort of finding prey items. However, I observed when two or three individuals approached the prey items simultaneously, one individual could follow another one's dragline to reach the prey item. This could facilitate reduction in search efforts when groups foraging is employed, but it did not reflect on the overall searching durations. Therefore, the interactions among group members cannot assist an individual to reach the prey item faster, but their interactions can assist an individual to stay feeding longer according to my results of per capita feeding rate studies.

The cost of approaching prey item was lowered in groups of two or three. In the results of CA, the chance of feeding was higher in groups of two and three. Although the data did not show any group size that would cause failure of foraging for all group members. Unlike a predator-prey system in which the predator is the only consumer of the prey, the host has potential to detect the kleptoparasites and either to chase them away, or to attack and even to kill them. I did not directly measure the risk of being detected and attacked by the host because it was difficult to quantify the risk of the

kleptoparasites being detected by the host. However, the higher success rate of feeding in small groups reflects their lower risk of attack by the host.

When group size got larger, instead of gathering to the prey all at once, *Argyrodes* took turns to feed and tolerated two to three individuals foraging at hub of web. This showed mutual tolerance of the individuals in larger groups. The feeding durations among the individuals in larger groups did not show significant differences no matter how fast of an individual reaching and feeding on the prey. This indicates that no dominant individuals monopolized the feeding in this lab setting. When an *Argyrodes* approached prey simultaneously with others, the moves of one *Argyrodes* could distract the host so the ones that had already reached the prey could stay feeding. This alternating feeding behavior caused distraction to the host and elongated the feeding bouts of larger groups (Figure 3). These integrated outcomes of the taking turn behavior and host distracting behavior could be the main reason that I saw the increase of per capita feeding rate. These results indicate that the group foragers of *A. miniaceus* not only show mutual tolerance of group members, i.e., possibly by avoiding competition, but they also actively help each other to forage.

Game theory-based models which do not assume kinship, such as the group foraging model of Packer and Ruttan (1988), are often use to explain the adaptive function of a group-living behavior. These social foraging models apply to any gregarious organisms, as long as the gregarious foragers show interdependency of their costs and benefits (Giraldeau and Caraco 2000). There are four roles in Packer and Ruttan's (1988) model of cooperative hunting: cooperator, cheater, scavenger, and solitary. I did not include cheater and scavenger because in my system because: (1) The

penalty for being a cheater, defined as an individual that never leaves the prey item reaching it, is extremely high. In my experiments I observed only two out of 202 individuals that did not take turns after they reached the prey item. They were both attacked and eaten by their hosts. From my video records, most individuals leave the prey item after feeding for a while and most individuals come back and forth to feed on the prey item. (2) This system does not allow scavengers because the host will consume the whole prey item by the end of the experimental trial. I also omitted the parameter "prey encounter rate" in Packer and Ruttan's (1988) model because there was only one prey item provided in each experimental trial. To apply Packer and Ruttan's (1988) model, we had to re-parameterize their equations to fit my system.

My modified model is shown in Table 1. I modified the payoff of being a cooperator in a cooperative group as the shared prey value (V/n) weighted by the group foraging success rate (H_n). I deducted the risk of being attacked by host when feeding (C_n), and deducted the cost of searching in host web (S_n). The payoffs to a cooperator in a non-cooperative group, and payoffs to a non-cooperative individual in a non-cooperative group are the same. It is because they both can be viewed as solo foragers in this system. The payoff of solo foraging is the prey value (V) weighted by solo foraging success rate (H_1), minus the risk of being attacked when feeding and the solo searching cost (S_1). According to game theory, cooperative kleptoparasitism would be favored if the payoff to a cooperator in a cooperative group is larger than the payoff to individuals (whether cooperative or non-cooperative) in a non-cooperative group.

Based on my modified model and the results I have shown, being a cooperator is favored in the group-living system of *A. miniaceus*. This is because: (1) When group size

is one, the solitary foraging rate, H_1, is significantly lower than that of cooperators in small groups (see result of per capita feeding rate). (2) When group size was two or three, the group foraging rates, H_2 and H_3, were significantly larger than those of any other group size (see results of per capita feeding rate and CA). (3) The cost of searching does not differ for individuals in groups of different sizes (see the result of search duration). (4) The value of the prey item would not be a limiting factor when group is small because a 0.3 g prey is much heavier than an *Argyrodes* (~0.02 g). Thus the terms V/n and V are approximately the same if n is small, e.g., n=2 or 3 in this study. Therefore, the term $H_n V/n - C_n - S_n$ would be larger than $H_1 V - C_1 - S_1$ at least when the group size is two or three. For group sizes larger than three, i.e., n=4 to 7, the prey value and length of feeding bout could become limiting factors and cause the per capita feeding rate to decrease to the same level as n=1. In addition, the risk in approaching the prey (and host) is likely to be lower in a group because, because the chance of being detected by the host and chased is diluted (the result of CA). From these inferences, the payoff to a cooperator would be larger than the payoff to a solitary forager (Table 1); thus group-living behavior is the ESS in this system.

Natural groups of *A. mineaceus* in the field usually include adult females, males and juveniles, and the observed group sizes were usually larger than the optimal group size of 2 or 3 determined for my lab populations. In the field, the mean of observed group sizes is 6.1±5.5 individuals, including adult males and juveniles as well as adult females (see Chapters 3 and 4). This observation could be attributed to two factors. The first is that the weight of prey items captured in the field might be significantly larger than the prey items I provided in the lab. The daily average weight of prey captured by an adult

female *N. pilipes* in tropical areas is 0.0382 gram, and the average weight per prey item is 0.0205 gram; large but rare prey items weighed on average 0.2 gram (Robinson and Robinson, 1973). The prey items I provided were ~0.3 gram, which is equivalent to the rarer large prey items in the field. Therefore, the hypothesis that *N. pilipes* in the field capture larger prey, and thus can tolerate more kleptoparasites in the web, can be rejected. The second hypothesis is based on social foraging theory (Giraldeau and Caraco 2000). This theory predicts the natural group size will usually exceed the optimal group size because the per capita benefit would still be higher than the per capita benefit of single individuals when the group size is only moderately higher than the optimal group size. When the group size becomes too large and the per capita benefit decreases to a level equal or lower than the per capita benefit of single individuals, the group size of a social foraging organism would stop increasing. However, this hypothesis remains to be tested in my *Nephila/Argyrodes* system.

Another factor influencing group size is the possibility of repeated interactions among individuals. In these experiments I used only unrelated individuals (collected from different host webs) and assembled new groups of kleptoparasites for each feeding trial. However, my measurements of feeding trial duration and feeding bout duration as a function of group size showed that the host took longer to finish the prey and discard it when kleptoparasites were more numerous. In nature, over several feeding episodes, this longer "feeding window" might be important, particularly for juveniles.

I conclude that the group-living behavior in *A. miniaceus*, at least at the level of foraging, has the adaptive function of cooperation; using a game theory model, the payoff of being cooperator in a group outweighs being solitary. This system should be attractive

to sociobiologists because it allows direct manipulation and observation in the laboratory of parameters such as prey size, group size, search effort, relatedness of group members, etc., that are important in theoretical models. However, this system also raises many new questions. The mechanism(s) of communication among group members is not clear. In field observations, I rarely find different Argyrodinae species in the same web even if they occur in the same forest, but mechanisms for recognizing and responding to non-conspecifics are not known. In addition, because Argyrodinae is sister to other social Theridiidae, which have highly developed maternal care, whether the social interactions of *A. miniaceus* are rooted in kin selection as well as social foraging should be tested. In future research, I will address the effect of kinship among group members on foraging success, investigate environmental factors such as resource size that may affect cooperative group size, and document fine-scale population genetic structure that may result from group-living behavior. In particular, I plan to contrast these variables between group-living species and solitary species to determine if cooperation only appears in naturally group-living species, and does not occur in normally solitary species when placed in similar contexts. To address these questions, future studies will employ the use of population genetics, population ecology, and phylogenetic comparative methods.

Chapter 3

Kleptoparasitic spiders form larger groups in small webs than predicted by simple aggregation at resources

Abstract

Kleptoparasitic spiders in the subfamily Argyrodinae (Theridiidae) make their living by feeding on prey captured in the webs of larger host spiders; host webs constitute discrete resource patches for the kleptoparasites. Though most kleptoparasitic Argyrodinae live solitarily in the webs of their hosts, a few species live in groups within the webs of their hosts. We investigated the relationship between the size of resource patches (host webs), environmental variables at the web, and the dispersion of solitary and group-living kleptoparasites to answer two questions: (1) Do solitary and group-living species differ in their response to size of resource patches or environmental variables? (2) Are the groups formed by group-living species larger, or smaller, than be predicted based on the size of resource patches alone? I conducted surveys of potential host webs and kleptoparasites along transect lines 150 to 600 m in length for five group-living species (*Argyrodes fissifrons*, *A. flavescens*, *A. kumadai*, *A. miniaceus*, and *A. lanyuensis*) and two solitary species (*A. fasciatus* and *Neospintharus trigonum*). All potential host webs on a transect line were noted. For each web we recorded GPS coordinates, the number of kleptoparasites present, and a series of environmental parameters: illumination (in Lux), relative humidity, temperature, height of the web above the ground, host species, distance to nearest host, distance to nearest kleptoparasites, and – for those host species that had orb webs with three-dimensional tangle webbing – tangle web length. The dispersion index of host webs was calculated for each transect. For each kleptoparasite species, Poisson regression analysis was used to find the set of resource-related and environmental variables that best predicted the

number of kleptoparasites in host webs. I used double logarithmic plot of group size ratios and their corresponding host web area ratios to find the observed slope (the simple linear regression coefficient of group size ratio on web area ratio). By comparing the observed slope to the slope predicted by a continuous input ideal free distribution null model, I determined if observed group sizes were significantly deviated from that would be predicted by size of the resource patches alone. The dispersion indices showed that the hosts of all the Argyrodinae studied were clustered in distribution. The Poisson regression analysis showed that size of resource patches (web area), was positively associated with group size in all group-living species except *A. flavescens*, but did not predict presence or number of solitary kleptoparasites in a host web. None of the other variables showed significant predictive value either for all group-living or all solitary species. The observed group size exceeded the group size predicted by the continuous input model of ideal free distribution in small host webs but group size is smaller in larger host webs for all species. I suggest that social interactions and kinship among group members may cause the deviation from ideal free distribution models in group-living species. In solitary species, environmental factors may play more important role in predicting number of individuals in a host web.

Introduction

Aggregations of conspecific animals are very common in natural populations. The simplest explanation is that resources are often patchily distributed and animals are attracted to the resource patches (Begon et al. 1996). Individuals in these groups may compete with each other in order to gain more resources and to increase their individual fitness (termed "dispersion economy" by Giraldeau and Caraco (2000)). This behavior results in a strong correlation between group size and size of the resource patch occupied.

Social interactions are another possible cause of aggregation (termed "aggregation economy" by Giraldeau and Caraco (2000)). Aggregations may have a number of social functions, such as parent offspring aggregations (Amos et al. 1993), aggregations for mating (Thornhill 1980), group hunting (Scheel and Packer 1991), guarding of resources, or defense against predators (Hass and Valenzuela 2002), which result in benefits to group members. In these cases, group size is not necessarily predicted by size of resource patches, or only partially predicted by size of resource patches.

The kleptoparasitic Argyrodinae (Araneae: Theridiidae) are good systems in which to examine dispersion in response to resources. Of 238 species in the subfamily Argyrodinae, most are kleptoparasites in the webs of other spiders, though some are predators of web-building spiders and others are free-living in their own webs. Like most spider groups, the majority of Argyrodinae are solitary, but about 20 species have been observed living in groups or "gangs" in the webs of other spiders (Whitehouse 2011), mostly species in the genera *Argyrodes* (primarily Asian species) and *Faiditus* (primarily New World species). Observed gangs range in size from 5 or fewer in *Argyrodes incursus*, *Faiditus atopus* and *F. dracus* to as many as 46 in *A. miniaceus* (see Table 2).

The turnover of individuals in and out of host webs is not exceedingly high, so that group sizes are relatively stable (Whitehouse 1988, Tso and Severinghaus 2000, Kao 2008), and the groups are of a size that is easily counted, usually fewer than 40 individuals in a web (Elgar 1993, Whitehouse 2011). Resource patches, i.e., the host webs, have clear boundaries (Agnarsson 2011) and continuous input of resources, in the form of insect prey.

The variables that predict the size of kleptoparasitic groups have been studied in nine of the 20 known group-living Argyrodinae species (Cangialosi 1990c, Grostal and Walter 1997, Tso and Severinghaus 2000, Miyashita 2002, Agnarsson 2003, Koh and Li 2003, Hénaut et al. 2005, Kerr 2005, Agnarsson 2010; see Table 2), but only a single solitary species was studied (Larcher and Wise 1985). This probably reflects the fact that (based on my field experience) populations of solitary kleptoparasites tend to be small and individuals are hard to locate; it is much easier to detect the presence of the group-living species in large host webs.

For the group-living species that have been studied, the major predictor of group size is the size of the host web, which is positively correlated with availability of prey (Elgar 1993). Studies in *A. elevatus* and *F. americanus* (see Agnarsson 2010), *A. flavescens* (see Miyashita 2002), *A. argentatus* (see Kerr and Quenga 2004), *A. globosus* (see Hénaut 2000), and *A. fissifrons* (see Tso and Severinghaus 2000) showed strong correlations between number of kleptoparasites per web and size of the webs of their hosts, *Cyrtophora*, *Argiope* (Araneidae), and *Nephila.* In *A. antipodianus* web size is a good predictor of number of kleptoparasites, although the correlation is weak (Grostal and Walter 1997). For *F. ululans*, which are kleptoparasites of social spiders such as

Anelosimus eximius (Theridiidae), larger cooperative host webs hosted more kleptoparasites than smaller webs (Cangialosi 1990b). However, *F. ululans* also prey on their social hosts (araneophagy) as one of their main foraging strategies. Based on these studies, one might infer that larger resources predict larger groups in Argyrodinae, and Argyrodinae gangs are merely aggregation of conspecifics around resource patches. Indeed, Elgar (1993) and Agnarsson (2003, 2010) suggested that the distribution of kleptoparasites per web for group-living species simply followed the expectations of an ideal free distribution (Fretwell and Lucas 1970).

The effect host web size on typically solitary species has only been investigated in *Neospintharus trigonum*. Solitary *N. trigonum* use several foraging strategies; they may catch prey in their own webs, kleptoparasitize in other spider webs, or prey on their hosts. However, there is no evidence that a larger host webs predict the presence of more *N. trigonum* (Larcher and Wise 1985).

In the host-kleptoparasite system, spatial distribution exists on two levels: distribution of the host, and distribution of the kleptoparasites within host webs. Hosts may be clustered, uniformly distributed or randomly distributed. The kleptoparasites may also be clustered, uniformly distributed or randomly distributed, but this distribution will be superimposed on the distribution of host web. For example, if host webs are distinctly clustered, kleptoparasites could be randomly distributed among the clustered webs, uniformly distributed (for example, one kleptoparasite per web) or clustered, with aggregations of kleptoparasites occurring in some host webs. Host webs are the primary resource for the kleptoparasites; group-forming Argyrodinae are typically found in large

webs produced by large-bodied hosts, such as species of *Nephila* (Nephilidae) or *Cyrtophora* (Araneidae). Solitary Argyrodinae are often found in smaller host webs.

What determines the distribution and number of kleptoparasites in host webs? Do the factors influencing the distribution and group size of solitary and group-living kleptoparasites differ in any fundamental way? Do group-living kleptoparasites form groups larger or smaller than would be predicted simply by size of the host web alone? Dispersion of both solitary and group-living kleptoparasites could be explained purely by the size of the resource patches (size of individual host webs, size of clusters of host webs, and/or distance to neighboring webs), by physical factors (e.g., microclimates preferred by the kleptoparasites) and/or by social interactions among kleptoparasites that confer added benefits to individuals in groups. Here we investigate the dispersion and size of host webs, and the dispersion of group-living and solitary kleptoparasites among host webs.

I determine the dispersion of host webs by calculating a dispersion index, or the variance/mean ratio of host webs per unit area of habitat (Ludwig and Reynolds 1988). I use a Poisson linear regression approach (Cameron and Trivedi 1998) to test the utility of a variety of resource-related variables and environmental variables in predicting the presence and group size of solitary and group-living kleptoparasites in the webs of their hosts.

To detect whether social factors, or other factors, may also be involved in determining the size of kleptoparasitic "gangs" of the group-living species, I compare observed group sizes to those predicted by an ideal free distribution model (Fretwell and Lucas 1970). The IFD model states that if individuals can distinguish the quality of

habitats and can migrate freely among them, they will disperse according to the sizes of resource patches to maximize the individual intake rate. As a result, the ratio of two group sizes (G_i/G_j) is equal to the ratio of the sizes of their corresponding resource patches (k_i/k_j) (Fretwell and Lucas 1970). That is:

$$\frac{G_i}{G_j} = \frac{k_i}{k_j} \qquad \text{Equation 1}$$

G's are the group sizes at habitat patches i and j; and k's are the sizes of resources at habitats i and j. Fagen (1987) developed a method named habitat matching rule to quantify the deviation from ideal free distribution model. He took the power function form and added two free parameters to equation 1,

$$\frac{G_i}{G_j} = b\left(\frac{k_i}{k_j}\right)^a \qquad \text{Equation 2}$$

where a is the sensitivity parameter used to access response of G_i/G_j when k_i/k_j changed, the b is bias parameter used to access whether the individuals in a population favor one site or another without taking resource size into account (i.e., under the condition that $k_i/k_j=1$, or $\log(k_i/k_j)=0$). By transferring equation 2 into log terms, this equation becomes a linear relationship,

$$\log\left(\frac{G_i}{G_j}\right) = a\log\left(\frac{k_i}{k_j}\right) + \log b \qquad \text{Equation 3}$$

Because IFD prediction is $G_i/G_j = k_i/k_j$, in order to get the equality on both sides of this equation, the expected values of a and b are both one. Therefore, in a population, by taking the log values of pairwise group size ratios G_i/G_j and the log values of the corresponding pairwise resource size ratios k_i/k_j and fit this two log ratios in a simple

linear regression equation, the slope of this equation is the estimation of *a*, and the intercept of this equation is the estimation of *b*. This provides a quantitative test of deviation from IFD model. If the observed slope of simple linear regression equation is larger than one, this indicates the individuals in the population over-use large resource and under-use small resource. This is termed over-matching. On the other hand, if the slope is smaller than one, this indicates the individuals in the population under-use large resource and over-use small resource. This is termed under-matching (Fagen 1987).

In a review of habitat matching rule, Kennedy and Gray (1993) found systematically underuse of larger resources and over-use of smaller resources in 44 of the 52 species (mean slope of these species is 0.7). This pattern of consistent under-matching of IFD prediction has been explained by the individuals have perceptual limit of habitat quality (e.g., Abrahams 1986), travel costs among resource patches (e.g., Korona 1990), interference competition (e.g., Sutherland 1983), unequal competitive abilities (e.g., Milinski 1979), and — for social organisms – kinship among group members (e.g., Morris et al. 2001).

I tested whether the observed group sizes per web are larger (or smaller) than those predicted by the IFD model for both group-living and solitary Argyrodinae using web size as the predictor. In addition to web size, I also measured other host related predictors and environmental predictors and compared whether the predictors of group size in group-living species is different from the predictors in solitary species.

Material and methods

Field site selection and surveys – From 2007 to 2010, I surveyed: (1) a population of *A. miniaceus* in Huoyenshan, Taiwan (June 2007); (2) a population of *A. fissifrons* on Orchid Island, Taiwan (July, 2007); (3) a population of *A. lanyuensis* on Orchid Island, Taiwan (July, 2007); (4) a population of *A. flavescens* at Labrador Park, Singapore (December 2009); (5) two populations of *A. kumadai* in Lienhwachi, Taiwan (December 2010) (6) a population of *A. fasciatus* on Pulau Ubin, Singapore (December 2009); and (7) a population of *N. trigonum* in Lawrence, Kansas, USA (August 2010). The time and sites of these surveys were conducted during the season when both Argyrodinae and hosts were abundant in these field sites. In total, I surveyed five group-living species, *A. fissifrons*, *A. kumadai*, *A. flavescens*, *A. miniaceus*, and *A. lanyuensis*, and two solitary species, *A. fasciatus* and *N. trigonum*.

I know from our field experience that many host species are distributed linearly along trails, which form light gaps in the forests, and so linear transects were used for field surveys. I determined the length and width of each transect based on conditions of the local populations of hosts, though I kept the transect lines within one kilometer in length and within 0.1 kilometer in width. On each transect, I searched exhaustively to find all potential host webs (with and without kleptoparasites), determined the position of each web by hand-held GPS (Garmin eTrex Summit HC) and counted the number of kleptoparasites present in each web. I also measured a number of parameters related to size of resource and microclimate at each web site (see below).

Dispersion of host webs – I first examined the dispersion of host webs in each population surveyed. Each transect line was partitioned into blocks of 20 to 50 m in

length (see Figure 6). The number of blocks on a transect line depended on the length of the transect. The counts of the number of host webs in each block were used to calculate the mean number of webs and variance per block. The dispersion index, or variance/mean ratio, was calculated to characterize the dispersion of host webs: clustered, random, or uniform. Dispersion indices significantly larger than one indicate a clustered distribution; values significantly smaller than one indicate uniform distribution, and values equal to one indicate random distribution. I calculated dispersion indices for webs of all host species and tested whether they were significantly different from one using the chi-square value with degree of freedom (n-1). The chi-square values were obtained by multiplying (dispersion index)(n-1), where n is the number of blocks on a transect line (Ludwig and Reynolds 1988).

Predictors of kleptoparasite group size – I also examined variables that might predict the number of kleptoparasites per web (hereafter group size). The putative predictors fall into two categories: host related predictors and environmental predictors. I measured these predictors for every web on each transect (with and without kleptoparasites), with all measurements for a transect taken within the same day. All sampling on a transect line was done on a clear day, and within a single, four hour time interval. The host-related predictors were: web area (square web radius×π), and sum of host web areas if the hosts built communal webs (communal webs are defined as a group of webs interconnecting with each other). Distances to the nearest host web, and distances to the nearest host web with kleptoparasites were determined from the GPS data. As for the predictor host species, hosts were coded from largest to smallest, e.g., if I found *Nephila* (the large), *Argiope* (medium), and species of Theridiidae (the smallest) in a transect, I ranked

Nephila as one, *Argiope* as two and Theridiidae as three in the data matrix. For those host species that had orb webs with three-dimensional tangle webbing, I measured the tangle web length, which is the length from the sheet web to the highest point of the web.

The environmental predictors were height of the host web (the distance from web center to the ground in cm), light intensity at the web (lux), humidity (%), and temperature (°C). I used a hand-held mini-environmental meter (AGM, USA) to measure these environmental factors. For kleptoparasites, I recorded the numbers of females, males, and juveniles in each web; the sum of females, males, and juveniles is the group size in a web. The sample size, i.e., number of host webs, was at least 30 in each population. After the survey, I collected the kleptoparasites of each web and preserved them in 95% EtOH for identification and as specimens for my fingerprinting project (see Chapter 3).

Poisson regression of predictors of group size – I used Poisson regression analyses to find the best set of predictors for the number of kleptoparasites in host webs for each Argyrodinae species. The Poisson regression is often in the case when the response variable is counts. The first assumption of the Poisson regression is that the predictors (or independent variables) in a model have linear relationships with the log values of response variable (or dependent variable). The model can be written as log (Y) = intercept $+b_1X_1+b_2X_2\ldots+b_nX_n$, in which b_1, b_2, …are the regression coefficients and X_1, X_2, … are the independent variables in this model. An offset variable is often included in this model as an adjustment when the count data are collected from subpopulations of different sizes. This is to eliminate the effect of larger counts from a larger subpopulation. Because I counted all the individuals in the webs in each population, inclusion of this variable was

not necessary in my study. The second assumption is that the response variable follows a Poisson distribution, such that the ratio of the mean to the variance is one. I used the deviance, or the likelihood ratio test statistic, as chi-square value to test if my response variable, group size, fits a Poisson distribution using degree of freedom = (number of observations – number of parameters in the model). If the count data are Poisson distributed, the deviance/degree of freedom ratio would be close to one. If the data of group size deviated from Poisson distribution, in my cases some of the deviance/*DF* ratios were larger than one, termed over-dispersion, I then used negative binomial distribution instead of Poisson distribution to conduct my analyses. I carried out these analyses in SAS 9.22 using the **proc genmod** command using either Poisson distribution or negative binomial distribution. The SAS program performed the analyses of maximum likelihood parameter estimates. The test statistic I used to determine the significance of parameter estimates is Wald chi-square.

Test of ideal free distribution model of group size – For group-living Argyrodinae species, I compared observed group sizes to those predicted under the ideal free distribution model in order to determine if groups were larger or smaller than expected, given the size of the resource patch. I used web area to represent the size of resources, as many studies have shown that web area is highly correlated with prey capture rate (Enders 1975, Rypstra 1982, 1985, Higgins 1995, Herberstein and Tso 2000). I used the log values of k_i/k_j as the predictor to predict the change of the log values of G_i/G_j (Equation 3) in a given transect. I used the IFD predicted slope (slope = one) as the null hypothesis, and compared this to the observed slope, i.e., the regression coefficient of the simple linear regression line. If the observed ratio is significantly larger than one, it

indicates that the large webs contain more kleptoparasites than the number predicted by IFD and small webs contain fewer kleptoparasites than IFD prediction (over-matching). In contrast, if the observed slope is smaller than one, it indicates that the host large webs housed fewer kleptoparasites than expected under the IFD prediction and the individuals in this population over-used the smaller webs (under-matching). If the slope is not significantly different from the predicted ratio of IFD, the distribution of kleptoparasites in host webs is predicted by kleptoparasite response to the sizes of host webs. We used **proc reg** command in SAS (SAS 9.22) to find the simple linear regression equation and I used the command **Test a=1** to test if the IFD predicted slope is significantly different from the regression coefficient.

Results

Distribution of host webs – The distributional patterns of host webs and the relative number of kleptoparasites per web are shown in Figure 6. All hosts of both solitary and group-living kleptoparasites show a clustered or patchy dispersion, as indicated by dispersion indices significantly greater than one (Figure 6).

The dispersion index of *A. flavescens* was 9.29 (x^2=55.74, *DF*=6, p<0.001) from total 30 webs (eight webs are communal) on a 250 m transect (Figure 6a). The spatial index of *A. fissifrons* was 2.50 (x^2=15.00, *DF*=6, p=0.002) from total 39 webs on a 150 m transect (Figure 6b). For *A. kumadai*, I surveyed 32 webs from two near by populations (distance in between 3km). There were 23 webs on the first transect (250 m in length) and the dispersion index was 3.07 (x^2=15.35, *DF*=5, p=0.014). The second transect had nine webs and I did not calculate the dispersion index because of the small number of

sample size (Figure 6 c and d). The spatial index of *A. lanyuensis* was 2.50 (x^2=15.00, *DF*=6, *p*=0.002) with total 39 webs on a 150 m transect (Figure 6e). The dispersion index of *A. miniaceus* is 3.72 (x^2=40.97, *DF*=12, *p*<0.001) from total 66 webs on a 600 m transect (Figure 6f). The spatial index of *A. fasciatus* was 2.51 (x^2=17.57, *DF*=7, *p*<0.001) with 19 webs (42 communal webs) on a 520 m transect (Figure 6g). The spatial index of *N. trigonum* was 8.54 (x^2=85.40, *DF*=10, *p*<0.001) from total 42 webs on a 550 m transect (Figure 6h).

Poisson regression of predictors of kleptoparasite group size – Results of the Poisson regression are shown in Table 3 (showing regression statistics) and Table 4 (summarizing positive and negative predictors of group size). Web area was the variable with widest predictive value. The Poisson regression coefficient was positive for all group-living species and negative for all solitary species, though not all estimates were significant. Web area was a significant, positive predictor of kleptoparasite group size for all group-living species except *A. flavescens*; the negative correlations between web area and the presence of the solitary species were not significant.

For group-living *A. flavescens*, the distance to the nearest neighboring host web was positively and significantly correlated with group size, but distance to the nearest neighboring host web with kleptoparasites in it was negatively correlated with group size. That is, the more isolated a web was from neighboring webs, the larger the group of kleptoparasites occupying that web was likely to be, while proximity to other webs with kleptoparasites was a good predictor that the group in the focal web would be smaller. In contrast, distance to the nearest neighboring web with kleptoparasites was a significant positive predictor of group size in group-living *A. miniaceus*. Group size in two of the

group-living species, *A. kumadai* and *A. lanyuensis*, was also predicted by some of the environmental variables: light intensity and height of the web above the ground were significant positive predictors of group size in *A. kumadai*, and relative humidity was a significant positive predictor of group size for *A. lanyuensis*.

For solitary species, none of the variables related to host size or spacing were significant; only environmental variables were significant predictors of the presence of solitary kleptoparasites in a host web. Height of the web above the ground positively predicted the presence *of A. fasciatus*, while humidity and temperature positively predicted the presence of *N. trigonum*.

Comparisons of Predicted and observed group sizes in kleptoparasites – The observed simple linear regression coefficients (*a*'s) were compared to the predicted slope one under the IFD model. The comparisons of observed kleptoparasite group sizes to those predicted under the IFD model showed a systematically under-use of large host webs and over-use of small host webs for all species I tested (Figure 7 a to g). The simple linear regression equation of solitary species *A. fasciatus* showed that the intercept is significantly larger than zero (Figure 7f). This indicated there are other factors, besides web area, can affect the habitat selection of this species.

Discussion

The factors promoting group-living in animals range from the simple to the complex. Among the simplest explanations is that when resources are patchily distributed, the organisms exploiting those resources will also be patchily distributed across the landscape. This assumes that individuals can assess the size of a resource patch, travel

among resource patches, and compete with individuals sharing the same resource patch to ensure they obtain a sufficient share of resources. Such a population is assumed to follow an ideal free distribution (IFD), in which exploitation competition is the only kind of interaction among group members and there is no benefit gained from social interaction among individuals in a group (Giraldeau and Caraco 2000). In the case of kleptoparasitic spiders in host webs, a continuous input model of IFD is appropriate (Fretwell and Lucas 1970, Tregenza 1994) because the host webs keep catching prey items and the prey items are rapidly consumed by kleptoparasites or by the host. The number of individuals in a resource patch is determined by size of the resource and amount of the resource required per capita—the "dispersion economy" of Giraldeau and Caraco (2000). The expected group size (G) in a resource patch or web is predicted by resource size (k) as described by the ratio of $G_i/G_j = k_i/k_j$ (Fretwell and Lucas 1970). This process results in a strong correlation between group size and size of the resource patch occupied.

The spatial distribution of kleptoparasitic spiders is subject to structuring at multiple levels. Because the kleptoparasites occupy and forage in the webs of larger host spiders, the spatial dispersion of kleptoparasites is first structured by the distribution of host webs. My survey of host webs showed that the webs of all host species examined — both those hosting solitary kleptoparasites and those hosting group-living kleptoparasites—were significantly clustered in distribution.

The second level of dispersion concerns the distribution of kleptoparasite populations in the available host webs. Dispersion of solitary kleptoparasites, such as *A. fasciatus* and *N. trigonum* examined in this study, consists essentially of presence or absence in host webs. I found that size of the host web had little value in predicting the

number of solitary kleptoparasites in host webs. Though the host webs occupied by solitary species of kleptoparasites could be as large as 1 meter in diameter, close to the size of host webs occupied by group-living species, I rarely found multiple individuals of a "solitary" species in a host web. On the other hand, some environmental parameters had significant predictive value (Table 3 and 4). These two results show that solitary versus group-living behavior of these kleptoparasites cannot be chalked up to differences in the dispersion of host webs, nor to the typical size of hosts and host webs used.

The dispersion of group-living species in host webs is more interesting. My studies agreed with those of several other authors (Cangialosi 1990c, Grostal and Walter 1997, Tso and Severinghaus 2000, Miyashita 2002, Agnarsson 2003, Koh and Li 2003, Hénaut et al. 2005, Kerr 2005, Agnarsson 2010) in showing that host web area was a highly significant predictor of the number of kleptoparasites in a host web. Host web area was a significant predictor of group size in all group-living species examined except *A. flavescens* (Table 3). Given this relationship, most authors who have considered the question assume that groups of kleptoparasites are simply following an ideal free distribution (IFD). However, until now, no one has tested whether observed group sizes actually fit the predictions of the IDF model.

Most behavioral or ecological studies of Argyrodinae have focused on group-living species (Grostal and Walter 1997, Tso and Severinghaus 2000, Miyashita 2002, Agnarsson 2003, Kerr and Quenga 2004, Hénaut et al. 2005, Agnarsson 2010). These species show strong correlations between size of the host web and size of the kleptoparasite group. Agnarsson (2003, 2010) have suggested that the distribution of these kleptoparasites in hosts webs follows and ideal free distribution. This inference

simply assumes that per capita occupancy of web area is the only predictor of group size. Agnarsson ignored the interaction among group members and does not test to see if the amount of resources (host web area) per kleptoparasite is constant over the population, as would be predicted by the IFD model. In this study, I compared observed kleptoparasite group sizes to those predicted by the IFD model. I found that in all five group-living species and two solitary species, the individuals in a population under-used larger host webs and over-used smaller host webs. This under-matching pattern implies that in these species, group size is not determined solely by size of the resource patch and other factors, such as interaction among group members, need to be considered (Figure 7 a to e).

Under the IFD model, distribution of individuals into patches is governed by exploitation competition for resources, and per capita access to resources should be constant across the population. Given the distributions observed in group-living species, if kleptoparasites only compete to the resource, then the group sizes of kleptoparasites should conform the IFD prediction. However, from our field observations, although mutual tolerance exists among group members in a web, the group members in a web actively expel the kleptoparasites coming from other webs, which is a form of interference competition. The same pattern of interference competition has also been found in group-living species *A. flavescens* and *A. bonadea* in Japan (Miyashita 2001). In addition, the group members in group-living species are likely to be related individuals at least in *A. miniaceus* and *A. kumadai* (see the results of Chapter 4). I therefore argue that in group-living species, interference competition and kinship could be the reasons of under-using large webs and over-using small webs.

I found the same pattern of under-using large webs and over-using small webs in two solitary species. In my field observations, the individuals are extremely aggressive to conspecifics. This suggested strong interference competition among conspecific individuals. According to my results of Poisson regression, web area does not predict the number of individuals in a host web; instead, environmental predictors are better predictors. This indicates the environmental factors play more important role than resource size to number of individuals in a web in solitary species. However, because I only observed two solitary species, further observations of habitat selection should be conducted.

Both solitary and group-living species are found among the kleptoparasitic Argyrodinae. I conclude that group-living behavior is not a result of clustered distribution of host webs, as the hosts of both solitary and group-living Argyrodinae show clustered distribution. Likewise, kleptoparasite groups do not form simply because the webs they occupy are large and can support more kleptoparasites. Solitary species may occupy host webs as large as those occupied by group-living species, yet multiple "solitary" kleptoparasites are almost never found in a single host web, and size of the host web is not a significant predictor of the number of solitary kleptoparasites in a web. Size of the host web is a significant predictor of group-size for four of the five group-living Argyrodinae examined, but group size is not determined solely by the per capita resources (capture web area) available in webs of different sizes. For all studied species, the size of aggregations in host webs significantly exceeded the group-size predicted by the continuous input ideal free distribution model in small host webs and the group size is significantly smaller then predicted. I argue that, for group-living species, social

interactions among group-members, as well as kinship among group members (see Chapter 4) provide additional benefits that favor individuals that remain in groups thus cause the under-matching phenomenon. The distribution of individuals of solitary may be governed by environmental factors. However, for both solitary and group-living species, the experimental studies of habitat selection should be conducted.

Chapter 4: Population genetic structure of kleptoparasitic spiders

– A comparison between group-living and solitary species –

Abstract

Most species of *Argyrodes*, *Faiditus* and *Neospintharus* (Theridiidae: Argyrodinae) are solitary kleptoparasites in the webs of their host. However, some species in *Argyrodes* and *Faiditus* live in groups in the webs of their hosts. These group-living kleptoparasites approach the host and feed on large prey that their host is feeding upon. Experimental studies in the lab indicate that this is a form of cooperative behavior, as kleptoparasites in groups or "gangs" were able to feed for a longer period of time than single kleptoparasites and take turns feeding on the prey item. In this study, I investigated whether members of kleptoparasitic gangs in natural populations are composed of related individuals by comparing population structure of two group-living kleptoparasitic species, *Argyrodes miniaceus* and *Argyrodes kumadai*, and two solitary kleptoparasitic species, *Argyrodes fasciatus* and *Neospintharus trigonum*. For each species, I mapped and collected all specimens found along transects of 400 to 500 meters. I carried out DNA finger-printing for each individual using the TE-AFLP method (three-endonuclease amplified fragment length polymorphisms), and used spatial autocorrelation analyses to assess the relatedness of gang members and to compare phenotypic similarity as a function of distance in group-living and solitary species. I found that**:** (1) in both group-living species, relatedness is highest among spiders sharing the same host web (gang members) and declines steeply with increasing distance, and (2) in both solitary species, collected over a similar geographic scale, there was no structure at all. There were no significant relationships between pairwise distance and pairwise similarity at any scale. These results, along with observations on reproductive behavior, suggest that population genetic structure in the group-living species is caused by limited dispersal of group

members; thus individuals in the same web are likely to be relatives. In contrast, the absence of genetic structuring in populations of solitary species suggests a high level of dispersal of individuals. These results suggest that it is possible for group-living kleptoparasitic spiders to gain inclusive fitness benefits when cooperating with other kleptoparasites in a host web.

Introduction

Group-living is not widespread in spiders, primarily because most spiders are predatory and cannibalistic. None-the-less, there are a variety of types of group-living and social systems among the spiders (Buskirk 1981, D'Andrea 1987 , Bilde and Lubin 2011). Two of the best-studied types are cooperative societies (also called permanent non-territorial societies; Aviles 1997) and communal societies (also called colonial or territorial societies; Aviles 1997).

Cooperative behavior is rooted in extended maternal care, and is characterized by suppression of dispersal by the young, retention of the mutual tolerance usually shown by early instar spiderlings, an inbred mating system, and cooperative behavior among colony-mates (Smith and Hagen 1996, Agnarsson et al. 2007, Bilde and Lubin 2011). This type of group-living could be favored by kin selection, i.e., cooperative behavior could evolve if the inclusive fitness benefits of cooperation outweighed the costs (Hamilton 1964), and also by group selection (Smith and Hagen 1996) if it resulted it better survivorship, reproduction and new colony initiation for cooperative groups compared to groups composed of both altruists and cheaters.

Communal or colonial behavior has received less attention. Communal groups originate from aggregations of individuals at valuable resources, such as nesting sites, web-building sites or food resources (Uetz and Hieber 1997). They typically exhibit extensive dispersal of immatures and little or no cooperation among group members. Groups may be ephemeral or long-lasting, or in a few cases, obligate.

The kleptoparasitic spiders in the subfamily Argyrodinae (Theridiidae) exhibit another group-living syndrome that is fundamentally different from the cooperative and

communal systems. Kleptoparasites steal prey items caught and prepared by host spiders; this also entails the risk of being killed by the host while foraging in the hosts' webs (Whitehouse 1997). For some Argyrodinae species living in groups or "gangs," social kleptoparasitism enhances foraging success, at least when gangs are small (see Chapter 2 and an observation in Whitehouse et al. 2002).

Group-living behavior in kleptoparasitic Argyrodinae may have origninated with extended maternal care, with aggregtions of individuals at rich resources, or both. Maternal care in Argyrodinae consists simply of placing the egg-sac in or adjacent to a host web. However, a recent revision of the family Theridiidae based on morphological data showed that the sub-family Argyrodinae is sister to a clade containing many subsocial and cooperative species that have extended maternal care (Agnarsson 2004). Maternal care in these theridiids includes regurgitating food for hatchlings, providing small pieces of prey, providing small intact prey, or allowing immatures to feed on large prey alongside the mother (Ruttan 1991). The spiderlings show mutual tolerance and may even cooperate in the capture of small prey (Kim et al. 2005). Mutual tolerance in subsocial species ends when the spiderlings disperse, but is retained through adulthood in cooperative species (Bilde et al. 2005, Salomon and Lubin 2007).

Agnarsson (2004) has suggested that maternal care might be ancestral for both Agyrodinae and the sister clade that includes subsocial and cooperative species. He also proposed that extended maternal care and persistant tolerance among siblings led to the evolution of cooperative behavior in the sister clade to Argyrodinae, while in the Argyrodinae, mutual tolerance is retained, but the host assumes the role of "web and food provider". In this situation, the form of sociality in group-living Argyrodinae has the

same root in maternal care as the cooperative behavior in their sister Theridiidae, and group members may consist of related individuals.

The origin of group-living in Argyrodinae may also have been influenced by aggregation of individuals (related or not) at rich resources — in this case the resource is host webs. The webs of host species may be considered as long-lasting, but still dynamic, habitat patches that provide stable resources for these kleptoparasites (Cangialosi 1990b, Elgar 1993, Hénaut 2000). Clustered spacing patterns of hosts have been observed across many host-parasite combinations in Argyrodinae, e.g., *Anelosimus eximius* hosting *A. uluans* (Cangialosi 1990b), *Nephila clavipes* hosting *A. elevatus* and *A. caudatus* (Agnarsson 2003), and *N. clavipes* hosting *Argyrodes* spp. (Rypstra 1985). Usually, larger and more clustered host webs are occupied by more Argyrdinae than smaller or isolated host webs (Agnarsson 2010).

These observations suggest that members of kleptoparasite groups are aggregating at rich, patchy resouces. Depending on the level of migration among webs, members of these aggregations might be related or unrelated individuals. If members in group-living Argyrodinae are primarily unrelated, their social foraging behavior may have originated from reciprocal altruism and can be explained best by game theory-based predictions (Trivers 1971).

In this study, I (1) reveal the genetic relationship among kleptoparasitic gang members, and (2) contrast the population genetic structure of group-living species to that of solitary Argyrodinae species.

All Argyrodinae show female egg-sac guarding behavior, and at least some group-living Argyrodinae complete their life cycles in host webs (Whitehouse 1988, Cangialosi 1990a). These group-living species forage, find mates, and lay egg-sacs in the host web. Based on my field observations of *A. miniaceus* and *A. fissifrons* (a closely related species to *A. kumadai*), the spiderlings utilized their host web immediately upon emerging from the egg-sac. My lab observations on two group-living species, *A. miniaceus* and *A. kumadai*, and two solitary species, *A. fasciatus* and *N. trigonum*, show they all have four instars before molting to adulthood. In field observations of group-living species, I observed that a "gang" usually includes both sexes and multiple instars of the kleptoparasites. It is possible that some individuals might stay in the same web with their siblings from hatching to adulthood.

Thus, understanding dispersal among host webs and the resulting relatedness among group members is essential for understanding the costs and benefits associated with group-living in kleptoparasitic Argyrodinae. Direct field observation of dispersal among host webs by individual Argyrodinae is almost impossible because their adult body size is only about two to four mm. Several studies have instead labeled the individuals in a web and counted the daily immigration of unmarked individuals and emigration of marked individuals in a web (Tso and Severinghaus 2000, Kao 2008). These studies showed the among web migration rate of group-living Argyrodinae is lower than that of the solitary species *N. trigonum*, which was the only species has been studied (Wise 1982). Defense of the host web resource by group members and competition with intruders appear to be the reason for low migration rate among the webs for group-living Argyrodinae (Miyashita 2002). Because the webs of host spiders are not

permanent resource patches, the founder kleptoparasites in a host web could gain the most fitness if they can defend the web from intruders, reproduce and establish a kin group before the host dies or moves its web.

I investigated these questions by using DNA fingerprinting to compare population structure and relatedness in both solitary and group-living kleptoparasites in the subfamily Argyrodinae. I used the population genetic structure of solitary species as the baseline to test if the population of group-living species is more genetically structured. I expect very little genetic structure in populations of solitary kleptoparasites, because they are obligated to leave the host web to find new resources and mates. I also investigated population structure for juveniles, sub-adult and adult females, and sub-adult and adult males in the group-living Argyrodinae, to assess which class of individuals was most likely to disperse. If kleptoparasitic gangs consist of related individuals, then cooperation among gang members can have positive effects on fitness both directly, through increased foraging success, and indirectly, through inclusive fitness effects. If members of kleptoparasitic gangs are no more closely related than a random selection of individuals from the population, then the benefits of group-living would consist solely of advantages to individuals in foraging and survival.

Material and methods

Natural History – I included two group-living species, *A. miniaceus* and *A. kumadai*, and two solitary species, *A. fasciatus* and *N. trigonum*, in my analyses. I observed the creep-up-and-share (see Chapter 2) behavior in the two group-living species in this study. All the developmental stages in *A. miniaceus* possess this behavior. For *A. kumadai*, this

foraging strategy only appears in the spiderling stage through the third instar. Host specialization is different between group-living species and solitary species. Group-living *A. kumadai* is a specialist of *Cyrtophora* and *A. miniaceus* is a specialist of *Nephila*. For solitary species in my study, *A. fasciatus* is often found in the webs of Psechridae, but they also utilize the webs of Theridiidae, *Cyrtophora*, and *Nephila*. The second solitary species in my study, *N. trigonum*, is a generalist. They utilize the webs of *Argiope*, *Agelenopsis*, *Araneus*, Linyphiidae, and Theridiidae.

Sample collection –The population of *A. miniaceus* was collected from Huoyenshan, Taiwan (N 24° 21' 51", E 120° 44' 20") in July 2007. Their host is the orb-weaver *Nephila pilipes* (Nephilidae). The population of *A. kumadai* was collected from Lienhuachih, Taiwan (N 23° 54' 51.1", E 120° 53' 17.3") in December, 2009. Their host is the orb-weaver *Cyrtophora moluccensis* (Araneidae). The population of *A. fasciatus* was collected on Pulau Ubin Island, Singapore (N 1° 24' 24", E 103° 57' 58") in December 2009. *Neospintharus trigonum* were collected from Lawrence, KS (N 38° 56' 58.2", W 95° 16' 7.3"). All specimens collected from a host web were stored in 95% ethanol for later identification of instar stages and DNA fingerprinting. All spiders collected from a single host web were kept together. I chose a forest with a large number of host webs and exhaustively surveyed a transect about 0.5 kilometer long and 50 meters wide to locate all host webs (see the distribution of the hosts of these species in Chapter 2). The position of each web was determined using hand-held GPS (Garmin eTrex Summit HC). I then converted the positions of the host webs from the geographic coordinates format (longitude and latitude) into Universal Transverse Mercater (UTM) coordinates for subsequent analyses.

Identification of instar stages – Each kleptoparasitic spider collected during surveys was identified to instar in the lab under a microscope. Based on the lab rearing of Argyrodinae, I were able to classify specimens into five stages: stage I, spiderlings– legs I are not elongated; stage II, 2nd instar–legs I are elongated, body size is close to that of spiderlings; stage III, 3rd instar–slightly swollen palps in males, body size significantly larger than spiderling and 2nd instar; stage IV, 4th instar–genitalia of both sexes distinguishable but without detailed structures; and stage V, mature adult–with complete genitalia.

DNA extraction – I extracted DNA from the whole spider when the specimens were spiderlings, 2nd instar, or 3rd instar. For 4th instars and adults, I extracted DNA from legs and cephalothorax using Sigma-Aldrich DNA GeneElute kit (GN350, USA). The tissue was homogenized in lysis T buffer provided by the manufacturer, and incubated at 55 °C for 24 hours. I then followed the commercial protocol for extraction of genomic DNA from each specimen. The DNA samples were stored at -20 °C until proceeding to the next step.

DNA fingerprinting- I carried out DNA fingerprinting using the three-endonuclease amplified fragment length polymorphism (TE-AFLP) method of van der Wurff et al. (2000). DNA fingerprints were collected for all individuals in one population of each species to examine population structure. I repeated fingerprints of 10 % of the individuals from each population to test for reliability of fingerprints. I used three restriction enzymes — *Xba*I*, Bam*HI and *Rsa*I — to digest DNA from each specimen. The resulting DNA fragments were ligated to adaptors with sticky ends complementary to the *Xba*I and *Bam*H1 sticky ends of the DNA fragments. Each digestion/ligation reaction contained

1.0 µL of DNA extract, 2.0 µL of 10×ligase buffer, 2.0 µL of 500mM NaCl, 7.5 units ligase (NEB, USA), 1.25 units *Xba*I (Promega, USA), 6 units *Bam*HI (NEB, USA), 1 unit *Rsa*I (Promega, USA), 4.0 µL of *Bam*H1 adaptor (1 picoM/µL concentration), 4.0 µL of *Xba*1 adaptor (1 picoM/µL concentration), and enough water to make a 20 µL reaction (see van der Wurff et al. 2000 for sequence of the adaptors).

A subset of the DNA fragments were PCR amplified using primers complementary to the adaptors plus additional arbitrary bases; I used primer combination *Xba*I-CC and *Bam*HI-C The *Xba*I-CC primer is complementary to one strand of the XbaI adaptor plus the two arbitrary bases "CC", while the BamHI-C primer is complementary to one strand of the BamHI plus the arbitrary base "C" (see van der Wurff et al. 2000 for sequence of the primers). Each 12.75 µL PCR reaction contained 0.5 µL of DNA sample, 2.5 µL of 5×PCR buffer, 0.75 µL of 25mM $MgCl_2$, 0.25 µL of *Bam*HI-C florescence-labeled primer (10pmol/ µL), 0.25 µL of *Xba*I-CC primer (10pmol/ µL), 0.125 µL of Taq polymerase (Gotaq, Promega, USA), and 0.25 µL of 10 mM dNTPs. I followed the thermal profile described by van der Wurff et al (2000), which had 3 min denaturation at 95 °C, followed by 95 °C for 30 sec, 70 °C for 30 sec, and 72 °C for 60 sec for 10 cycles; 95 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 60 sec for 40 cycles; and, finally, 72 °C for 20 min and stopping at 4°C. One microliter of PCR product was diluted with 29 ul of water before fragment sizing. The total number of individuals I did for TE-AFLP fingerprinting and the number of individuals I successful got the TE-AFLP fingerprints were shown in Table 5.

Fragment sizing – The diluted PCR fragments were sized using a Beckman CEQ 8000 automatic sequencer and the resulting data were imported to the program GeneMapper *v* 4.0 for scoring peak heights. The settings used in GeneMapper *v* 4.0 were: fragment size range, 50bp to 600 bp; no normalization; common alleles deleted; thresholds value type=absolute, and threshold value for inclusion in data set = 100.0 relative fluorescence units (rfu). After sizing the fragments in GeneMapper *v* 4.0, I collected the raw peak height data for each locus from all the individuals.

Signal normalization – I followed Whitlock et al. (2008) to conduct peak height signal normalization and phenotype calling for each fragment. The raw data of peak height that I screened in GeneMapper *v* 4.0 were imported in AFLPscore 1.4b (Whitlock et al. 2008), an interactive scripting program written in R. To normalize the raw peak height data, the sum of fluorescence intensity, *i* (in rfu), of every peak from each individual spider's fingerprint is calculated. The program then calculates the median of fluorescence intensity, *m*, across the whole data table (individual × loci). The ratio of *m*/*i* was used as the normalization factor and all the peak height values in the data table were multiplied by this normalization factor. This generates a new data table with normalized peak height values, corrected for individual reactions of different intensities.

Testing reliability of data- After normalizing the peak height of each locus in a species' TE-AFLP fingerprints, I tested the mismatch rate between my formal data and repeated samples. Depending on the actual mean height of peaks in the data table for each species, I tried a series of peak height selection thresholds and locus selection thresholds to find the combination that generated the lowest mismatch rate for repeated data (i.e., two TE-AFLP fingerprints generated for the same individual). I aimed to retain the largest

number of loci in my data matrices while still keeping the mismatch rate as low as possible and mismatched loci from the same individual were eliminated from the data matrix. Whitlock et al. (2008) suggest that a mismatch rate below 3 to 4 % is adequate.

Phenotype calling - After finding the optimal combination of peak height selection threshold and locus selection threshold, I used this combination of selection thresholds to conduct phenotype calling, i.e., sort the peak heights of the loci in data matrices into "0" (peak absent) and "1" (peak present), and generate a phenotype table. Each peak, representing a piece of amplified DNA of a particular size, was considered to correspond to one particular gene locus. I eliminated from my data matrix any loci that showed a peak present in only one individual or showed a peak present in every individual except one (singletons). The optimized phenotype tables were then used for the following analyses.

Spatial Autocorrelation Statistics –Spatial autocorrelation was used to compare the population genetic structure of group-living and solitary Argyrodinae. This procedure plots the autocorrelation coefficient ***r***, a measure of pairwise genetic or phenotypic similarity between pairs of individuals, as a function of the pairwise spatial distance between them.

Pairwise phenotypic distance between individuals was calculated from the phenotype tables described above. The loci scored were treated as binary data from diploid individuals (presence of a peak or band indicates the individual is homozygous or heterozygous for the allele "peak present"; absence of a peak indicates the individual is homozygous for the allele "peak absent"). Because the TE-AFLP loci are scored as

binary markers I refer to this as phenotypic data, to distinguish it from codominant markers such as microsatellites.

Calculation of phenotypic distance between pairs of individuals followed the method of Huff et al. (1993), in which any loci that are in the same state in both individuals (i.e., 1 and 1 or 0 and 0) are given a value of 0, and any loci that differ in state between two individuals (i.e., 0 and 1, or 1 and 0) are given a value of 1. Pairwise phenotypic distance is the sum of scores across all loci. Pairwise geographic or spatial distance between individuals was calculated from the Universal Transverse Mercator positions calculated for each individual from the GPS coordinates recorded at the time of sample collection. Both pairwise similarity and pairwise spatial distances were calculated in the Excel-based program GenAlEx6.4 (Peakall and Smouse 2006).

Spatial autocorrelation analyses were also performed in GenAlEx6.4. The autocorrelation coefficient, ***r***, can take values from -1 to +1, and is a measure of genetic similarity of pairs of individuals separated by specified linear distances (Peakall et al. 2003). Calculated values of ***r*** were plotted as a function of the specified distance classes (see Figures 1-3). Separate analyses were performed for each species, using the "single population" option, as I have data from only a single population of each species, and the "variable distance classes" option so that I could specify distance classes appropriate to the size of host webs, web clusters and collection transects (see Table 6). I did not allow empty distance classes (i.e., classes in which there were few or no pairs of individuals separated by that range of distances); as a result I used different distance values for *A. miniaceus*, *A. kumadai* and the two solitary species. For the group-living species *A. miniaceus* I used the distance classes 0 to1 m, >1 to 25 m, >25 to 50 m, >50 to 100 m,

>100 to 200 m, and >200 to 400 m. Distances of 0-1 m correspond to kleptoparasites in the same host web, because the host web is about 1 m in diameter. Individuals separated by distances >1 m to 25 m represent individuals in neighboring webs in a cluster. The other distances compare individuals from more widely separated webs. For the group-living species *A. kumadai*, I used the distance classes 0 to1 m, >1 to 10 m, >10 to 50 m, >50 to 100 m, and >100 to 200 m. For solitary species, I used distances as close to those used for group-living species as possible, while avoiding distance categories with few or no pair-wise distance samples.

For each of the group-living species I also did separate analyses of data from different age/sex classes to determine at which stage that these kleptoparasites were likely to disperse. I divided my samples of *A. miniaceus* into sub-adult and adult females, sub-adult and adult males, and juveniles; I subdivided my samples of *A. kumadai* into sub-adults and adults *versus* juveniles.

Two statistical tests were performed to test the null hypothesis of no genetic structure: bootstrap estimates of ***r*** that generate a 95% confidence interval around the observed estimate of ***r*** in each distance class, and a permutation procedure that generates a distribution of ***r*** values under the assumption of no spatial structure (both described in detail in Peakall et al. 2003).

Within each distance class, 1000 bootstraps were performed by drawing pairwise distances (with replacement) from the set of pairwise distances in that distance class. The 1000 bootstrap estimates of ***r*** were ranked, and the 25th and 975th values were used to define the 95% confidence interval around the observed estimate of ***r***. If the 95%

bootstrap confidence interval around an estimate of ***r*** does not span zero, one can infer significant spatial structure.

The permutation procedure produces the upper and lower bounds of a 95% confidence interval around ***r*** = 0 at each distance class, ***r*** = 0 being the expected value under the assumption of no geographic structure. I used 1000 permutations in which pairwise phenotypic distances were randomly shuffled among spatial locations to generate the distribution of ***r*** under the hypothesis of no spatial structure, ranked the values of ***r*** obtained by permutations and used 25th and 975th values to define the upper and lower boundaries of a 95% confidence interval around the hypothesis of no structure distances (Peakall et al. 2003). If a value of ***r*** estimated from the data falls outside of this range, then significant spatial structure can be inferred.

In this study, I considered estimates of ***r*** for a particular distance class to be significant and biologically meaningful if both the estimated value of ***r*** and the 95% bootstrap confidence interval around the value of ***r*** fell outside the 95% confidence interval around ***r*** = zero determined by permutation.

Results

The parameters used in phenotype calling, the resulting number of individuals and loci retained in my data matrices, and the level of peak-calling mismatches between repeated samples are presented in Table 5. Results and sample sizes of spatial autocorrelation analyses are shown in Figures 8-10 and Tables 6a-6c.

Both group-living species showed significant spatial structuring, with highest ***r*** values at distances of 0-1 m, the distance corresponding to individuals in the same host

web (***r*** = 0.085 for *A. miniaceus*, ***r*** = 0.068 for *A. kumadai*). In the *A. miniaceus* population, values of ***r*** are lower but still significant up to 50 m. In *A. kumadai* no values of ***r*** were significant at distances greater than 1 m (Figure 8, Tables 6a and 6b).

Figure 9 shows spatial autocorrelation results for adult and sub-adult females, adult and sub-adult males, and juveniles of *A. miniaceus*. In each age/sex class, results are similar: The highest values of ***r*** are found at 0-1 m. In sub-adult and adult females, the pattern seen at 25 and 50 m is the same as in the total population sample (observed ***r*** values are greater than the upper limit of r expected under the null hypothesis of no population structure), but the confidence interval around ***r*** at 25 m overlaps the upper limit of ***r*** expected under the null hypothesis of no population structure. In the sub-adult and adult males, values of ***r*** are not significant at 25 m. At 50 and 100 m values of r are larger but the confidence intervals around the ***r*** values overlap the upper limit of ***r*** expected under the null hypothesis of no population structure. In juveniles, no significant values of ***r*** are detected at distances greater than 1 m.

Figure 10 shows spatial autocorrelation results for adult and sub-adult females, adult and sub-adult males, and juveniles of *A. kumadai*. As was the case for the total population sample, the only significant values of ***r*** were at pairwise distances of 1 m or less — occupants of the same web.

In contrast to the group-living species, there was absolutely no signal of population structure in either of the solitary species (Figure 8, Table 6c).

Discussion

This study is the first to examine population structure in any argyrodine spider, and the first to compare population structure in solitary and group-living web kleptoparasites. The results of my DNA fingerprinting and spatial autocorrelation study showed clear genetic structure in the two group-living species, *A. miniaceus* and *A. kumadai*, associated with the population subdivision of the kleptoparasites in different host webs. This pattern was not observed in the two solitary species, *A. fasciatus* and *N. trigonum*.

The two species of solitary kleptoparasites, *A. fasciatus* and *N. trigonum,* utilize a variety of host webs. Typically only one, or occasionally two of these kleptoparasites are found in a single host's web. The lack of significant genetic structure in these populations is consistent with extensive dispersal — by immatures emerging form the egg-sac and/or by older individuals moving among host webs.

In both group-living species, pairwise phenotypic similarity was highest among kleptoparasites occupying the same host web, and declined more or less steeply with increasing distance among individuals. This indicates that the gangs of kleptoparasitic *A. miniaceus* or *A. kumadai* occupying a host web include at least some related individuals. However, there were also clear differences between the population structures of *A. miniaceus* and *A. kumadai*. In *A. miniaceus,* from the population I collected the samples, significant signals of relatedness were still detected at 50 m, while in *A. kumadai* the only significant values of the autocorrelation coefficient, ***r***, were for individuals occupying the same host web.

In *A. miniaceus*, there are clear differences in the spatial autocorrelation patterns of sub-adult and adult females on one hand, and juveniles (from hatchling to 3rd instar) on the other (Figure 9). Autocorrelation coefficients at 1 m are higher for females than juveniles, and the signal of relatedness for females persists to a point between 50 and 100 m, while the signal for juveniles drops to insignificant at some point between 1 and 25 m. For the second group-living species, *A. kumadai*, there were no differences in spatial structure of adults versus juveniles.

These observations are consistent with lab and field observations on the behavior of the two group-living species. *Argyrodes miniaceus* occupy the webs of *Nephila pilipes*; these host webs often last for more than two weeks and up to six weeks (see the natural history of *N. pilipes* in Robinson and Robinson 1973). In the lab populations, the development time from a female laying egg-sac to emerging of hatching is about five days, and from hatchling to adult can be as short as 10 days, implying that only one to two generations can be completed in a host web before the kleptoparasites need to relocate. In addition, the number of young produced per female can be large: each adult female can produce about 40 spiderlings per egg sac and two to three egg sacs in her lifespan. Even the forty to 120 offspring of one female are apparently too many for a single host web to support, as gangs that large are never found in natural populations, so the proportion of juveniles that disperse is expected to be large.

The genetic structure of *A. miniaceus* should be strongly influenced by the first individuals locating and occupying a host web. The rapid reproduction and quick maturation of offspring could allow offspring of the founder females to occupy the host web while it lasts. Among juveniles there is no signal of pair-wise similarity at distances

greater than 1 m, while positive signals of pair-wise similarity between females are detectable at 50 m. This leads us to hypothesize that juveniles disperse widely once they leave the natal web, but most do not successfully enter and mature in other occupied webs, while later dispersing females may successfully move to nearby webs. Further study of the dispersal and web invading behavior of juveniles, and replicate studies of other *A. miniaceus* populations are necessary to determine if this scenario is correct.

The autocorrelation coefficients of adult and juvenile *A. kumadai* in the same webs (0.073 and 0.078, respectively) are nearly identical; not significant values of ***r*** were found for any of the other pairwise distance classes examined. Field observations show that the life history of *A. kumadai* differs from that of *A. miniaceus*. The group-living *A. kumadai* specializes on three-dimensional webs, especially the webs of *Cyrtophora* spp.; these webs can last for up to three months. As a result, *A. kumadai* are very likely to complete multiple generations in a single host web. In contrast to *A. miniaceus*, in which the adults can participate in creep-up-and-share strategy (see Chapter 2) to steal food from the host, we did not observe any cooperative behavior by adults of *A. kumadai,* even though multiple adults occur in a single host web and exhibit mutual tolerance. The adult *A. kumadai* scavenge small insects captured in the tangle web area of the three-dimensional host webs. They rarely stay on the horizontal orb web where their host usually feeds. Only the juvenile *A. kumadai* have been observed to use the creep-up-and-share strategy, which means juveniles in a web feed together with their host while their host is feeding on a large prey. I hypothesize that the kleptoparasite "tenants" in a long-lasting host web consist of extended families, which occupy a web until the web is destroyed or relocated.

Because the community of the hosts is dynamic in the forest, the social groups of *A. miniaceus* have fundamental differences to other social spider systems. The main difference is the individuals in a host web could only complete very few life cycles in a host web. Because once the host web was destroyed or moved, the group in that web would either extinct or the members have to migrate. Therefore, completely loss of the ability to disperse is not an adaptive strategy for *A. miniaceus*.

I conclude that in group-living *Argyrodes*, group members are significantly more closely related than groups of individuals drawn randomly from the local population; this is in contrast to solitary Argyrodinae, which show no genetic structure at the spatial scales examined. The group-living life style was the cause of genetic structure in *A. miniaceus* and *A. kumadai*. These results, along with observations on reproductive and foraging behaviors of *A. miniaceus* (see Chapter 2), suggest that group members can gain inclusive fitness benefits by cooperating with group members, who are potentially kin. However, this hypothesis remains to be tested in the group-living *A. kumadai*, especially in their juvenile stage. The ability of group-living Argyrodinae to cooperate with both kin and non-kin conspecifics allows manipulation of the parameters both of mutualism and kinship. The future study should address if the kin groups have better performance in cooperation than non-kin groups.

Chapter 5: Host use and evolution of group-living behavior in a group of kleptoparasitic spiders: Molecular phylogeny of the Argyrodinae (Araneae: Theridiidae).

Abstract

Spiders in the subfamily Argyrodinae are known for their associations with other spiders. These associations include predation (araneophagy), web usurpation, and kleptoparasitism. Although the majority of the 238 described species are solitary, ~20 species live in groups in the webs of their hosts. I constructed a molecular phylogeny of argyrodine genera and species in order to investigate the evolution of web-invasion, araneophagy and kleptoparasitism, and the evolution of group-living behavior and its association with particular types of hosts. I investigated the phylogeny of 42 Asian and American species representing the six recognized genera of Argyrodinae, using partial sequences of six genes: mitochondrial cytochrome oxidase I (COI), NADH dehydrogenase subunit I (NDI), and 16S ribosomal RNA (16S) and nuclear 28 ribosomal RNA (28s), 18S ribosomal RNA (18S), and Histone 3 (H3). In total I used 3048 base pairs in the DNA sequence data matrix. I used likelihood methods to reconstruct the ancestral states of three behavioral characters: group-living behavior, specialization on large hosts, and kleptoparasitism. I tested for correlated evolution of group-living behavior and specialization on large hosts using reversible-jump Markov chain Monte Carlo methods. The molecular phylogenetic analyses support the monophyly of the Argyrodinae. Reconstruction of ancestral behavioral character states demonstrated strong support for a series of evolutionary transitions from araneophagy to kleptoparasitism, and then to group-living kleptoparasitism. I found that the evolution of group-living behavior is strongly correlated with specialization on the use of large hosts, which provide a larger food resource than smaller hosts.

Introduction

Spiders in the subfamily Argyrodinae are known for their associations with other spiders, though a few species spin their own webs and capture their own insect prey. These associations include predation on other spiders (araneophagy), web usurpation, and kleptoparasitism. Although the majority of the 238 described species (Platnick 2012) are solitary, ~20 species live in groups in the webs of their hosts.

In the Argyrodinae, the genera *Ariamnes* and *Neospintharus* use araneophagy as their main foraging strategy. *Rhomphaea* employs araneophagy and occasionally kleptoparasitism. Very little is known about the foraging strategies or habits of *Spheropistha* (Elgar 1993, Whitehouse et al. 2002). The genera *Argyrodes* and *Faiditus* use kleptoparasitism as their main foraging strategy.

Several authors have proposed scenarios for the origin of kleptoparasitic behavior in the Argyrodinae. Free-living behavior (typical of the vast majority of spiders) is considered the basal condition in the lineage. In one scenario, proposed by Agnarsson (2004), web invasion and kleptoparasitism is the next behavior to arise, followed by web usurpation (with or without eating the host), and finally araneophagy. Other authors have proposed the reverse; from a basal, free-living lineage, one or more lineages of araneophages arose, with skills to enter webs of other spiders and prey upon them. Finally, some araneophages gave rise to kleptoparasites that enter webs of larger spiders and steal food items, and even feed alongside the host. These hypotheses are discussed in Smith Trail (1980), Whitehouse et al. (2002) and Agnarsson (2004), but have never been tested in a phylogenetic comparative context because until now, robust genus and species level phylogenetic estimates have been unavailable for the Argyrodinae.

Interactions between host spiders and their kleptoparasitic Argyrodinae have drawn the most research attention and multiple species have been studied (Vollrath 1979, Smith Trail 1980, Tanaka 1984, Whitehouse 1988, Cangialosi 1990, Grostal and Walter 1997, Hénaut 2000, Miyashita et al. 2004, Baba et al. 2007). The few discussions of group-living behavior in Argyrodinae have tended to focus on proximate causes: factors that might predispose them to group-living and the ecological costs and benefits of the behavior. Whitehouse et al. (2002) described host-distracting behavior by groups of some argyrodine kleptoparasites, and suggested that group-forming behavior in Argyrodinae enhances the effectiveness of kleptoparasitism. A requirement of group-forming behavior is mutual tolerance; Whitehouse (2011) discussed three factors that could predispose these spiders to mutual tolerance: (a) a genetic predisposition to tolerance of conspecifics stemming from maternal care; (b) a benefit from group foraging; and (c) a trade-off between predation risk and occupancy of a food resource.

A genetic or phylogenetic predisposition to tolerance of conspecifics could originate in maternal care and the resulting prolonged association of juvenile siblings. This idea has been proposed by many authors, beginning with Kullmann (1972), to explain the origins of mutual tolerance and social behavior in spiders (see reviews in Buskirk 1981, D'Andrea 1987 , Aviles 1997, Bilde and Lubin 2001). All argyrodine species possess egg-guarding behavior, which is a form of maternal care. However, based on my observations of multiple species, the maternal care of Argyrodinae does not extend to the juvenile stage. Game-theory based models of group hunting or group foraging have been applied to vertebrate and invertebrate foragers (Packer and Ruttan 1988) and were discussed in more detail in Chapter 2.

The proposed trade-off between predation risk and occupancy of food resource suggests that an individual kleptoparasite might benefit by being the sole kleptoparasite in the host web or might at least benefit from having fewer conspecifics in the web, as this would increase its share of food resources in the host web. However the aggressive interactions needed to expel conspecifics from the web could make all the resident kleptoparasites more conspicuous to the host, and increase their probability of being killed or expelled.

Here, I address the evolution of group-living behavior from a phylogenetic perspective. The first question is quite basic: **is there a single origin of group-living behavior in the Argyrodinae or has this behavior evolved multiple times**? Other questions address the association of group-living behavior with other traits or characteristics. For example, if group-living is favored because it increases foraging success of argyrodine kleptoparasites, then **group-living behavior should arise in a species only after kleptoparasitism has become the major foraging strategy**.

Females of solitary argyrodines (at least those whose reproductive behavior is known) tend to place their egg-sacs away from their host webs. Nearly all group-living argyrodines occupy the webs of large orb-weaving hosts such as *Nephila* (Nephilidae), *Argiope* (Araneidae), or *Cyrtophora* (Araneidae), and the females deposit their egg-sacs in the large and long-lasting host webs (Su, personal observations), presumably ensuring the hatchlings will have access to food resources without engaging in dangerous dispersal activities. This requires use of hosts with webs that last long enough for the kleptoparasite's eggs to hatch and the spiderlings to emerge and feed; these will typically be large host species. **Accordingly, I hypothesize that there is a significant association**

between group-living behavior and the use of extremely large hosts, and that use of large hosts preceded the evolutionary origin of group-living.

These hypotheses or scenarios cannot be evaluated without a robust phylogeny of the Argyrodinae. This group has long been considered a particularly difficult group for phylogenetic studies, and as a result the taxonomy of the group has never been stable (see the discussions in Exline and Levi 1962, Yoshida 2001, Agnarsson 2004). Currently six genera are recognized: *Argyrodes, Ariamnes, Faiditus, Neospintharus, Rhomphaea*, and *Spheropistha*.

Simon (1893) described *Argyrodes* as a genus and treated the genera, *Argyrodes*, *Ariamnes*, and *Rhomphaea* as a tribe named Argyrodeae. Exline and Levi (1962) revised the New World species and lumped the species that were in *Argyrodes*, *Ariamnes*, and *Rhomphaea* into a single genus, *Argyrodes*. Within this genus, Exline and Levi (1962) recognized six species groups: *Argyrodes, Ariamnes, Cancellatus, Cordillera*, *Trigonum*, and *Rhomphaea*. Tanikawa (1998) added *Spheropistha* to the genus *Argyrodes.* This system was maintained until early 2000's although either morphologically or behaviorally the species in this genus are extremely diverse. In 2001, Yoshida elevated Exline and Levi's one genus system to a subfamily level. In this subfamily Argyrodinae, Yoshida (2001) retained the genus *Argyrodes* and resurrected *Ariamnes*, *Rhomphaea*, and *Spheropistha.* Agnarsson's (2004) morphological phylogeny of Theridiidae strongly supported the monophyly of the Argyrodinae. A molecular phylogeny of the Theridiidae showed a similar result (Arnedo et al. 2004). Agnarsson's 2004 morphological study also supported Yoshida's (2001) genera. Agnarsson (2004) also elevated the *Cancellatus* and

Cordillera species groups to a single genus, *Faiditus*, and elevated *Trigonum* species group to genus *Neospintharus*. This formed the current six genera system of Argyrodinae.

In this study, I estimated the first molecular phylogeny of argyrodine genera and species in order to investigate the evolution of araneophagy and kleptoparasitism, and the evolution of group-living behavior and its association with particular types of hosts. I sampled species of all six genera in Argyrodinae with emphasis on Southeast Asian species, mostly *Argyrodes*. This sampling strategy allowed me to test the monophyly of the subfamily and each genus, to examine the evolution of araneophagy and kleptoparasitism, and to investigate the evolutionary history of group-living behavior and its association with specialization on large hosts.

Material and methods

Taxon sampling – I included 42 species of Argyrodinae in the analyses: 25 species in *Argyrodes*, six species in *Faiditus*, five species in *Rhomphaea*, two species in *Neospintharus,* two in *Ariamnes,* and two in *Spheropistha*. I collected 37 of these species from 2006 to 2011, and I used DNA sequence data of five species from Arnedo et al. (2004). The list of species, collection sites and time of collection are presented in Table 7. Freshly collected specimens were preserved in 95% ethanol at – 20 °C until they were used in DNA extraction. The North American species were identified to species whenever possible according to Exline and Levi (1962). The Asian specimens were identified by comparison to the specimens in Raffles Museum for Biodiversity Research, Singapore and The Naturmuseum Senckenberg, Germany. If I could not identify a specimen to species, the closest species name was listed and indicated as confer, cf., in

Table 7. This indicates that they appear to be closely related to a named species. If a specimen could not be identified as being close to any named species, I identified them to genus and numbered the species according to the order in which they were collected.

Outgroups – For the outgroup selection for Argyrodinae, I chose closely related subfamilies in Theridiidae, following the morphological phylogeny of Agnarsson (2004) and the molecular phylogeny of Arnedo et al. (2004). The sister clade to Argyrodinae, the "*Anelosimus* + Theridiinae" clade, was used as the primary outgroup. From this clade I sampled *Anelosimus studiosus*, *An. eximius, An. rupununi*, *Kochiura rosea, Echinotheridion otlum*, *Theridion calcynatum*, *T. nigroannulatum, and Coleosoma acutiventer.* I also included *Enoplognatha caricis* in Pholcommatinae, *Spintharus flavidus* and *Moneta* sp. in Spintharinae, *Latrodectus mactans* in Latrodectinae, and *Dipoena hortoni* in Hadrotarsinae as outgroups to the "Argyrodinae+Anelosimus+ Theridiinae" clade. Sequence data for these outgroup taxa are taken from Arnedo et al. (2004) and Agnarsson et al. (2007). In addition, I sequenced *Coleosoma* cf. *blanden* as one of the outgroups.

DNA extraction – I extracted DNA from legs and cephalothorax of the specimens using Sigma-Aldrich DNA GeneElute kit (GN350, USA). The tissue was homogenized in lysis T buffer provided by the manufacturer and incubated at 55 °C for 24 hours. I then followed the commercial protocol for extraction of genomic DNA from each specimen. The DNA samples were stored at -20 °C until proceeding to the next step.

Molecular markers – I used three mitochondrial and three nuclear genes to reconstruct the molecular phylogeny of Argyrodinae. Partial sequences of mitochondrial cytochrome oxidase I (COI), NADH dehydrogenase subunit I (NDI), and 16S ribosomal RNA

(16S), and nuclear 28 ribosomal RNA (28s), 18S ribosomal RNA (18S), and Histone 3 (H3) were sequenced.

DNA amplification – The following primer combinations were used in polymerase chain reaction (PCR): LCO-J-1490 and HCO-N-2198 (Folmer et al. 1994) as preliminary primers for cytochrome oxidase I partial sequence and COI-F and COI-r as internal primers; LR-N-12945 (Hedin 1997) and N1-J-12581 for 16S and NDI partial sequence (Hedin and Maddison 2001); 18S-4F paired with 18S-9R (Giribet et al. 1996) for 18S; 28Sc and 28So (Whiting et al. 1997) for 28S; and H3aF and H3aR (Colgan et al. 1998) for H3. See Table 8 for the sequences of primers. DNA fragments were amplified by direct polymerase chain reaction.

The reactants were initially denatured for 3 min at 95 °C, followed by 30 cycles of 60 s at 95 °C, annealing 60 s at either 45 °C for 16S+NDI, 28S, H3, or 48 °C for COI and 18S, 60 s at 72 °C, and then a final extension at 72 °C for 10 min. PCR products were assayed by electrophoresis on a 1.0% agarose gel with TBE buffer and were visualized under UV light after ethidium bromide staining.

PCR products were prepared for sequencing using ExoSapit (USB) following manufacturer's instructions, and sequenced at the Idaho State University Core Molecular Research Facility and Genetic Sequencing Facility in Natural History Museum at the University of Kansas. The purified PCR products were sequenced using the BigDye terminator cycle sequencing kit and analyzed on an ABI 3100 or 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Sequence alignment – The DNA sequences and their chromatograms were first checked using the sequence editor, Geneious Pro 5.5.5 (Biomatters, USA). I assembled two

complementary strand sequences of each individual to check the accuracy of the DNA sequence data. The edited and assembled sequences were exported into a single file for multiple alignments. All sequences of COI, H3, NDI sequences were aligned automatically using ClustalW (Thompson et al. 1994) in Geneious Pro 5.5.5 (USA) with default gap opening/gap extension penalty ratio (15/5) because the alignments of these protein-coding sequences did not require gaps. For 16S, 18S, and 28S sequences, multiple alignments were carried out with varied gap opening/ gap extension penalties over a range including the ratio 8/2, 20/2, 40/2, and 90/3. The alignments were then optimized manually and converted to FASTA format.

Model test – I used jModelTest 1.0 (Guindon and Gascuel 2003, Posada 2008) to find the best model for each gene. This program uses maximum likelihood method to find the best-fit model for a gene. I used both Akaike information criterion (AIC) and Bayesian information criterion (BIC) to find the best-fit model. In the phylogenetic analyses, I used the model selected by BIC.

Phylogenetic tree reconstruction – Phylogenetic trees were reconstructed using parsimony method in PAUP*, *v.*4b10 (Swofford 2002), likelihood method in GARLI *v.*2.0 (Zwickl 2006), and Bayesian method in MrBayes *v.*3.1.2 (Ronquist and Huelsenbeck 2003). I performed these analyses for each gene separately and also for the combined data matrix of six genes.

Equally-weighted parsimony analyses were first performed with a heuristic search, and trees were obtained by random addition with 1000 replications and with tree-bisection-reconnection (TBR) branch swapping algorithm. In this analysis gaps were treated as missing data and the Maxtree setting was set at 50000. Only minimal-length

trees were saved. For bootstrap support, I performed 1000 replications with 10 iterations of random addition of taxa in each replication. I then used 50% majority rule to get the support of each node.

Maximum likelihood (ML) method was performed in GARLI *v.*2.0 (Zwickl 2006). I specified the model of sequence evolution by setting the ratematrix, nucleotide equilibrium state frequencies, alpha shape parameter, and proportion of invariable sites. For bootstrap support, I performed 500 replications in ML method. Fifty percent majority rule was utilized to evaluate the support for each node.

Bayesian analysis was performed in MrBayes 3.20 (Huelsenbeck and Ronquist 2001). I conducted this analysis for each gene as well as the combined sequence data matrix. The combined data matrix was partitioned and the best-fit model (obtained from jModeltest 1.0) of each gene was assigned. The analyses started with random tree and default prior in MrBayes 3.1.2 because there was no solid information of this phylogeny. The number of generations I ran was set depending on the preliminary test of each data matrix of a gene with two Markov chain Monte Carlo (MCMC) chains with sampling frequency of every 100 generations. I terminated analyses if the standard error difference between two chains close to 0.001 and determined the the appropriate number of burn-in generations using a test analysis before the formal analysis.

Comparative analyses – Two behavioral characters, group-living and specialization on large hosts, were coded according to data provided in Whitehouse (2011), Elgar (1993), and my field records of >700 webs of collected host-kleptoparasite specimens. I coded the absence of group-living behavior as "0" and the presence of group-living behavior as "1", for each species included in phylogenetic analyses. The second character,

specialization on large hosts (here I define large hosts as *Argiope*, *Cyrtophora*, and *Nephila*), was coded as "1"; others, i.e., generalists, specialists of small hosts, and free-living species, were coded as "0" (Table 9).

I coded a third character, foraging strategy, according to Elgar (1993). I coded species using both kleptoparasitism and predation on other spiders, or the species that only use predation (K&P) as "0", and the species using only kleptoparasitism (K) as "1" (Table 9). To visualize the character switching events, I reconstructed the ancestral states of these characters using the likelihood method, implemented in Mesquite 2.75 (Maddison and Maddison 2011).

The correlated evolution between two binary characters, group-living and specialization on large hosts, was tested using Pagel and Meade's Bayesian Analysis of Correlated Evolution of Discrete Characters (Pagel and Meade 2006). Pagel and Meade's method has two advatages compared to other methods. First, it takes the uncertainty of phylegenetic tree topologies into account using Bayesian Markov chain Monte Carlo method. The hypothesis of correlated evolution of characters is tested by summing over possible trees instead of using a "best" or a favored tree to trace the character state switching events of two characters (Pagel and Lutzoni 2002). Second, it chooses the best-fitting model of trait evolution using reversible-jump Markov chain Monte Carlo (RJ-MCMC, (Green 1995) method. This particular form of Bayesian analysis avoids selecting the best model by generating large numbersof pairwise tests of alternative models. Instead, the RJ-MCMC method explores the universe of possible models, and visits them in propotion to the posterior probabilities of different models (see Pagel and Meade 2006 for the application of this method to character evolution). Because of these two

advantages, Pagel and Meade's method simultaneously accounts for the uncertainty of phylogenetic tree topologies and uncertainty of character evolution to select best-fitting model among the possible models of correlated evolution of characters.

I compared the fit of the independent model (i.e., the evolution of the two charaters is not correlated) and the fit of dependent model (i.e., the evolution of these two characters is correlated) using Pagel and Meade's method. In the independent model, each character has two trasition rate parameters that accout for the transitions between two character states, 0 and 1, of one character. The trasition rate parameter is not dependent on the state of the other character, therefore there are four parameters in this model ($q_{\alpha 1}$, $q_{\alpha 2}$, $q_{\beta 1}$, and $q_{\beta 1}$, Figure 11a). The dependent model, or correlated model, has eight transiton parameters. They account for the transition rates when two states of a character depend on the two states of the other character. This results in four different combinations of states, i.e., {0,0}, {0,1}, {1,0}, and {1,1} and two transition rates between each pair of combinations of states (Figure 11b).

I used the RJ-MCMC method implemented in BayesTraits 1.0 (Pagel and Meade 2006) to test whether group-living in Argyrodinae is correlated with specialization on large hosts. To take phylognenetic uncertainty into account, I used the 42 argyrodine ingroup taxa to re-run the Bayesian trees in MrBayes 3.2 (Huelsenbeck and Ronquist 2001). I sampled a tree every 100,000 generations to avoid autocorrelation of tree topologies among sampled trees and had the chain stopped at 11,000,000 generations with burn-in value=10. This generated 100 trees from each run, thus 200 trees in total for the analyses of correlated evolution of characters. The trees were rooted and tree file

format was converted into the required nexus format for BayesTraits 1.0 using BayesTrees (Meade 2012).

In BayesTraits 1.0, I ran the analyses of correlated evolution of characters for 10,000,000 iterations with sampling frequence of 1,000 iterations for their log likelihood scores of the models. I excluded the first 2,500,000 (25% of total) iterations as burn-in. The harmonic means of log likelihood scores of each run were reported at the end of each analysis in BayesTraits 1.0. For a conservative interpretation, I ran analyes of the independent model and the dependent model five times each, and I took the smallest difference of log likelihood score between the independent and dependent models.

To interpret the diffenece between independent and dependent models, I calculated the Bayes factors (BF) (Kass and Raftery 1995) that was expressed on a logarithmic scale using the formula 2ln(BF) (Pagel and Meade 2006). That is, I took the difference of harmonic mean log likelihoods, which are the outputs of BayesTraits 1.0, of dependent and independent models, and multiplied it by two. According to Kass and Raftery's (1995) criteria, values between two to five on the logrithmic scale are positive evidence of correlated evolution of characters. BF values greater than five are strong evidence of correlation, and a value greater than 10 is very strong evidence. Finally, following the recommendations of Pagel and Meade (2006), I controlled the acceptance rate of iteration with 20 to 49 % using ratedev = five to 20 and I ran RJ-MCMC under exponential distribution.

Results

Sequences – I sampled 3048 bps of sequence data from six genes. The length of the gene fragment and preferred model of sequence evolution for each gene are shown in Table 10. I used the gap opening/ gap extension ratios of 90/3 for 16S, 18S, and 28S to minimize the number of gaps in the data matrices. All sequences will be deposited in GenBank upon the submission for publication of this research result. The numbers listed in Table 7 are the serial PCR reaction numbers in the Smith lab.

Phylogenetic trees – I conducted phylogenetic tree reconstructions using Bayesian, maximum likelihood, and parsimony methods for the data matrix of each gene and the combined sequence data matrix. A summary of tree statistics from three tree reconstruction methods is shown in Table 10.

The molecular phylogeny of Argyrodinae based on the combined data matrix of six genes is presented in Figure 12; Figure 13 shows the summary of the results of Bayesian analyses of each gene tree. All three phylogenetic methods indicate that Argyrodinae is a monophyletic clade (Bayesian posterior probability =1.00, bootstrap value of ML =100, and bootstrap value of MP =97). Four of the named genera — *Ariamnes*, *Neospintharus*, *Rhomphaea,* and *Spheropistha*—are each supported as monophyletic clades, while the other two—*Faiditus* and *Argyrodes*—are not. *Rhomphaea* + *Neospintharus* form a monophyletic clade which is sister to all the other genera. Within the sister clade to *Rhomphaea* + *Neospintharus*, *Ariamnes* is sister to all the other taxa. *Faiditus* species from the New World (*F. chickeringi*, *F.* cf. *chickeringi*, *F. amplifrons*, *F. cancellatus* and *F. globosus*) form a sister to the clade including *Argyrodes*, *Spheropistha* and the SE Asian species *F. xiphias* thus rendering the genus *Faiditus* as currently

constituted paraphyletic. My data suggest that New World *Faiditus* may form a monophyletic clade, but more extensive sampling of *Faiditus* species is required to confirm this. The specimens of *F. xiphias* collected from Singapore, Malaysia, Thailand, Taiwan, and a specimen similar to *F. xiphias* collected from China form a monophyletic clade, as do the two species of *Spheropistha*. However both of these clades are nested within *Argyrodes*.

There are multiple well-supported species groups within *Argyrodes* (including *F. xiphias* and *Spheropistha* clades) but the relationships among these groups are not resolved by my analyses of separate or combined data. The *A. elevatus* species group (see Figure 13), which includes three species from the New World and five species from Asia, is a monophyletic clade with strong statistical support. The *A. fissifrons* species group includes five species that specialize on hosts with three-dimensional webs. The *A. miniaceus* species group includes six species. The *A. nasutus* species group, which includes *F. xiphias*, contains most of the solitary species (Figures 12 and 13).

I visualized the reconstruction of ancestral states of the three focal behavioral characters. I included 19 group-living species and 22 solitary species in the analyses. Group-living behavior appeared to have seven character state switching events on the combined data Bayesian tree. Similarly, there are seven character state switching events for specialization on large hosts. The Bayes factors of five runs of correlated character evolution analyses ranged from 29.97 to 32.75 (Table 11). Even under the most conservative interpretation, using the smallest Bayes factor (29.97), the evolution of group-living behavior and specialization on large hosts are strongly correlated.

I observed a single origin of use of kleptoparasitism alone. Within this clade, there is a reversal from kleptoparasitism only to kleptoparasitism + predation on the branch of the *A. fissifrons* species group and a reversal event in *A. elevatus*. This analysis provides strong statistical support for the interpretation that group-living behavior and specialization on large hosts exclusively evolved after the foraging strategy switched from kleptoparasitism + predation to kleptoparasitism only.

Discussion

Although this is the first study of group-living behavior among kleptoparasitic spiders, many other examples of aggregations and group-living behavior are known among the spiders. Subsocial behavior, or maternal care, is widespread across the order Araneae. In more developed social systems, siblings may remain together and cooperate in some aspects of nest construction or prey capture until they attain sexual maturity and disperse, as seen in the orb-weavers *Parawixia bistriata* (Araneidae; Fowler and Gobbi 1988, Fernández Campón 2007) or the crab spider *Diaea ergandros* (Thomisidae; Evans 1999, Evans and Goodisman 2002). A mother and several cohorts of offspring may remain together, as in the huntsman *Delena cancerides* (Yip et al. 2009). Many orb-weavers (Araneidae, Nephilidae, Tetragnathidae and Uloboridae), and cellar spiders (Pholcidae) are known to form aggregations of prey capture webs joined by support lines; individuals in these groups are territorial on their webs, and generally do not show any cooperative behaviors aside from mutual tolerance of conspecifics in close proximity. This behavior is variously called communal (Smith Trail 1980, Buskirk 1981, Smith 1982), colonial (Uetz and Hieber 1997), or territorial social behavior (Aviles 1997).

Several authors have suggested that these social systems originate from aggregations of individuals at particularly rich resource patches (Kullmann 1972, Uetz and Hieber 1997).

The rarest, but perhaps best studied spider social behavior is variously known as quasisocial (Brach 1977), cooperative (Smith and Hagen 1996), permanent non-territorial (Aviles 1997) or simply social behavior (Lubin and Bilde 2007). Cooperative spiders are characterized by cooperative web-construction and prey capture, sharing of prey, female-biased sex-ratios, small clutch size, and cooperative or at least indiscriminate care of the colony's young by females. The breeding and dispersal patterns are particularly unusual: juvenile dispersal is suppressed, and mating typically takes place among colony mates, leading to high levels of genetic similarity among colony mates and low levels of genetic diversity in the species (Roeloffs and Riechert 1988, Smith and Engel 1994, Smith and Hagen 1996, Smith et al. 2009). Any dispersal out of the colony usually occurs post-mating. Many authors have proposed that cooperative species arose from ancestors with prolonged maternal care, in which the dangerous juvenile dispersal phase was completely eliminated (Aviles 1997, Agnarsson et al. 2006, Bilde and Lubin 2011).

Fewer than 40 of the 40,000 named spider species are currently known to exhibit cooperative behavior, yet it has evolved independently at least 18 times (Aviles 1997, Bilde and Lubin 2011) including eight to nine independent origins in the family Theridiidae (Agnarsson et al. 2006), five in the theridiid genus *Anelosimus* (Agnarsson et al. 2007), and three independent origins in the eresid genus *Stegodyphus* (Johannesen et al. 2007). These, along with general observations on the taxonomic placement of other cooperative species, show that the sister taxa of cooperative spiders are generally subsocial, not cooperative species. This stands in sharp contrast to the situation in most

social insects: a social species, particularly a eusocial species such as an ant, termite, honey bee, bumble bee or stingless bee, is usually embedded in a large clade of social or eusocial species (Wilson 1975).

These observations highlight two related puzzles about cooperative behavior in spiders: why is cooperative behavior in spiders so rare if it has evolved so many times? Why has evolution of cooperation not produced any radiations of cooperative species? There are fundamentally two explanations for the rarity of cooperative behavior and the absence of large radiations of cooperative species: either speciation rate is low or extinction rate is high in cooperative spider species.

Agnarsson et al. (2006) and Johannsen et al. (2007) argued that because cooperative species have strongly inbred populations and low genetic variation, their niche is relatively narrow. This could make speciation more difficult, or extinction more likely. Agnarsson et al. (2006), based on phylogenetic analyses of the genus *Anelosimus* (Theridiidae), favor the hypothesis that cooperative spiders are subject to rapid extinction. They argued that cooperative sociality in spiders is a transient condition resulting from repeated origin and rapid extinction of social species. They described this process as a balance between the short-term benefits of sociality and the long-term costs of inbreeding.

Johannsen et al. (2007) carried out a phylogenetic analysis of the genus *Stegodyphus* (Eresidae), which includes three independent origins of cooperative behavior. Their analysis suggested that each of the three cooperative social species was quite old relative to the other species in the genus. Their study favors the hypothesis that

speciation rates of cooperative species are lower than those of non-cooperative species (assuming they have not diversified into unrecognized cryptic species).

My observations on the behavior, ecology and genetics of the group-living argyrodines (Chapters 2-4 of this work) indicate that their behavior is unique, but most similar to cooperative behavior. Like cooperative spiders, multiple adults as well as juveniles of the group-living kleptoparasites coexist in a single web, but in this case it is the web of the host, not a web cooperatively built by group members (Chapter 3, this work). Like the cooperative spiders, group-living kleptoparasites share prey, though it is captured by the host, not by the group members (Chapter 2, this work). And like the cooperative spiders, the kleptoparasites sharing a web are more closely related than a random assemblage of spiders drawn from the local population (Chapter 4, this work). However, unlike the cooperative species, maternal care consists only of egg-sac guarding behavior and does not extend to care of the juveniles (Su, unpublished observations). Also unlike cooperative spiders, juvenile dispersal does not appear to be suppressed, genetic differentiation among social groups is not as extreme as in the cooperative spiders (Chapter 4), and there is no evidence to indicate that the primary sex-ratio is significantly different from 1:1.

The results of my studies on the group-living argyrodines are important for three reasons: First, the group-living kleptoparasitic argyrodines, whose behavior, ecology and genetics are described in more detail in the Introduction and Chapters 2-4 of this work, present a fundamentally different type of cooperative sociality in spiders and another system for testing hypotheses about the evolution and ecology of sociality. Second, the phylogenetic study shows a pattern different from that observed in the *Anelosimus* and

Stegodyphus (see below); thus the social Argyrodinae may help solve the two puzzles of spider cooperative behavior. And finally, my results resolve the sequence in which araneophagic and kleptoparasitic behavior evolved in the Argyrodinae.

The phylogeny obtained in this study shows a minimum of three origins of group-living kleptoparasitism in Argyrodinae (Figure 14): a minimum of two origins in *Argyrodes* + *Spheropistha* lineage and a minimum of one in the New World *Faiditus.* Because my analysis does not include all (or even a majority) of the species in Argyrodinae, I must be cautious in interpreting my results. Even so, my analysis shows multiple examples of speciation in group-living lineages, particularly in the genus *Argyrodes*. *Argyrodes*, with 93 currently valid species (Platnick 2012), is my best-sampled genus; I include 12 named species (13% of the total), plus 10 new species (*Argyrodes* sp. 1-10) and 3 probable new species morphologically close to named species.

The distribution of group-living behavior on the phylogenetic tree of Argyrodinae is different from other social spiders in that there are more group-living species in the *A. elevatus*, *A. miniaceus*, and *A. fissifrons* species groups than there are solitary species. This suggested that once a lineage has evolved group-living behavior, subsequent diversification within these clades occurs frequently.

My correlated character analysis demonstrates that the origin of evolution of group-living behavior in the kleptoparasitic argyrodines is strongly correlated with specialization on large hosts (Table 11). This indicates that evolution of group-living behavior in the argyrodines is linked to specialization on large resource patches, that is,

on large host webs, rather than an extension of maternal care, as is seen in cooperative species.

I suggest that specialization on large hosts provided access to large quantities of insect prey and allowed coexistence of multiple kleptoparasites in a web. However the mode of feeding employed by group-living kleptoparasites, in which they creep up and share prey directly with the host, may require specialization on a single host or a small number of host species with similar web structure and similar behavior. Thus specialization on different host species could interrupt gene flow between different groups of specialists and lead to speciation. The placement of egg-sacs directly in long-lasting and predictable webs of large hosts could be considered a form of maternal care that extends to the juvenile stage, so I cannot exclude a possible association between group-living behavior and maternal care. However, at this time I cannot pursue this line of investigation due to insufficient observations on maternal care and placement of egg-sacs deposition behavior, especially for solitary species.

My field observations and lab studies of group-living kleptoparasites raise the possibility that aspects of group-living behavior may differ among the various lineages of in which it appears. For example, in *A. miniaceus* adult group members conduct a foraging behavior that I term creep-up-and-share: multiple adults approach the host while it is feeding on a wrapped prey item, and feed simultaneously. In doing so, group members gain more foraging benefit than solitary individuals (see Chapter 2). However, *A. kumadai* and *A. fissifrons,* members of a different group-living lineage, perform this behavior only in the first and second instar stages, not as adults. I observed the group-living behavior of *A. nephilae* in the lab and found they rarely use the creep-up and-share

strategy. Mostly, they scavenge small insects and food debris that are ignored by the host, which implies that their type of group-living may simply be an aggregation of independently-acting individuals, thus they are similar to the communal societies of orb-weavers and cellar spiders. Thus, though I coded all these behaviors as group-living in the comparative analyses, the form of group-living behavior in different species groups may be different.

Another persistent question in the biology of the Argyrodinae is the sequence of evolutionary steps leading to araneophagy and kleptoparasitism. Whitehouse et al. (2002) summarized four possible pathways to kleptoparasitism and araneophagy in Argyrodinae. The first model is no phylogenetic relationship between araneophagy and kleptoparasitism, second model is araneophagy has evolved from kleptoparasitism, the third model is kleptoparasitism has evolved from araneophagy (supported by Smith Trail 1980), and the fourth model is both araneophagy and kleptoparasitism have evolved from a common ancestor (supported by Whitehouse unpublished data). My data support Smith's (1980) hypothesis, or model 3 in Whitehouse et al. (2002), that kleptoparasitism in the Argyrodinae has evolved from araneophagy. This hypothesis suggests that the web-invading behavior of araneophagic species pre-adapted Argyrodinae to sense the activities of the host. Species derived from the araneophagic ancestors then became specialized to use kleptoparasitism as their main foraging strategy. My data show that the araneophagic genera *Neospintharus* and *Rhomphaea* are "basal" to the other clades. *Ariamnes*, which includes free-living araneophagic species, is "basal" to the clade containing *Argyrodes*, *Spheropistha*, and *Faiditus,* in which kleptoparasitism, and then group-living kleptoparasitic behavior arose (Figure 14). Therefore, I conclude that the

evolutionary pathway of web invading behaviors in Argyrodinae is araneophagy to kleptoparasitism, and then group-living kleptoparasitism.

Finally, my results also suggest that the generic and species level taxonomy of the Argyrodinae should be revised. *Spheropistha*, which contains four named species and one new unnamed species, should be included in the genus *Argyrodes*. The Southeast Asian species *Faiditus xiphias* should be transferred to *Argyrodes* as well. From the species I collected, I identified 11 taxa that are unknown to science. However, more extensive taxon sampling is needed before a revision of the Argyrodinae can be completed. In general, more solitary species (which are much harder to collect) will be necessary and more sampling is needed particularly in the New World, Africa and India.

Chapter 6 Conclusions

This is the first detailed study of group-living behavior in kleptoparasitic Argyrodinae, and the first molecular phylogenetic analysis of the Argyrodinae (Araneae: Theridiidae). In this study I did: (1) Field observations for about 30 species of Argyrodinae mainly in SE Asia and America. (2) Field measurements of dispersion of five group-living species and two solitary species, and group size for the group-living species. (3) Laboratory manipulations of group size and the effect of group size on foraging success. (4) Genetic analysis of genetic similarity among group-members, and population structure of both solitary and group-living species. (5) Molecular phylogenetics of Argyrodinae and comparative analyses of group-living behavior in this subfamily. The combined results of these different approaches make it possible to address several questions in the biology of the Argyrodinae.

Is group-living in the argyrodines a form of cooperative behavior, or are the groups simply collections of competitors exploiting large resource patches? Many earlier studies of group-living argyrodines showed that size of the host web is a good predictor of size of the resident kleptoparasite group (Cangialosi 1990c, Grostal and Walter 1997, Tso and Severinghaus 2000, Miyashita 2002, Agnarsson 2003, Koh and Li 2003, Hénaut et al. 2005, Kerr 2005, Agnarsson 2010). These authors assumed that individuals these groups were simply competitors, and that group size was determined by size of the resource patch; thus group sizes should thus conform to an ideal free distribution model. The work presented in Chapter 3 on the relationship between the size of the host web and the size of kleptoparasite groups showed that the distribution of group sizes deviates from the expectations of the ideal free distribution. Specifically,

kleptoparasites over use small webs based on size of the resource alone. Or to put it another way, considering the number of host webs and their sizes, the kleptoparasites could have spread out a bit more and faced less competition on resource. This suggests that group membership provides indirect or direct fitness benefits that make it possible for webs to support more kleptoparasites than predicted in poorer resource patch. I propose that one possible benefit of group life is that it enables these kleptoparasites to extract more resources from prey than a solitary kleptoparasites can.

To determine if the kleptoparasite's group-living behavior is a form of cooperation, it is first necessary to define cooperation. I used the definition of West (2007): "a behavior which provides a benefit to another individual (the recipient), and which is selected for because of its beneficial effect on the recipient." In the case of Argyrodinae, one behavior that fits definition of cooperation is mutual tolerance, a trait emphasized by early researchers on spider sociality (Krafft 1982, Darchen and Delagedarchen 1986). While most argyrodine species do not tolerate the presence of conspecifics in their host web (with the exception of mates or young juveniles) group-living argyrodines share the host web with adults of both sexes as well as juveniles, often in large numbers (up to ~40 individuals in a web), even though this means food resources will be shared among many individuals. The behavior of group-living *A. miniaceus* fulfills the requirements of this definition of cooperation. Laboratory and field observations showed that only a small number of kleptoparasites (2-4 in the case of *A. mineaceus*) feed with the host at any one time no matter how large the group is; refraining from feeding and allowing others to feed first may reduce disturbance to the host, allowing a longer feeding session for all members.

The results of laboratory studies in which the size of *A. mineaceus* groups was artificially manipulated (see Chapter 2) and analyses of relatedness among group members (Chapter 4) showed that both direct fitness benefits from reciprocal altruism and inclusive fitness benefits from cooperating with related individuals may be involved in the origin and maintenance of group kleptoparasitism. A comparison of time spent feeding per individual in groups ranging in size from one to seven unrelated *A. mineaceus*, showed that individuals in groups were more successful (spent more time feeding) than solitary individuals. The analyses of DNA fingerprint data from two group-living species, *A. mineaceus* and *A. kumadai*, showed that group members are significantly more similar than randomly assembled groups. Thus in nature, group members are likely to be cooperating with relatives, adding an inclusive fitness component to the benefits of group-living. .

Many questions about the biology of the Argyrodinae can only be answered in the context of phylogeny. The phylogenetic analysis in Chapter 5, based on three mitochondrial and three nuclear genes, is the first phylogenetic analysis of this subfamily based on molecular data. This analysis included species from all six currently recognized genera of the subfamily Argyrodinae, but the majority of species included are from the genus *Argyrodes*. With 93 currently recognized species, *Argyrodes* is the largest genus in the subfamily, and includes the largest number of species known to have group-living behavior. The results of this phylogenetic analysis support the broad outlines of argyrodine systematics: Argyrodinae is a monophyletic lineage, and our results are consistent with monophyly of the genera *Neospintharus*, *Rhomphaea*, and *Ariamnes* (few species are sampled in from these genera). The genus *Faiditus* is paraphyletic; at least

one species currently placed in *Faiditus* is grouped within Argyrodes. The small genus *Spheropistha*, which currently includes four named species, is also grouped within *Argyrodes*. *Argyrodes* as currently conceived is paraphyletic, but could be made monophyletic by transferring *F. xiphias* and *Spheropistha* to *Argyrodes*. The relationships among species within *Argyrodes* are not resolved although several species groups are strongly supported.

Group-living kleptoparasitic behavior appears in species currently placed in the genera *Argyrodes, Faiditus*, and *Spheropistha*. **Has group-living behavior evolved once in the Argyrodinae, once each in *Argyrodes*, *Faiditus* and *Spheropistha,* or multiple times in each genus?** The phylogenetic analyses in Chapter 5 showed several independent origins of group-living behavior in the Argyrodinae, including a minimum of two origins in *Argyrodes* + *Spheropistha* and a minimum of one origin in *Faiditus*. The comparative analyses indicated that specialization on large hosts is correlated with the evolution of group-living behavior.

The phylogeny of group-living kleptoparasites differs in one striking way from the phylogenies of two well-studied genera with cooperatively social species: *Stegodyphus* (Eresidae) and *Anelosimus* (Theridiidae). In each of these two genera there are multiple cooperative species, but each appears to have evolved cooperative behavior independently; there is no evidence of radiations of group-living species. In contrast, the phylogeny of the Argyrodinae shows strong evidence of speciation in group-living lineages.

The group-living kleptoparasitic Argyrodinae are found in the webs of large, orb-weaving hosts in the families Nephilidae and Araneidae. A test of correlated evolution showed that group-living and specialization on large hosts are strongly correlated.

The phylogenetic analysis also can be used to address a long-standing question concerning the evolution of foraging strategies in Argyrodinae: **Did kleptoparasites evolve from araneophagic ancestors, did araneophages evolve from kleptoparasitic ancestors, or did kleptoparasites and araneophages each evolve independently from free-living ancestors?** The results of this analysis clearly favor the origin of kleptoparasites from araneophagic ancestors.

Future directions

These four research chapters should not be considered the end of the argyrodine story. Instead, each chapter is the initial step in a line of research that can be greatly expanded.

1. Future phylogenetic research.

The phylogeny presented in this thesis did not resolve the relationships among species groups in *Argyrodes,* and the sampling is largely biased to Southeast Asian species. To produce a more comprehensive phylogeny and to further investigate the evolution of social behavior in Argyrodinae, additional taxon sampling is needed, particularly from Africa and South America. This will require field observations of the species' hosts and foraging behavior. This is necessary for studying the evolution of kleptoparasitism and group-living because a species natural history is often unclear from the literature, especially for solitary Argyrodinae. In addition, there are undoubtedly

many new species still to be discovered; of the 42 species of Argyrodinae included in this study, 10 appear to be unnamed.

2. Comparative behavioral ecology.

The results of studies on group size, foraging behavior and relatedness in *A. miniaceus* indicate that their group-living behavior is a form of cooperative sociality. Both reciprocity and inclusive fitness appear to play a role in the maintenance of group kleptoparasitism in *A. miniaceus*, and these factors are likely to be important in other species of group-living Argyrodinae. However, the form of group-living and the relative importance of kin selection and reciprocity may differ among species. One avenue for future research is comparative behavioral ecology of group-living Argyrodinae. This work would expand my approach of behavioral study to different solitary and group-living species, ideally covering all argyrodine genera and species groups, and including species from different continents. This is surely challenging, but potentially very rewarding. Because group-living behavior has apparently evolved independently several times in the Argyrodinae, I can explore these groups for differences in behavior and ecology, and determine whether cooperation is a common feature of all the group-living kleptoparasites.

3. A new model system for evolution of cooperation.

This newly discovered social behavior is worthy of attention from sociobiologists as a new model system, because it allows direct manipulation of kinship and reciprocity

in one system. This provides the opportunity to test theoretical models of cooperation empirically.

A. Effect of kinship on cooperation in groups. In the laboratory studies of foraging behavior in *A.* miniaceus, group size was varied from 1 to 7, but relatedness among individuals in a group was held (more or less) constant, by using wild-caught individuals collected from different host webs. The next step in this line of research is to manipulate relatedness among individuals in a foraging group. Because these spiders can be bred and reared in the laboratory, and because they have very short generation times, it will be feasible to assembly groups of full siblings, or different ratios of siblings and non-siblings, to test the effect of relatedness on cooperative behavior. For example, future studies should address whether sibling groups have better foraging performance in cooperation than non-kin groups.

B. Behavioral interactions among siblings. Argyrodinae is sister to a clade containing other cooperatively social Theridiidae with highly developed maternal care; no direct maternal care of juveniles has been observed among Argyrodinae, though too few species have been observed in enough detail to say with certainty maternal care does not exist. However, cooperative interactions among siblings and other cohorts of young are also important in the evolution of cooperative behavior. Behaviors such as mutual tolerance, cooperative prey capture and prey sharing observed among hatchlings are retained in cooperatively social species (see review in Bilde and Lubin 2011). Thus social interactions in sibling groups should be investigated in both solitary and group-living species, to see if antecedents to cooperative behavior are present, and to see if the immature spiders discriminate between siblings and non-siblings.

C. Role of cheaters. By using game theory based approach, I have shown that the benefits of cooperative foraging are greater than the benefits of solitary foraging in *A. miniaceus*. In future research, the possible role of cheaters in the group kleptoparasitism system should be addressed. Cheaters would be individuals that reap benefits of group life but do not reciprocate in the expected way. One form of cheating behavior observed in *A. miniaceus* conducting the creep-up-and-feed strategy is for an individual to stay feeding on the prey item without taking turns with other group members. Two individuals performed this behavior, and both were eaten by the host. By manipulating features of the kleptoparasitic group, such as number of individuals, relatedness of group members, even species composition of groups (creating, for example, a group composed of one individual of a solitary species and several individuals of a group-living species) and features of the environment (prey size, prey number, frequency of feeding) it may be possible to detect factors that permit or suppress cheating.

D. Communication among group members. The mechanisms of communication among group members are not clear. In field observations from Southeast Asian species, I rarely found different Argyrodinae species in the same web even if they occur in the same forest. The mechanisms for recognizing and responding to conspecifics and kin are not known. From the studies of host-kleptoparasite interaction in Argyrodinae, vibration (Vollrath 1979) and chemotactile (Suter et al. 1989) information can be used by the host or kleptoparasite to detect each other's presence or activities. The communication among members of kleptoparasitic group may use the same means. Future research should approach this question through studies of the physiology of signaling among group

members, and behavioral assays to test the kleptoparasites abilities to distinguish conspecifics from non-conspecifics, and siblings from non-siblings.

References

Abrahams, M. V. 1986. Patch choice under perceptual constraints: a cause for departures from an ideal free distribution. Behavioral Ecology and Sociobiology **19**:409-415.

Agnarsson, I. 2003. Spider webs as habitat patches: the distribution of kleptoparasites (*Argyrodes*, Theridiidae) among host webs (*Nephila*, Tetragnathidae). Journal of Arachnology **31**:344-349.

Agnarsson, I. 2004. Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). Zoological Journal of the Linnean Society **141**:447-626.

Agnarsson, I. 2010. Habitat patch size and isolation as predictors of occupancy and number of argyrodine spider kleptoparasites in *Nephila* webs. Naturwissenschaften **38**:1-5.

Agnarsson, I., L. Aviles, J. A. Coddington, and W. P. Maddison. 2006. Sociality in Theridiid spiders: Repeated origins of an evolutionary dead end. Evolution **60**:2342-2351.

Agnarsson, I., W. P. Maddison, and L. Aviles. 2007. The phylogeny of the social *Anelosimus* spiders (Araneae : Theridiidae) inferred from six molecular loci and morphology. Molecular Phylogenetics and Evolution **43**:833-851.

Arnedo, M. A., J. Coddington, I. Agnarsson, and R. G. Gillespie. 2004. From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. Molecular Phylogenetics and Evolution **31**:225-245.

Amos, B., C. Schlotterer, and D. Tautz. 1993. Social structure of pilot whales revealed by analytical DNA profiling. Science **260**:670.

Aviles, L. 1997. Causes and consequences of cooperation and permanent-sociality in spiders. Pages 476-498 *in* J. C. Choe, B. J. Crespi, editor. The evolution of social behavior in insects and arachnids. Cambridge University Press.

Baba, Y. G., R. J. Walters, and T. Miyashita. 2007. Host-dependent differences in prey acquisition between populations of a kleptoparasitic spider *Argyrodes kumadai* (Araneae: Theridiidae). Ecological Entomology **32**:38-44.

Begon, M., J. L. Harper, and C. R. Townsend. 1996. Ecology: individuals, populations, and communities. Wiley-Blackwell.

Bilde, T. and Y. Lubin. 2001. Kin recognition and cannibalism in a subsocial spider. Journal of Evolutionary Biology **14**:959-966.

Bilde, T. and Y. Lubin. 2011. Group living in spiders: cooperative breeding and coloniality. Pages 275-298 *in* M. E. Herberstein, editor. Spider behavior: Flexibility and versatility. Cambridge University Press, Cambridge.

Bilde, T., Y. Lubin, D. Smith, J. M. Schneider, and A. A. Maklakov. 2005. The transition to social inbred mating systems in spiders: role of inbreeding tolerance in a subsocial predecessor. Evolution **59**:160-174.

Brach, V. 1977. *Anelosimus studiosus* (Araneae: Theridiidae) and the evolution of quasisociality in theridiid spiders. Evolution:154-161.

Buskirk, R. 1981. Sociality in the Arachnida. Pages 281-367 *in* H. R. Hermann, editor. Social insects. New York: Academic Press.

Cameron, A. C. and P. K. Trivedi. 1998. Regression analysis of count data. Cambridge Univ Pr.

Cangialosi, K. 1990a. Life cycle and behavior of the kleptoparasitic spider, *Argyrodes ululans* (Araneae, Theridiidae). Journal of Arachnology **18**:347-358.

Cangialosi, K. R. 1990b. Kleptoparasitism in colonies of the social spider *Anelosimus eximius* (Araneae, Theridiidae). Acta Zoologica Fennica **190**:51-54.

Cangialosi, K. R. 1990c. Social spider defense against kleptoparasitism. Behavioral Ecology and Sociobiology **27**:49-54.

Cangialosi, K. R. 1997. Foraging versatility and the influence of host availability in *Argyrodes trigonum* (Araneae, Theridiidae). Journal of Arachnology **25**:182-193.

Cobbold, S. M. and Y. C. Su. 2010. The host becomes dinner: possible use of Cyclosa as a nuptial gift by Argyrodes in a colonial web. Journal of Arachnology **38**:132-134.

Colgan, D., A. McLauchlan, G. Edgecombe, J. Macaranas, G. Cassis, M. Gray, S. Livingston, and G. Wilson. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology **46**:419-437.

D'Andrea, M. 1987. Social behaviour in spiders (Arachnida Araneae). Ital. J. Zool. N.S. Monogr. **3**:1-156.

Darchen, R. and B. Delagedarchen. 1986. Societies of spiders compared to the societies of insects. Journal of Arachnology **14**:227-238.

Eberhard, W. G. 1979. *Argyrodes attenuatus* (Theridiidae): a web that is not a snare. Pshyche **86**:407-413.

Elgar, M. 1993. Inter-specific associations involving spiders: kleptoparasitism, mimicry and mutualism. Memoirs of the Queensland Museum **33**: 411-430.

Elgar, M. A. 1989. Kleptoparasitism: a cost of aggregating for an orb-weaving spider. Animal behaviour **37**:1052-1055.

Enders, F. 1975. Effects of prey capture, web destruction and habitat physiognomy on web-site tenacity of *Argiope* spiders (Araneidae). Journal of Arachnology **3**:75-82.

Evans, T. A. 1999. Kin recognition in a social spider. Proceedings of the Royal Society of London. Series B: Biological Sciences **266**:287-292.

Evans, T. A. and M. A. D. Goodisman. 2002. Nestmate relatedness and population genetic structure of the Australian social crab spider *Diaea ergandros* (Araneae: Thomisidae). Molecular Ecology **11**:2307-2316.

Exline, H. and H. Levi. 1962. American spiders of the genus *Argyrodes* (Araneae, Theridiidae). Bulletin of the Museum of Comparative Zoology. **127**:75-202.

Fletcher, J. A. and M. Doebeli. 2010. Assortment is a more fundamental explanation for the evolution of altruism than inclusive fitness or multilevel selection: reply to Bijma and Aanen. Proceedings of the Royal Society B: Biological Sciences **277**:677-678.

Fretwell, S. D. and H. L. Lucas. 1970. On territorial behavior and other factors influencing habitat distribution in birds. Acta biotheoretica **19**:16-36.

Fernández Campón, F. 2007. Group foraging in the colonial spider *Parawixia bistriata* (Araneidae): effect of resource levels and prey size. Animal behaviour **74**:1551-1562.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3**:294-299.

Fowler, H. and N. Gobbi. 1988. Cooperative prey capture by an orb-web spider. Naturwissenschaften **75**:208-209.

Giraldeau, L. A. and T. Caraco. 2000. Social foraging theory. Princeton Univ Pr.

Giribet, G., S. Carranza, J. Baguna, M. Riutort, and C. Ribera. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Molecular Biology and Evolution **13**:76-84.

Green, P. J. 1995. Reversible jump Markov chain Monte Carlo computation and Bayesian model determination. Biometrika **82**:711-732.

Greenacre, M. J. 2007 Correspondence analysis in practice. Chapman & Hall.

Grostal, P. and D. E. Walter. 1997. Kleptoparasites or commensals? Effects of *Argyrodes antipodianus* (Araneae: Theridiidae) on *Nephila plumipes* (Araneae: Tetragnathidae). Oecologia **111**:570-574.

Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology **52**:696-704.

Hamilton, W. D. 1964. Genetical evolution of social behaviour I. Journal of Theoretical Biology **7**:1-16.

Hass, C. C. and D. Valenzuela. 2002. Anti-predator benefits of group living in white-nosed coatis (*Nasua narica*). Behavioral Ecology and Sociobiology **51**:570-578.

Hedin, M. 1997. Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: Nesticus): inferences from geographic-based sampling. Evolution **51**:1929-1945.

Hedin, M. and W. Maddison. 2001. A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). Molecular Phylogenetics and Evolution **18**:386-403.

Hénaut, Y. 2000. Host selection by a kleptoparasitic spider. Journal of Natural History **34**:747-753.

Hénaut, Y., J. Delme, L. Legal, and T. Williams. 2005. Host selection by a kleptobiotic spider. Naturwissenschaften **92**:95-99.

Herberstein, M. E. and I. M. Tso. 2000. Evaluation of formulae to estimate the capture area and mesh height of orb webs (Araneoidea, Araneae). Journal of Arachnology **28**:180-184.

Higgins, L. E. 1995. Direct evidence for trade-offs between foraging and growth in a juvenile spider. Journal of Arachnology **23**:37-43.

Huff, D., R. Peakall, and P. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. TAG Theoretical and Applied Genetics **86**:927-934.

Johannesen, J., Y. Lubin, D. R. Smith, T. Bilde, and J. M. Schneider. 2007. The age and evolution of sociality in *Stegodyphus* spiders: a molecular phylogenetic perspective. Proceedings of the Royal Society B: Biological Sciences **274**:231-237.

Kao, C. 2008. Host selection of red abdomen *Argyrodes* (*Argyrodes miniaceus*). In *Department of Ecology and Evolutionary Biology*, vol. Master, pp. 63. Taipei: National Taiwan University.

Kass R.E., and A.E. Raftery. 1995. Bayes factors. Journal of the American Statistical Association **90**:773-795.

Kennedy M. and R.D. Gray. 1993. Can ecological theory predict the distribution of foraging animals? A critical analysis of experiments on the ideal free distribution. Oikos **68**:158-166.

Kerr, A. M. 2005. Behavior of web-invading spiders *Argyrodes argentatus* (Theridiidae) in *Argiope appensa* (Araneidae) host webs in Guam. Journal of Arachnology **33**:1-6.

Kerr, A. M. and A. S. Quenga. 2004. Population variation of web-invading spiders (Theridiidae: *Argyrodes* spp.) on host webs in Guam, Mariana Islands, Micronesia. Journal of Natural History **38**:671-680.

Kim, K. W., B. Krafft, and J. C. Choe. 2005. Cooperative prey capture by young subsocial spiders. Behavioral Ecology and Sociobiology **59**:92-100.

Koh, T. H. and D. Li. 2003. State dependent prey type preferences of a kleptoparasitic spider *Argyrodes flavescens* (Araneae: Theridiidae). Journal of Zoology **260**:227-233.

Korona, R. 1990. Travel costs and ideal free distribution of ovipositing female flour beetles, Tribolium confusum. Animal behaviour **40**:186-187.

Krafft, B. 1982. The significance and complexity of communication in spiders. Pages 15–66 *in* P. N. a. R. Witt, J. S., editor. Spider Communication: Mechanisms and Ecological Significance. Princeton University Press, Princeton, NJ.

Kullmann, E. J. 1972. Evolution of social behavior in spiders (Araneae; Eresidae and Theridiidae). American Zoologist **12**:419-426.

Larcher, S. F. and D. H. Wise. 1985. Experimental studies of the interactions between a web-invading spider and two host species. Journal of Arachnology:43-59.

Ludwig, J. A. and J. F. Reynolds. 1988. Statistical ecology: a primer on methods and computing. Wiley-Interscience.

Lubin, Y. and T. Bilde. 2007. The evolution of sociality in spiders. Advances in the Study of Behavior **37**:83-145.

Maddison, W. P. and D. R. Maddison. 2011. Mesquite: A modular system for evolutionary analysis. Version 2.75.

Meade, A. 2012. BayesTrees 1.3. http://www.evolution.reading.ac.uk/BayesTrees.html.

Milinski, M. 1979. An Evolutionarily Stable Feeding Strategy in Sticklebacks1. Zeitschrift für Tierpsychologie **51**:36-40.

Miyashita, T. 2001. Competition for a limited space in kleptoparasitic *Argyrodes* spiders revealed by field experiments. Population ecology **43**:97-103.

Miyashita, T. 2002. Population dynamics of two species of kleptoparasitic spiders under different host availabilities. Journal of Arachnology **30**:31-38.

Miyashita, T., Y. Maezono, and A. Shimazaki. 2004. Silk feeding as an alternative foraging tactic in a kleptoparasitic spider under seasonally changing environments. Journal of Zoology **262**:225-229.

Morris, D. W., P. Lundberg, and J. Ripa. 2001. Hamilton's rule confronts ideal free habitat selection. Proceedings of the Royal Society of London. Series B: Biological Sciences **268**:921-924.

Nowak, M. A. 2006. Five Rules for the Evolution of Cooperation. Science **314**:1560-1563.

Packer, C. and L. Ruttan. 1988. The evolution of cooperative hunting. American Naturalist **132**:159-198.

Pagel, M. and F. Lutzoni. 2002. Accounting for phylogenetic uncertainty in comparative studies of evolution and adaptation. Biological evolution and statistical physics **585**:148-161.

Pagel, M. and A. Meade. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. American Naturalist **167**:808-825.

Paquin, P. and N. Dupérré. 2001. On the Distribution and Phenology of *Argyrodes fictilium* (Araneae, Theridiidae) at Its Northern Limit of North America. Journal of Arachnology **29**:238-243.

Peakall, R., M. Ruibal, and D. B. Lindenmayer. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution **57**:1182-1195.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Platnick, N. 2012. The world spider catalog, version 12.5 American Museum of Natural History. http://research.amnh.org/iz/spiders/catalog/ .

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution **25**:1253-1256.

Robinson, M. H. and B. Robinson. 1973. Ecology and behavior of the giant wood spider *Nephila maculata* (Fabricius) in New Guinea. Simthisonian Contributions to Zoology **149:**1-84.

Roeloffs, R. and S. E. Riechert. 1988. Dispersal and population-genetic structure of the cooperative spider, *Agelena consociata*, in west African rainforest. Evolution **42**:173-183.

Ronquist, F. and J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**:1572.

Ruttan, L. M. 1991. Effects of maternal presence on the growth and survival of subsocial spiderlings (Araneae: Theridiidae). Journal of Insect Behavior **4**:251-256.

Rypstra, A. L. 1982. Building a better insect trap; an experimental investigation of prey capture in a variety of spider webs. Oecologia **52**:31-36.

Rypstra, A. L. 1985. Aggregations of *Nephila clavipes* (L.)(Araneae, Araneidae) in relation to prey availability. Journal of Arachnology **13**:71-78.

Salomon, M. and Y. Lubin. 2007. Cooperative breeding increases reproductive success in the social spider *Stegodyphus dumicola* (Araneae, Eresidae). Behavioral Ecology and Sociobiology **61**:1743-1750.

Scheel, D. and C. Packer. 1991. Group hunting behaviour of lions: a search for cooperation. Animal behaviour **41**:697-709.

Simon, E. 1893. Histoire naturelle das araignées, Paris **1**: 257-488.

Smith, D. 1982. Reproductive success of solitary and communal *Philoponella oweni* (Araneae: Uloboridae). Behavioral Ecology and Sociobiology **11**:149-154.

Smith Trail, D. 1980. Predation by Argyrodes (Theridiidae) on solitary and communal spiders. Psyche **87**:349-356.

Smith, D. and R. Hagen. 1996. Population structure and interdemic selection in the cooperative spider *Anelosimus eximius*. Journal of Evolutionary Biology **9**:589-608.

Smith, D., S. Van Rijn, J. Henschel, T. BILDE, and Y. Lubin. 2009. Amplified fragment length polymorphism fingerprints support limited gene flow among social spider populations. Biological Journal of the Linnean Society **97**:235-246.

Smith, D. R. and M. S. Engel. 1994. Population structure in an Indian cooperative spider, *Stegodyphus sarasinorum* Karsch (Eresidae). Journal of Arachnology **22**:108-113.

Suter, R. B., C. M. Shane, and A. J. Hirscheimer. 1989. Spider vs. spider: *Frontinella pyramitela* detects *Argyrodes trigonum* via cuticular chemicals. Journal of Arachnology **17**:237-240.

Sutherland, W. J. 1983. Aggregation and theideal free distribution. The Journal of Animal Ecology **52**:821-828.

Swofford, D. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tanaka, K. 1984. Rate of predation by a kleptoparasitic spider, *Argyrodes fissifrons*, upon a large host spider, *Agelena limbata*. Journal of Arachnology **12**:363-367.

Thornhill, R. 1980. Sexual selection within mating swarms of the lovebug, *Plecia nearctica* (Diptera: Bibionidae). Animal behaviour **28**:405-412.

Tregenza, T. 1994. Common Misconceptions in Applying the Ideal Free Distribution. Animal behaviour **47**:485-487.

Trivers, R. L. 1971. Evolution of reciprocal altruism. Quarterly Review of Biology **46**:35-57.

Tanaka, K. 1984. Rate of predation by a kleptoparasitic spider, *Argyrodes fissifrons*, upon a large host spider, *Agelena limbata*. Journal of Arachnology **12**:363-367.

Tanikawa, A. 1998. The new synonymy of the spider genus *Argyrodes* (Araneae: Theridiidae) and a description of a new species from Japan. Acta Arachnologica **47**:21-26.

Thompson, J., D. Higgins, and T. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research **22**:4673.

Trizzino, M., P. Audisio, G. Antonini, A. De Biase, and E. Mancini. 2009. Comparative analysis of sequences and secondary structures of the rRNA internal transcribed spacer 2 (ITS2) in pollen beetles of the subfamily Meligethinae (Coleoptera, Nitidulidae): potential use of slippage-derived sequences in molecular systematics. Molecular Phylogenetics and Evolution **51**:215-226.

Tso, I. M. and L. L. Severinghaus. 1998. Silk stealing by *Argyrodes lanyuensis* (Araneae: Theridiidae): a unique form of kleptoparasitism. Animal behaviour **56**:219-225.

Tso, I. M. and L. L. Severinghaus. 2000. *Argyrodes fissifrons* inhabiting webs of Cyrtophora hosts: Prey size distribution and population characteristics. Zoological Studies **39**:236-242.

Uetz, G. and C. Hieber. 1997. Colonial web-building spiders: balancing the costs and benefits of group-living. *Pages* 458-475 *in* J. C. Choe, B. J. Crespi, editor. The evolution of social behavior in insects and arachnids. Cambridge University Press.

van der Wurff, A. W. G., Y. L. Chan, N. M. van Straaien, and J. Schouten. 2000. TE-AFLP: combining rapidity and robustness in DNA fingerprinting. Nucleic Acids Research **28**. e105.

Vollrath, F. 1979. Behaviour of the kleptoparasitic spider *Argyrodes elevatus* (Araneae, Theridiidae). Animal behaviour **27**:515-521.

Vollrath, F. 1984. Kleptobiotic interactions in invertebrates. *Pages* 61-94 *in* C. J. Barnard editor. Producers and Scavengers: strategies of exploitation and parasitism. Chapman and Hall, New York.

West, S. A., A. S. Griffin, and A. Gardner. 2007. Evolutionary explanations for cooperation. Current Biology **17**:R661-R672.

Whitehouse, M. 1988. Factors influencing specificity and choice of host in *Argyrodes antipodiana* (Theridiidae, Araneae). Journal of Arachnology **16**:349-355.

Whitehouse, M. 1991. To mate or fight? Male-male competition and alternative mating strategies in *Argyrodes antipodiana* (Theridiidae, Araneae). Behavioural processes **23**:163-172.

Whitehouse, M. 1997. The benefits of stealing from a predator: foraging rates, predation risk, and intraspecific aggression in the kleptoparasitic spider *Argyrodes antipodiana*. Behavioral Ecology **8**, 663-667.

Whitehouse, M. 2011. Kleptoparasitic spiders of the subfamily Argyrodinae: a special case of behavioural plasticity.*in* M. E. Herberstein, editor. Spider Behaviour: Flexibility and Versatility. Cambridge Univ Pr.

Whitehouse, M. E. A. 1986. The foraging behaviours of *Argyrodes antipodiana* (Theridiidae), a kleptoparasitic spider from New Zealand. New Zealand Journal of Zoology **13**:151-168.

Whitehouse, M. E. A. and R. R. Jackson. 1993. Group structure and time budgets of *Argyrodes antipodiana* (Araneae, Theridiidae), a kleptoparasitic spider from New Zealand. New Zealand Journal of Zoology **20**:201-206.

Whitehouse, M. E. A. and R. R. Jackson. 1998. Predatory behaviour and parental care in *Argyrodes flavipes*, a social spider from Queensland. Journal of Zoology **244**:95-105.

Whitehouse, M., I. Agnarsson, T. Miyashita, D. Smith, K. Cangialosi, T. Masumoto, D. Li, and Y. Henaut. 2002. *Argyrodes*: Phylogeny, Sociality and Interspecific Interactions: A Report on the *Argyrodes* Symposium, Badplaas 2001. Journal of Arachnology **30**:238-245.

Whiting, M., J. Carpenter, Q. Wheeler, and W. Wheeler. 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Systematic Biology **46**:1-68.

Whitlock, R., H. Hipperson, M. Mannarelli, R. Butlin, and T. Burke. 2008. An objective, rapid and reproducible method for scoring AFLP peak height data that minimizes genotyping error. Molecular Ecology Resources **8**:725-735.

Wiehle, H. 1928. Beitrage zur Biologie der Araneen. Zeitschrift fur Morphologie und Okologie der Tiere **11**:115-151.

Wise, D. H. 1982. Predation by a commensal spider, *Argyrodes trigonum*, upon its host: an experimental study. Journal of Arachnology **10**:111-116.

Wilson, E. O. 1975. Sociobiology : the new synthesis Cambridge, Mass. : Belknap Press of Harvard University Press.

Yip, E., S. Clarke, and L. Rayor. 2009. Aliens among us: nestmate recognition in the social huntsman spider, *Delena cancerides*. Insectes Sociaux **56**:223-231.

Yoshida, H. 2001. The genus *Rhomphaea* (Araneae: Theridiidae) from Japan, with notes on the subfamily Argyrodinae. Acta Arachnologica **50**:183-192.

Yoshida, H., I. M. Tso, and L. L. Severinghaus. 1998. Description of a new species of the genus *Argyrodes* (Araneae: Theridiidae) from Orchid Island, Taiwan, with notes on its ecology and behavior. Acta Arachnologica **47**:1-6.

Zwickl, D. 2006. GARLI: genetic algorithm for rapid likelihood inference. http://www.nescent.org/informatics/download.php?software_id=4.

TABLES

Table 1. The payoffs for four combinations of group members. H is the foraging successful probability. V is the prey volume, n is the number of individuals in a group, C is the cost of being attacked by host, and S is the searching effort.

	Cooperator	Non-cooperator
Cooperator	$H_n {}^{V}/_{n} - C_n - S_n$	$H_1 V - C_1 - S_1$
Non-cooperator	$H_1 V - C_1 - S_1$	$H_1 V - C_1 - S_1$

Table 2. Known group-living species in *Argyrodes* and *Faiditus* (Theridiidae: Argyrodinae). The species list and distribution information come from Platnick (2012). Currently 12/93 species in *Argyrodes* and 5/56 species in *Faiditus* are known to be group-living.

Species	Major host(s)	Geographic area	Group	References
Argyrodes antipodianus	*Araneus, Nephila*	Australia*, New Caledonia, New Zealand	>10	(Elgar 1989)
Argyrodes argentatus	*Nephila, Argiope*	China, East Indies, Hawaii, Singapore*	9	(Kerr and Quenga 2004)
Argyrodes bonadea	*Nephila*, *Cyrtophora*	China, Korea, Taiwan, Japan, Thailand*	15	(Miyashita 2002)
Argyrodes elevatus	*Nephila, Argiope*	USA* to Argentina, Galapagos Is.	20	(Vollrath 1979)
Argyrodes flavescens	*Nephila, Argiope*	Sri Lanka to Korea, Japan, Singapore*	30	(Koh and Li 2003)
Argyrodes flavipes	Non-kleptoparasitic	Queensland	10	(Whitehouse and Jackson 1998)
Argyrodes fissifrons	*Cyrtophora, Agelena*	Sri Lanka to China, Taiwan*, Australia	>20	Su, field obs.
Argyrodes incursus	*Achaearanea*	New South Wales, Lord Howe Is.	5	(Elgar 1993)
Argyrodes kumadai	*Cyrtophora*	Japan, Taiwan	6-8	(Baba et al. 2007)
Argyrodes lanyuensis	*Nephila*	Taiwan*, Philippines*	10	Su, field obs.
Argyrodes miniaceus	*Nephila*	Japan, Philippines*, Taiwan* to Australia	<46	Su, field obs.
Argyrodes nephilae	*Nephila*	USA*, West Indies to Argentina, Galapagos Is.	"Several"	(Exline and Levi 1962)
Faiditus atopus	*Nephila*	Panama to Ecuador	5	(Elgar 1993)
Faiditus cancellatus	*Araneus, Nephila*	USA*, Canada, Bahamas	"Several"	(Exline and Levi 1962)
Faiditus caudatus	*Nephila*	USA, West Indies to Argentina	"Several"	Vollrath 1979
Faiditus dracus	*Nephila*	USA to Paraguay	5	(Elgar 1993)
Faiditus globosus	*Nephila*	USA* to Ecuador	"Several"	(Exline and Levi 1962)
Faiditus ululans	*Anelosimus*	Mexico to Brazil	>10	(Cangialosi 1990b)

* indicate the geographic areas that the authors observed the group sizes or did the surveys.

Table 3. Poisson regression of the variables that predict number of kleptoparasites in host web. The host related predictors, including web area, tangle web length, host species, distance to nearest neighboring host (NN-H), and distance to nearest host web with kleptoparasites (NN-K). The environmental predictors are light intensity (Lux), humidity (H %), temperature (T°C), and the distance from host web to ground (D to ground). The estimated Poisson regression coefficients (estimate), Wald x^2 values, and p values indicate the significance of predictor effect (* indicate $p<0.05$). Deviance is the ratio of mean and variance of response variable, which indicates the fit of Poisson distribution.

	----------Host related predictors----------					----------Environmental predictors----------			
	Web Area	Tangle web length	Host species	NN-H	NN-K	Lux	H%	T°C	D to ground
Group-living species									
Argyrodes fissifrons	37 webs, # of kleptoparasites = 111, Mean group size = 3.3 ± 2.6							Host*: Cyrtophora* sp.	
	Negative binomial distribution, Deviance/*DF* =1.37, *DF*=29								
Estimate	**0.0003**			-0.0543	-0.0296	-0.0002	0.0060	-0.0914	-0.0021
Wald x^2	**3.91**	a	b	0.07	0.03	0.11	0.01	0.04	0.62
p	**0.0479***			0.7899	0.8716	0.7356	0.9356	0.8445	0.4303
Argyrodes flavescens	26 communal webs, # of kleptoparasites = 97, Mean group size = 6.1 ± 5.4							Host*: Nephila antipodiana*	
	Negative binomial distribution, Deviance/*DF*=1.43, *DF*=18								
Estimate	0.0003	-0.0057		0.0801	-0.0853	-0.0006	0.0461	0.4849	-0.0119
Wald x^2	1.16	1.71	b	1.86	3.00	0.22	0.07	0.38	0.88
p	0.2807	0.1911		0.1729	0.834	0.6412	0.7984	0.5373	0.3480
Argyrodes kumadai	32 webs, # of kleptoparasites = 96, Mean group size = 4.2 ± 5.8							Host*: Cyrtophora moluccensis*	
	Negative binomial distribution, Deviance=1.57, *DF*=21								
Estimate	**0.0157**	-0.0090		0.0170	0.0046	0.0001	-0.0138	-0.0159	**-0.0117**
Wald x^2	**7.14**	0.32	b	0.44	0.08	2.53	0.03	0.01	**11.95**
p	**0.0075***	0.5699		0.5050	0.7787	0.1115	0.8702	0.9291	**0.0005***
Argyrodes lanyuensis	39 webs, # of kleptoparasites = 30, Mean group size = 2.5 ± 3.7							Hosts: *Nephila*, *Cyrtophora*, *Achaearanea*	
	Poisson distribution, Deviance= 1.19, *DF*=29								
Estimate	**0.0007**	0.0140	0.8139	0.1195	-0.0152	-0.0028	**-0.2877**	-0.9911	0.0017
Wald x^2	**10.51**	0.86	1.99	0.55	0.08	2.42	**4.82**	1.73	0.19
p	**0.0012***	0.3524	0.1589	0.4579	0.7721	0.1198	**0.0281***	0.1882	0.6606
Argyrodes miniaceus	66 webs, # of kleptoparasites = 215, Mean group size = 5.4 ± 6.2							Host: *Nephila pilipes*	
	Negative binomial distribution, Deviance=1.2233, *DF*=58								
Estimate	**0.0002**			-0.0111	-0.0463	0.0000	-0.0114	0.1004	-0.0014
Wald x^2	**23.09**	a	b	0.05	2.03	0.63	0.07	0.15	0.26
p	**<.0001***			0.8221	0.1539	0.4266	0.7870	0.7031	0.6112

Table 3 (Continue)

Solitary species									
Argyrodes fasciatus		19 communal webs[c], # of kleptoparasites = 20, Mean group size = 1.0 ± 0.4						Host: *Psechrus* sp.	
		Poisson distribution, Deviance =0.56, *DF*=9							
Estimate	-0.0004		0.9332	0.0693	-0.0311	-0.0009	-0.421	-4.2824	**0.0206**
Wald x^2	1.13	a	1.40	2.09	2.05	2.26	3.70	3.24	**5.14**
p	0.2875		0.2370	0.1483	0.1524	0.1326	0.0544	0.072	**0.0233***
Neospintharus trigonum		51 webs, # of kleptoparasites = 40, Mean group size = 1.7 ± 0.9						Host: Agelenopsis, *Argiope*, *Araneus*, *Micrathena*, theridiid	
		Distribution: NB, Deviance= 1.27, *DF*=41							
Estimate	-0.0002	0.0432	0.1859	-0.0192	0.0056	0.0000	0.10002	**0.2243**	-0.0038
Wald x^2	0.09	2.37	1.97	0.77	0.34	0.08	3.51	**3.97**	0.71
p	0.7680	0.1237	0.1606	0.3790	0.5604	0.7760	0.0610	**0.0464***	0.3994

a – No tangle web present in this species; b – all spiders used the same host species; [c] – there are 41 individual webs in the population of *A. fasciatus*

Table 4. Summary of the Poisson regression results of the variables that predict number of kleptoparasites in host web. + indicates significant positive predictor, - indicates significant negative predictor, and NS indicates non-significant predictor.

	----------Host related predictors----------					----------Environmental predictors----------			
Parameter:	Web Area	Tangle web length	Host species	NN-H	NN-K	Lux	H%	T°C	D to ground
Group-living species									
Argyrodes fissifrons	+	a	b	NS	NS	NS	NS	NS	NS
Argyrodes flavescens	NS	NS	b	NS	NS	NS	NS	NS	NS
Argyrodes kumadai	+	NS	b	NS	NS	NS	NS	NS	−
Argyrodes lanyuensis	+	NS	NS	NS	NS	NS	−	NS	NS
Argyrodes miniaceus	+	a	b	NS	NS	NS	NS	NS	NS
Solitary species									
Argyrodes fasciatus	NS	a	NS	NS	NS	NS	NS	NS	−
Neospintharus trigonum	NS	NS	NS	NS	NS	NS	NS	−	NS

a – No tangle web present in this species
b – all spiders used the same host species

Table 5. Results of mismatch rate test of repeatability between the original and repeated TE-AFLP data. The locus selection and phenotype calling followed the procedure of Whitlock et al. (2008).

Species	*A. miniaceus*	*A. kumadai*	*A. fasciatus*	*N. trigonum*
Mismatch rate (%)	7.12	3.43	7.64	1.53
Locus-selection threshold (rfu)	800	20	300	120
Phenotype calling threshold (rfu)	100	200	100	100
Normalized mean peak height (rfu)	538.00	191.52	360.92	235.76
Retained loci	60	147	131	208
Retained individuals/individuals sampled	153/156	92/96	30/31	39/40

Table 6a. Results of spatial autocorrelation analysis for the group-living argyrodine species *Argyrodes miniaceus* (153 individuals). The distance classes are 0 to 1 m, >1 to 25 m, >25 to 50 m, >50 to 100 m, etc. **n** = the number of pairwise comparisons in each distance class, ***r*** = mean relatedness index calculated for each distance class, and **Ur error** and **Ul error** = upper and lower error bars that span the 95% confidence interval about ***r*** as determined by bootstrap resampling. Analyses were carried out for the total sample, and for Females (sub-adult and adults), males (sub-adult and adults), and juveniles (which are difficult to sex).

Distance Class (m)	**0-1**	**25**	**50**	**100**	**200**	**400**
Total population						
n	966	2110	1386	200	3276	2010
r	0.085	0.019	0.033	0.038	-0.035	-0.001
Ur error	0.011	0.006	0.008	0.019	0.005	0.007
Lr error	0.016	0.008	0.010	0.026	0.005	0.007
Females						
n	87	186	121	15	360	192
r	0.158	0.029	0.044	0.034	-0.059	-0.022
Ur error	0.043	0.025	0.021	0.052	0.014	0.031
Lr error	0.048	0.028	0.023	0.081	0.016	0.030
Males						
n	56	107	100	30	168	193
r	0.180	0.009	0.046	0.079	-0.031	-0.023
Ur error	0.046	0.027	0.020	0.041	0.024	0.018
Lr error	0.055	0.029	0.027	0.048	0.020	0.022
Juveniles						
n	162	426	235	18	592	250
r	0.079	0.002	0.003	-0.029	-0.019	0.019
Ur error	0.027	0.013	0.016	0.052	0.010	0.015
Lr error	0.027	0.014	0.022	0.048	0.011	0.023

Table 6b. Results of spatial autocorrelation analysis for the group-living argyrodine species *Argyrodes kumadai* (91 individuals). The distance classes are 0 to 1 m, >1 to 25 m, >25 to 50 m, >50 to 100 m, etc. **n** = the number of pairwise comparisons in each distance class, ***r*** = mean relatedness index calculated for each distance class, and **Ur error** and **Ul error** = upper and lower error bars that span the 95% confidence interval about ***r*** as determined by bootstrap resampling. Separate analyses were carried out for the total sample, and for adults and juveniles. We did not analyze males and females separately to avoid small sample sizes in each distance class.

Distance Class (m)	**1**	**10**	**50**	**100**	**200**
Total					
n	444	232	965	216	183
r	0.068	0.007	-0.023	0.000	0.013
Ur error	0.009	0.012	0.006	0.012	0.016
Lr error	0.010	0.013	0.007	0.013	0.016
Adults					
n	80	28	122	35	60
r	0.073	0.010	-0.030	0.021	0.025
Ur error	0.028	0.039	0.022	0.037	0.026
Lr error	0.023	0.039	0.021	0.035	0.026
Juveniles					
n	138	108	393	76	34
r	0.078	-0.004	-0.024	-0.005	0.020
Ur error	0.014	0.019	0.009	0.014	0.043
Lr error	0.020	0.020	0.007	0.023	0.040

Table 6c. Results of spatial autocorrelation analysis for the solitary argyrodine kleptoparasites *Argyrodes fasciatus* (30 individuals) and *Neospintharus trigonum* (39 individuals). The distance classes are 0 to 1 m, >1 to 25 m, >25 to 50 m, >50 to 100 m, etc. **n** = the number of pairwise comparisons in each distance class, ***r*** = mean relatedness index calculated for each distance class, and **Ur error** and **Ul error** = upper and lower error bars that span the 95% confidence interval about ***r*** as determined by bootstrap resampling.

A. fasciatus					
Distance Class (m)	**10**	**50**	**100**	**150**	
n	115	78	114	128	
r	0.016	0.013	-0.013	-0.012	
Ur error	0.017	0.022	0.018	0.018	
Lr error	0.019	0.018	0.016	0.016	
N. trigonum					
Distance Class (m)	**10**	**50**	**100**	**150**	**200**
n	72	114	366	100	49
r	0.008	0.001	-0.006	0.011	-0.002
Ur error	0.020	0.014	0.009	0.016	0.022
Lr error	0.020	0.014	0.007	0.016	0.020

Table 7. The specimens, collection data, source of outgroups used in the phylogenetic analyses. Collection numbers are the numbers of the voucher specimens in Smith lab. The sequence numbers are the PCR numbers in Smith lab and will be converted into accession number after submission to GenBank. The most of the outgroups are from Arnedo et al. (2004) and Agnarsson et al. (2008) that were downloaded from GenBank.

Species	Country	Locality	Latitude/ longitude	Collection number	COI	16s	NDI	18s	28s	H3
Argyrodinae										
Argyrodes cf. *amboinensis*	Singapore	Pulau Ubin Is.	N 1°24'43.92" E 103°58'26.64"	SU587-2	r2040			r2095	r2098	r2092
Argyrodes cf. *amboinensis*	Singapore	Pulau Ubin Is.	N 1°24'5.60" E 103°58'13.00"	SU565	r2033	r1615	r1615	r1601	r1587	r1629
Argyrodes argentatus	USA	Hawaii		Arnedo et al. 2004	AY231032	AY203957		AY230900	AY231090	AY230992
Argyrodes bonadea	China	Wuhan	N 30°28'59.99" E 114°21'10.11"	SU531	r2036	r1613	r1613		r1585	r1627
Argyrodes bonadea	China	Sichuan	N 30°25'3.00" E 103°24'56.10"	SU530	r2047	r1612	r1612	r1598	r1584	r1626
Argyrodes cylindratus	Philippines	Samar	N 11°38'8.60" E 125°2'56.70"	SU162	r2051			r119	r1589	R52
Argyrodes elevatus	Colombia	S. Vincente de Chucuri		SU604	r2038	r109	r109	r123	r69	r56
Argyrodes fasciatus	Singapore	Pulau Ubin Is.	N 1°24'43.92" E 103°58'26.64"	SU587-1	r2039	r2099	r2099	r2094	r2097	r2091
Argyrodes fasciatus	Singapore	Pulau Ubin Is.	N 1°24'43.92" E 103°58'26.64"	SU593	r2048	r1616	r1616	r1602	r1588	r1630
Argyrodes fissifrons	Taiwan	Orchid Is.	N 22°0'34.60" E 121°34'15.10"	SU184	r1748				r57	r44
Argyrodes flavescens	Singapore	Labrador Park	N 1°15'55.32" E 103°48'13.02"	SU549	r2042	r1614	r1614	r1600	r1586	r1628
Argyrodes flavescens	Thailand	Ay Kos		SU613	r2065	r1922	r1922		r1952	r1982
Argyrodes kumadai	Taiwan	Lienhwachi	N 23°54'51.10" E 120°53'17.30"	SU605	r2043			r1604	r1590	r1632
Argyrodes lanyuensis	Taiwan	Orchid Is.	N 22°00'34.63" E 121°34'15.13"	A1	r2044	r110	r110	r124	r2096	r2093
Argyrodes miniaceus	Taiwan	Huoyenshan	N 24°21'51.00" E 120°44'20.00"	SU431	r2045	r100	r100		r60	r47
Argyrodes cf. *nasutus*	Philippines	Mindanao	N 7°1'8.10" E 122°1'54.50"	SU396	r2050	r104	r104	r118		r51
Argyrodes cf. *nasutus*	Malaysia	Gambak		SU622	r2067	r1924	r1924	r1894		r1984
Argyrodes nephilae	Peru	Madre de Dios	S 12°34'15.42" W 70°6'6.67"	SU730	r2080	r1937	r1937	r1907	r1967	r1997
Argyrodes nephilae	USA	Tampa, FL		SU731	r2081	r1938	r1938			r1998
Argyrodes nephilae	USA	Tampa, FL		SU731	r2082	r1939	r1939		r1969	r1999
Argyrodes nephilae	USA	Ft. Lauderdale, FL	N 26°09'14.18" W 80°07'48.40"	SU260	r2046	r99	r99		r59	r46
Argyrodes pluto	USA	Lawrence, KS	N 38°56'58.20" W 95°16' 7.30"	SU611	r2064	r1921	r1921	r1891	r1951	r1981
Argyrodes sp1	Thailand	Kungreban		SU606		r1619	r1619	r1605	r1591	r1633
Argyrodes sp2	Japan	Ryukyu Is.		SU692	r2072	r1929	r1929	r1899	r1959	r1989
Argyrodes sp2	Philippines	Bicol	N 13°39'47.50" E 123°19'56.60"	SU50	r2035	r103	r103		r63	r50
Argyrodes sp3	Philippines	Luzon	N 16°09'19.20" E 120°54'6.70"	SU296	r2034	r101	r101		r61	r48
Argyrodes sp4	Japan	Ryukyu Is.		SU693	r2073	r1930	r1930		r1960	r1990
Argyrodes sp5	Kenya	Nakuru Park		Smith		r108	r108			R55
Argyrodes sp6	Sri Lanka	Colombo		SU691	r2071	r1928	r1928	r1898	r1958	r1988
Argyrodes sp7	Thailand	Pohu Rua		SU717	r2075	r1932	r1932	r1902	r1962	r1992
Argyrodes sp8	Thailand	Phu Rua	N 16°35'28.10" E 100°57'37.70"	SU718	r2076	r1933	r1933	r1903	r1963	r1993
Argyrodes sp10	China	Yunnan	N 20°55'48.72" E 101°15'16.98"	SU529	r2057	r1611	r1611		r1583	r1625
Argyrodes tripunctatus	Philippines	Mindoro	N 13°30'20.10" E 120°54'43.50"	SU334	r2052			r120		R53
Ariamnes attenuatus	Guyana	S of Gunns Landing		Arnedo et al. 2004	AY231033	AY230946		AY230901	AY231078	AY230993
Ariamnes cylindrogaster	Taiwan	Huoyenshan	N 24°21'51.00" E 120°44'20.00"	SU601	r2054	r1617	r1617			r1631
Faiditus amplifrons	Peru	Madre de Dios	S 12°34'15.42" W 70°6'6.67"	SU729	r2079	r1936	r1936			r1996
Faiditus cancellatus	USA	Lawrence, KS	N 38°56'58.20" W 95°16'7.30"	SU610		r1920	r1920		r1950	r1980
Faiditus cf. *chickeringi*	USA	Dallas, TX		SU607	r1578	r1620	r1620	r1606	r1592	r1634
Faiditus chickeringi	Guyana	S of Gunns Landing		Arnedo et al. 2004	AY231043			AY230912	AY231081	AY231002
Faiditus globosus	USA	Ft. Lauderdale, FL	N 26°09'14.18" W 80°07'48.40"	SU256		r98	r98		r58	r45
Faiditus xiphias	Malaysia	Gunung Senyum		SU627	r2069	r1926	r1926	r1896		r1986
Faiditus xiphias	Malaysia	Gunung Senyum		SU629	r2070	r1927	r1927	r1897	r1957	r1987
Faiditus xiphias	Thailand	CoMai		SU726	r2077	r1934	r1934	r1904		r1994
Faiditus xiphias	Taiwan	Taipei	N 24°50'12.70" E 121°39'6.40"	SU694	r2074	r1931	r1931	r1901	r1961	r1991

Faiditus xiphias	Singapore	Catchment Center	N 1°22'54.78" E 103°48'56.70"	SU547	r2088	r1945	r1945	r1915		r2005
Faiditus cf. *xiphias*	China	Yunnan	N 20°55'48.72" E 101°15'16.98"	SU528	r2061	r1610	r1610	r1596	r1582	r1624
Neospintharus syriacus	Israel	Jerusalem	N 31°46'20.64" E 35°11'45.55"	SU728	r2078			r1905	r1965	r1995
Neospintharus trigonum	USA	Hawaii		Arnedo et al. 2004	AY231048	AY230945		AY230917	AY230177	AY231006
Rhomphaea fictilium	USA	Lawrence, KS	N 38°56'58.20" W 95°16'7.30"	SU609	r2058	r1622	r1622	r1608	r1594	r1636
Rhomphaea metaltissimus	Guyana	S of Gunns Landing		Arnedo et al. 2004	AY231052	AY230950		AY230921	AY231083	AY231009
Rhomphaea saganus	Taiwan	Chaiyi		SU608		r1621	r1621		r1593	r1635
Rhomphaea sinica	Taiwan	Orchid Is.	N 22°00'34.60" E 121°34'15.10"	SU524	r2060	r1609	r1609	r1595	r1581	r1623
Rhomphaea sp1	Philippines	Luzon	N 8°00'26.70" E 125°04'29.30"	SU325	r2091	r1948	r1948	r1918	r1978	r2008
Rhomphaea sp1	Philippines	Mindanao	N 6°58'31.40" E 122°4'9.70"	SU365	r2089				r1976	r2006
Spheropistha nigroris	Taiwan	Orchid Is.	N 22°00'34.63" E 121°34'15.13"	SU517	r2062	r1919	r1919	r1889	r1949	r1979
Spheropistha sp.	Philippines	Mindoro	N 13°30'20.10" E 120°54'43.50"	SU333	r2053	r102	r102		r62	r49
Outgroups										
***Anelosimus* + Theridiinae clade**										
Anelosimus rupununi	Argentina	Formosa	S 26°10'53.00" W 58°56'59.00"							EF050362
Anelosimus studiosus	USA	Louisiana		Agnarsson et al. 2007	EF050321	EF050156	EF050417	EF050200		EF050367
Anelosimus eximius	Ecuador	Napo		Agnarsson et al. 2007	EF050297	EF050167	EF050395	EF050191	EF050233	
Kochiura rosea	Chile	Osorno	N 40°46'1.20" E 72°16'59.99"					EF050195	EF050242	EF050353
Coleosoma acutiventer	Ecuador	Morona Santiago	S 2°59'46.30" W 78°27'20.90"	Agnarsson et al. 2007	EF050286	EF050181	EF050386		EF050222	EF050339
Coleosoma sp. PH	Philippines	Luzon	N 14°8'3.70" E 121°13'29.50"	SU425	r2032	r107	r107	r121	r67	R54
Echinotheridion otlum	Ecuador	Napo	N 1°4'1.20" E 77°37'1.20"	Arnedo et al. 2004				EF050188	EF050227	EF050344
Theridion calcynatum	Ecuador	Napo	S 0°35'26.52" W 77°52'55.84"	Agnarsson et al. 2007	EF050322					EF050368
Theridion nigroannulatum	Ecuador	Morona Santiago	S 2°55'21.72" W 77°24'28.44"	Agnarsson et al. 2007	EF050324	EF050178	EF050418	EF050201	EF050264	EF050369
Pholcommatinae										
Enoplognatha caricis	Japan	Tokyo		Arnedo et al. 2004	AY231040	AY230962		AY230907	AY231096	AY231000
Spintharinae										
Moneta sp.	Malaysia	Pahang	N 4°28'55.19" E 101°23'16.78"	Agnarsson et al. 2007	EF050307				EF050246	EF050356
Spintharus flavidus	USA	North Carolina		Arnedo et al. 2004	FJ607586	AY230973		AY231108	AY231108	AY231013
Latrodectinae										
Latrodectus mactans	USA	North Carolina		Arnedo et al. 2004	AY231046	AY230966		AY230915	AY231100	AY231004
Hadrotarsinae										
Dipoena cf. *hortoni*	Guyana	S of Gunns Landing		Arnedo et al. 2004	AY231038	AY230961		AY230907	AY231095	AY230998

Table 8.The list of sequences of the primers that we used in this study. The annealing temperatures were tested according to the lab conditions.

Gene	Primer name	Primer sequence	Annealing temperature (°C)	Reference
Mitochondrial genes				
COI	LCO-J-1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	45	Folmer et al. 1994
	HCO-N-2198	5'-TAAACTTCAGGGTGACCAAAAAAATCA-3'		Folmer et al. 1994
	COI-F	5'-GGGCTCCTGACATAGCCTTTCCTCGAA-3'	48~52	This study
	COI-r	5'-TCCTGCTAAAATTGCAAATACAGCCCC-3'		This study
16s+ND1	LR-N-12945	5'-CGA-CCT-CGA TGT-TGA-ATT-AA-3'	45	Hedin1997
	N1-J-12581	5'-CCTTTA ACG AAT TTG AAT ATA-3'		Hedin and Maddison 2001
Nuclear genes				
18s	18S-4F	5'-CCAGCAGCCGCGCTAATTC-3'	45	Giribet et al., 1996
	18S-9R	5'-GATCCTTCCGCAGGTTCACCTAC-3'		Giribet et al., 1996
28s	28Sc	5'-GGTTCG ATT AGT CTT TCG CC-3'	45	Whiting et al. 1997
	28So	5'-GAAACTGCTCAAAGGTAAACGG-3'		Whiting et al. 1997
H3a	H3aF	5'-ATGGCTCGTACCAAGCAGAC(ACG)GC-3'	45	Colgan et al. 1998
	H3aR	5'-ATATCCTT(AG)GGCAT(AG)AT(AG)GTGAC-3'		Colgan et al. 1998

Table 9. The binary coding list of character states for group-living behavior, specialization on large hosts, and foraging strategy. We used data from Whitehouse (2011) and Elgar (1993) to code the behavior, but the original literatures of the behavior and hosts in a particular species were cited. In addition, we used my filed records to code most of the behaviors and hosts of solitary species because there is very limited information from the literatures about the natural history of solitary species. The species possess group living behavior were coded as 1 and others were coded as 0. The species specialized to large host were coded as 1, and other was coded as 0. The species use kleptoparasitism only (K) were coded as 1, and the species use both kleptoparasitism and predation (K&P) were coded as 0. Missing data were coded as ?.

Species	Group living (yes=1, no=0)	Specialization to large host (yes=1, no=0)	K=1 K&P=0	Hosts (family or genus name)	References
Argyrodes cf. *amboinensis*	0	0	1	Theridiidae, *Psechrus*	Su field record
Argyrodes argentatus	1	1	1	*Argiope**, *Cyrtophora*, *Nephila**	(Robinson and Robinson 1973, Kerr and Quenga 2004)
Argyrodes cf. *bonadea*	1	1	1	*Nephila*	(Miyashita 2002)
Argyrodes cylindratus	0	0	1	*Gasteracantha*, *Nephila*, Theridiidae	Su field record
Argyrodes elevatus	1	1	0	*Argiope*, *Nephila*	(Vollrath 1979)
Argyrodes fasciatus	0	0	1	*Psechrus*	Su field record
Argyrodes fissifrons	1	1	0	*Cyrtophora**, *Linyphia, Psechrus, Theridiidae*	Tanaka 1984, Su field record
Argyrodes flavescens	1	1	1	*Nephila**, *Gasteracantha*, *Leucauge*	(Miyashita et al. 2004)
Argyrodes kumadai	1	1	0	*Cyrtophora*	(Baba et al. 2007)
Argyrodes lanyuensis	1	0	1	*Nephila*, *Cyrtophora*, *Gasteracantha*, *Leucauge,* Theridiidae	Su field record
Argyrodes miniaceus	1	1	1	*Nephila*	Robinson & Robinson 1973
Argyrodes cf. *nasutus*	0	0	1	Linyphiidae*, Psechrus,* Theridiidae	Su field record
Argyrodes nephilae	1	0	1	*Argiope*, *Cyrtophora*, *Gasteracantha*, *Neoscona*, *Nephila*	Exline & Levi 1962
Argyrodes pluto	0	0	1	*Argiope*, *Metepeira*, *Latrodectus*	Exline & Levi 1962
Argyrodes sp1	1	1	1	*Nephila*	Su field record
Argyrodes sp2	1	1	1	*Nephila*	Su field record
Argyrodes sp3	1	1	1	*Nephila*	Su field record
Argyrodes sp4	1	1	1	*Nephila*	Su field record
Argyrodes sp5	?	?	?		Smith collection
Argyrodes sp6	1	1	?	*Cyrtophora*	Su field record
Argyrodes sp7	1	1	?	*Cyrtophora*	Su field record
Argyrodes sp8	1	1	1	*Nephila*	Su field record
Argyrodes sp10	0	0	1	Small web of Araneidae	Chang per. com.
Argyrodes tripunctatus	0	0	1	*Leucauge*, *Nephila*, *Nephilengys*, *Pholcus*	Su field record
Ariamnes attenuatus	0	0	1	Free living	(Eberhard 1979)
Ariamnes cylindrogaster	0	0	1	Free living	Su field record
Faiditus amplifrons	0	0	1	Chang per. com.	Bennet per. com.
Faiditus cancellatus	1	1	1	*Nephila*	Vollrath 1979
Faiditus cf. *chickeringi*	0	0	1	*Leucauge*, small web of Araneidae	Su field record
Faiditus chickeringi	0	0	1		
Faiditus globosus	1	1	1	*Gasteracantha*, *Nephila*	Henaut et al. 2005
Faiditus xiphias	0	0	1	*Argiope*, webs of Araneidae	Su field record
Faiditus cf. *xiphias*	0	0	1	Small web of Araneidae	Chang per. com.
Neospintharus syriacus	0	0	1	Linyphiidae	Smith obs.
Neospintharus trigonum	0	0	1	Linyphiidae, Araneidae	(Larcher and Wise 1985, Cangialosi 1997)
Rhomphaea fictilium	0	0	1	Linyphiidae, *Philoponella*, *Argiope*	(Smith Trail 1980, Paquin and Dupérré 2001)
Rhomphaea metaltissimus	0	0	0		
Rhomphaea saganus	0	0	0	Small web of Araneidae, Juvenile webs of *Nephila*	Su field record
Rhomphaea sinica	0	0	0	*Nephila*, *Cyrtophora*, Small web of Araneidae	Su field record
Rhomphaea sp1	0	0	0	*Nephila*, *Nephilengys*, Small web of Araneidae	Su field record
Spheropistha nigroris	0	0	1	*Nephila,* webs of Araneidae	Su field record
Spheropistha sp.	1	1	1	*Argiope*	Su field record

Table 10. Statistics of parsimony method, likelihood method, and Bayesian method. The sequence length shown was the length of a gene data we used after multiple alignment. The best-fit model in both Akaike information criterion (AIC) and Bayesian information criterion (BIC) were listed. We used the model under BIC for analyses in likelihood and Bayesian method. For NDI data, we used GTR+G instead of TrN+G because TrN+G cannot be implemented in MrBayes 3.2 and GTR+G is the similar model of TrN+G.

	COI	NDI	16s	28s	18s	H3a	Total combined
Sequence length (aligned)	753	105	576	784	863	327	3408
Best model (AIC)	GTR+I+G	TrN+G	GTR+G	GTR+I+G	TrN+G	GTR+I+G	-
Best model (BIC)	GTR+I+G	TrN+G	GTR+G	GTR+I+G	K80+I+G	GTR+I+G	-
Statistics of parsimony methods							
Tree length	2100	262	1722	2498	953	644	7912
Consistency index (Trizzino et al.)	0.3081	0.416	0.406	0.4468	0.6464	0.2904	0.4291
Retention index	0.5678	0.6531	0.6451	0.7198	0.6596	0.6143	0.2347
Number of trees	>50000	>50000	17396	>50000	8431	1047	1499
Statistics of likelihood method							
ln L score	-9563.9388	-992.3484	-8129.9258	-11544.1499	-5998.6263	-3279.2207	-40003.7452
Statistics of Bayesian method							
Model used in MrBayes	GTR+I+G	GTR+G	GTR+G	GTR+I+G	K80+I+G	GTR+I+G	Partitioned
Number of generations	10,000,000	6,000,000	3,000,000	15000000	10000000	15000000	5,000,000
Burn-in	1000	1000	500	5000	1000	5000	1000
Final average standard deviation of split frequencies	0.013655	0.008407	0.009718	0.009896	0.040008	0.005291	0.033803

Table 11. The results of correlated character evolution test of group living behavior and specialization to large host in Argyrodinae. The harmonic mean log likelihood scores of dependent model and the independent model were compared and the Bayes factor (BF) of each run was calculated using the difference of log likelihood scores of dependent and independent models × 2. The smallest BF is 29.971606 indicating that the dependent model is strongly supported. According to Bagel and Meade's (2006) criterion, BF >10 is strong evidence of correlated evolution of two binary characters.

	Harmonic mean of log likelihood scores				
	run1	run2	run3	run4	run5
Dependent model	-42.429468	-42.36066	-42.632073	-42.976446	-43.948041
Independent model	-59.008302	-58.899453	-59.008274	-59.027361	-58.933844
logD-logI	16.578834	16.538793	16.376201	16.050915	14.985803
Bayes Factor	33.157668	33.077586	32.752402	32.10183	29.971606

FIGURES

Figure 1. The proportion of the individuals in *feeding*, *reaching*, and *no contact* categories. The filled bars show the percentage of *feeding* kleptoparasites in a group size. The hollow bars show the percentage of *reaching* individuals in a group size treatment. The dashed line bars are the percentage of *no contact* individuals. The number in each bar shows the actual number of individuals in that category under that group size treatment.

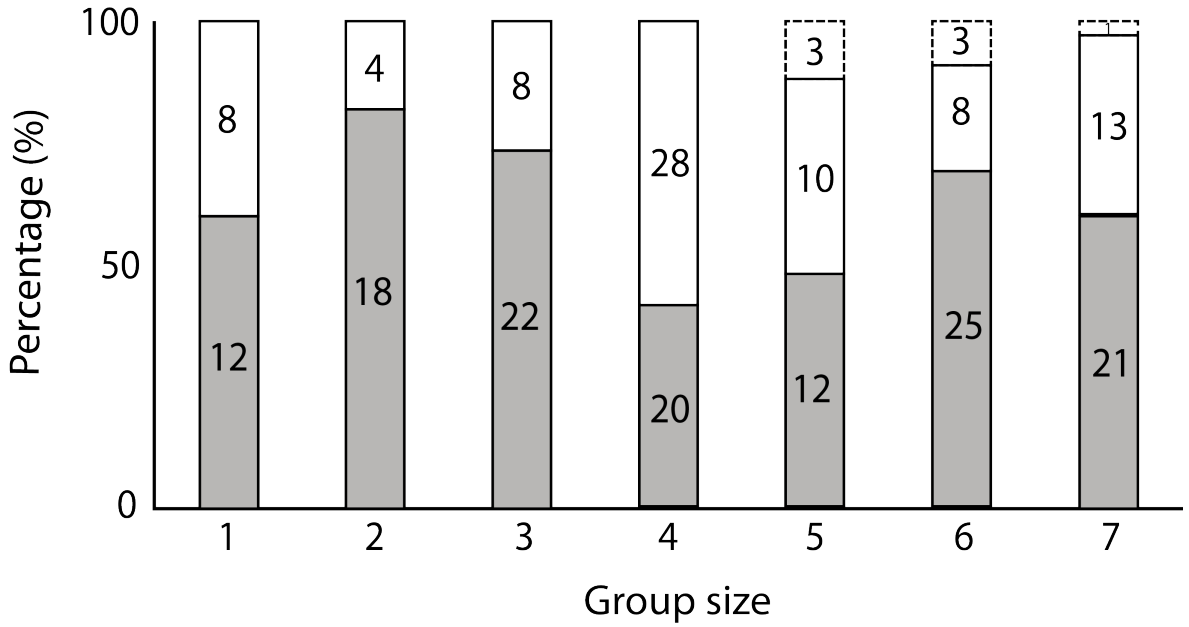

100
50
0
Percentage (%)
8
12
4
18
8
22
28
20
3
10
12
3
8
25
13
21
1
2
3
4
5
6
7
Group size

Figure 2. Correspondence Analysis of Foraging Success and Group Size. The circles indicate the dispersion of group sizes one through seven. The squares indicate the dispersion of three foraging success categories: *feeding*, *reaching* and *no contact*. The relatively close points on this two-dimensional graph, which has two components, indicate stronger association among these points. The group sizes two and three are closer to feeding category indicating individuals in groups of two or three are more likely to feed successfully. None of the group sizes are in the same quadrant of no contact category indicating no group size would cause completely failed of foraging.

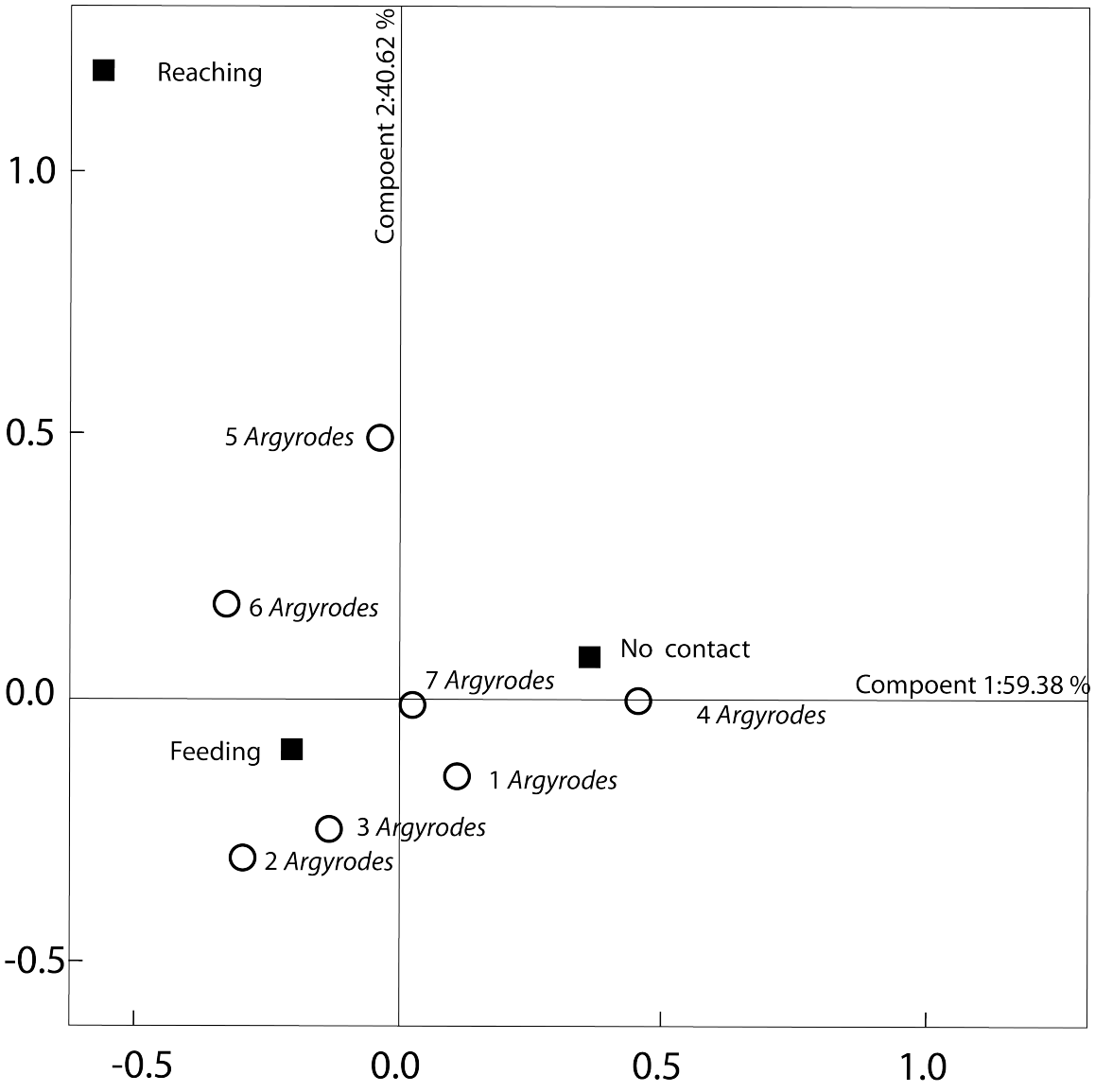

Reaching
Compoent 2:40.62 %
1.0
0.5
0.0
-0.5
5 Argyrodes
6 Argyrodes
No contact
7 Argyrodes
Compoent 1:59.38 %
4 Argyrodes
Feeding
1 Argyrodes
3 Argyrodes
2 Argyrodes
-0.5
0.0
0.5
1.0

Figure 3. ANOVA results of the effect of group size on feeding trials. The total heights of the stacked bars indicate the mean durations of the experimental feeding trials (from the time the host began wrapping prey until she discarded the prey) for groups of different sizes. The shaded portion of the bars indicates the mean length of time needed for the first *Argyrodes* to reach and touch prey. The unshaded portion of the bars are the mean durations of feeding bouts, from the time the first *Argyrodes* touched the prey until the host discards the prey. The error bars that are outside of the stacked bars are the standard errors (SE) of the mean length of experimental feeding trials. The error bars inside the shaded portion of the bars are the SE of the mean time elapsed until the first *Argyrodes* in the group touched the prey. The error bars inside the unshaded portion are the SE of feeding bouts. The letters, a, b, and c, next to the error bars indicate the Fisher's grouping. The letters a and b indicate the Fisher's grouping of the mean lengths of time needed for the first *Argyrodes* to reach and touch prey (the grouping of shaded portions). The letters c, d, and e indicate the Fisher's grouping of the mean feeding bouts (the grouping of unshaded portions). The letters f, g, and h indicate the Fisher's grouping of the mean durations of experimental feeding trials(the grouping of shaded+unshaded portions).

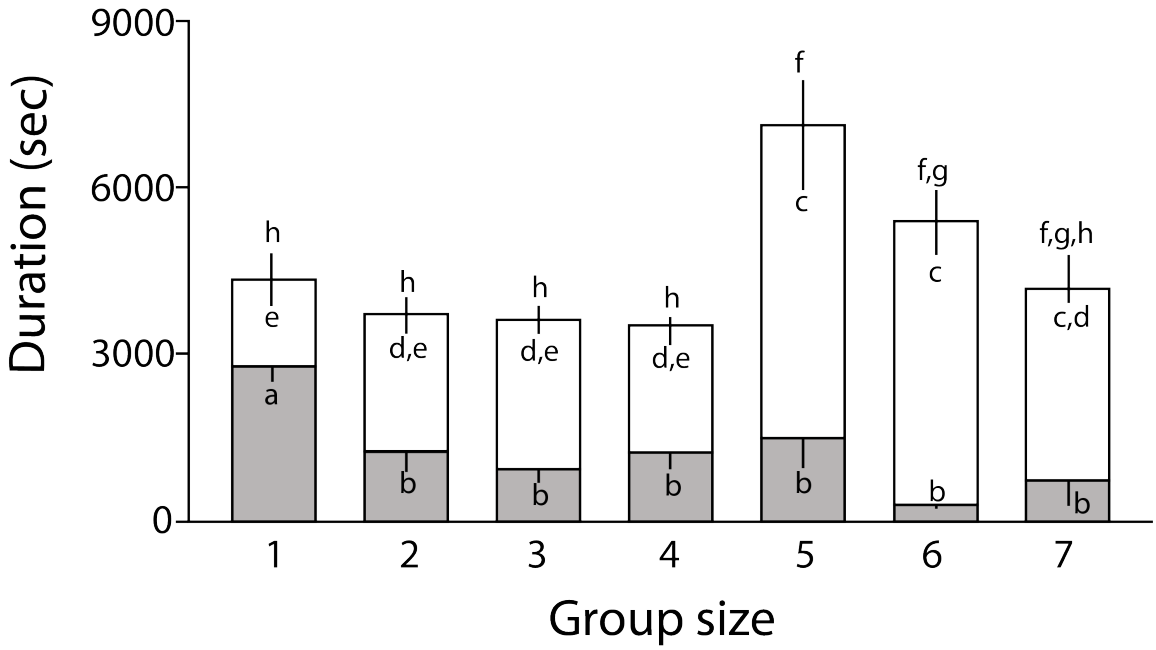

9000
6000
3000
0
Duration (sec)
h
e
a
h
d,e
b
h
d,e
b
h
d,e
b
f
c
b
f,g
c
b
f,g,h
c,d
b
1
2
3
4
5
6
7
Group size

Figure 4. The ANOVA results of (A) duration of time spent feeding and (B) per capita feeding rate. The letters, a, b, and c, next to the error bars indicate the Fisher's grouping. See Figure 1 for number of individuals used in each group size treatment.

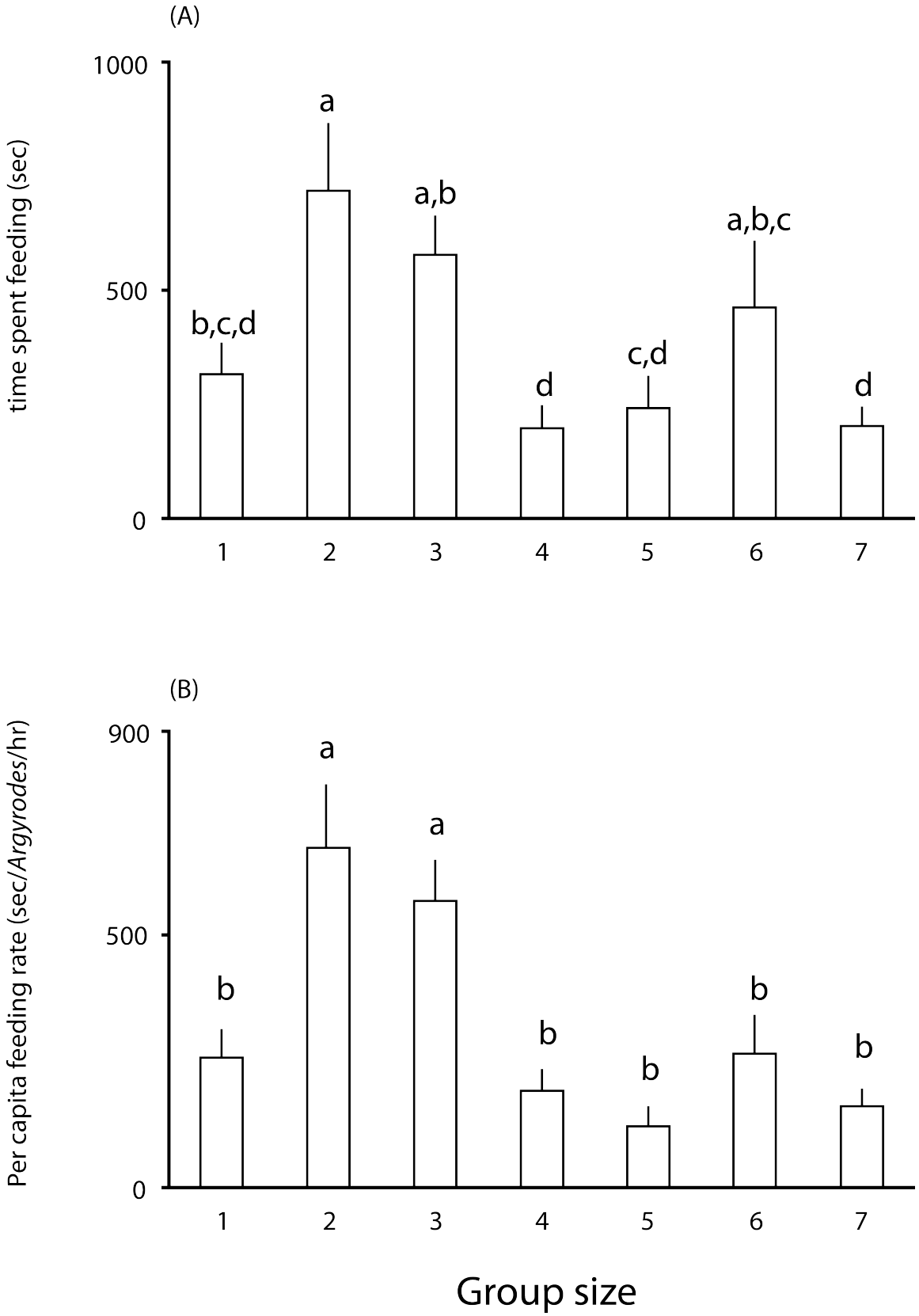

(A)
1000
500
0
time spent feeding (sec)
b,c,d
a
a,b
d
c,d
a,b,c
d
1
2
3
4
5
6
7
(B)
900
500
0
Per capita feeding rate (sec/*Argyrodes*/hr)
b
a
a
b
b
b
b
1
2
3
4
5
6
7
Group size

Figure 5. Time series plot of the number of kleptoparasites searching or feeding at hub of the host web. (a) to (d) are examples of the number of kleptoparasites at the hub in group size four to seven. The dashed lines are the observed numbers of kleptoparasites at hub. The solid lines are the smoothed line produced by the moving average model.

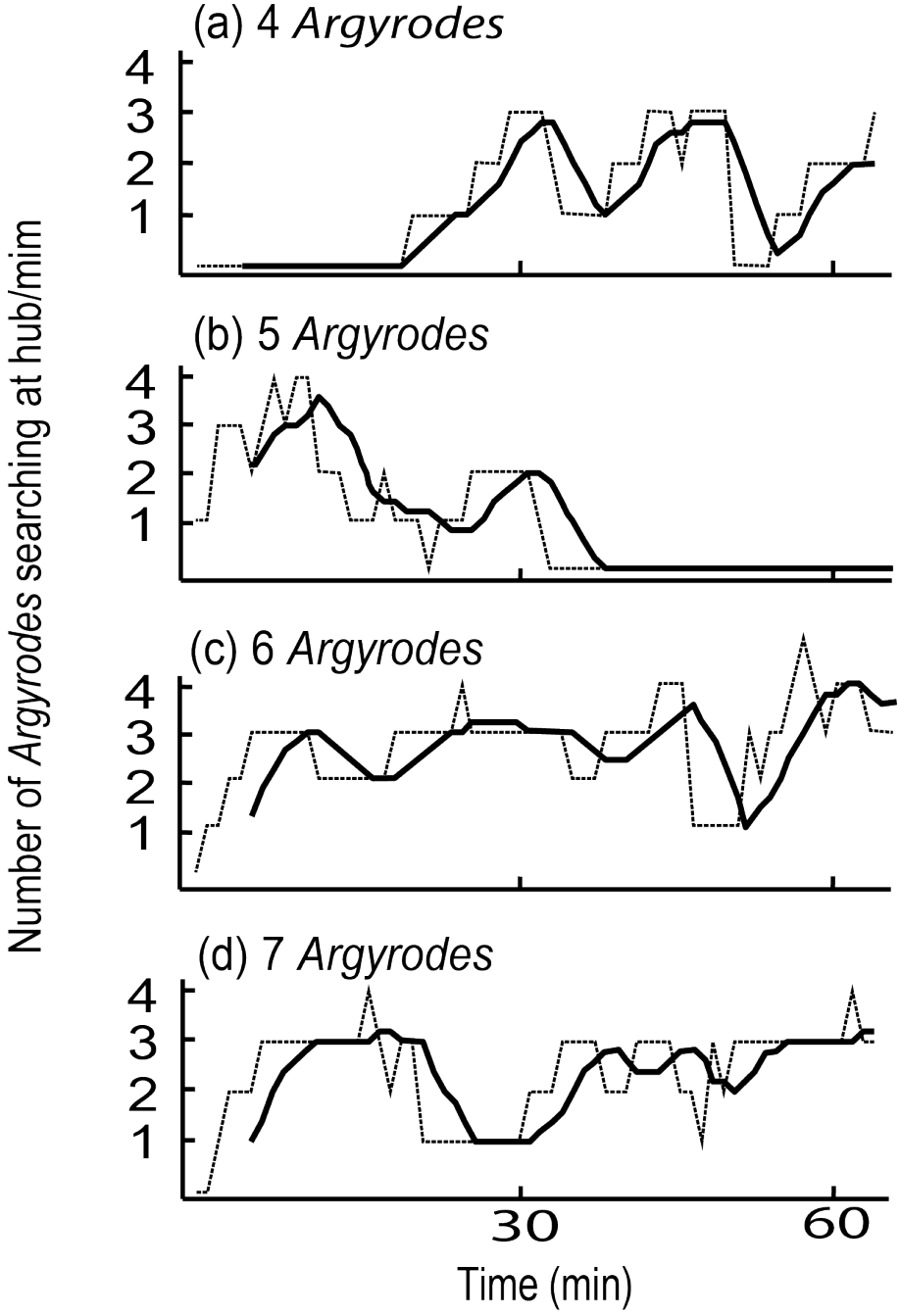
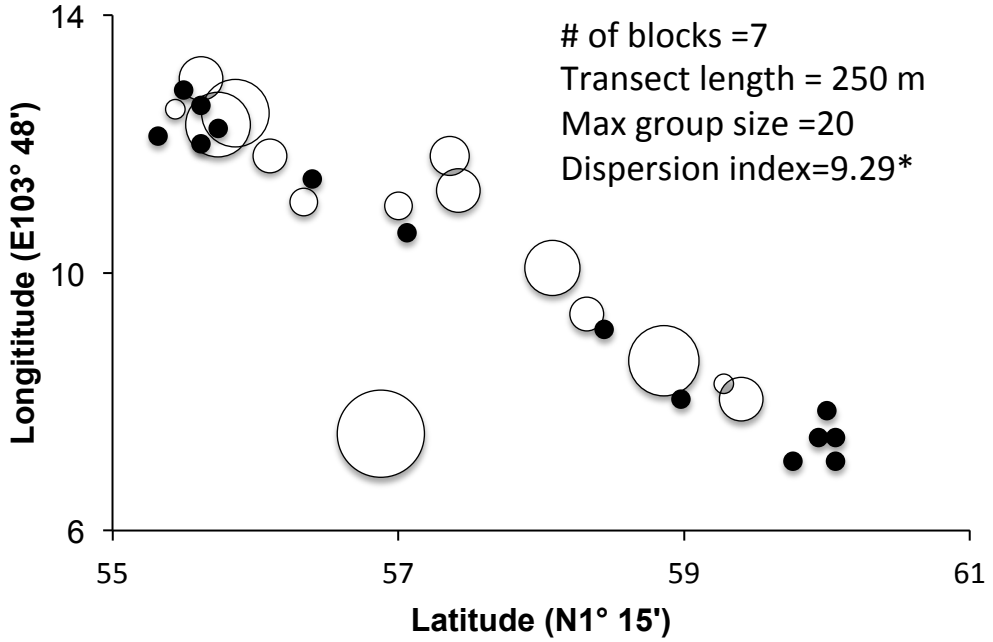
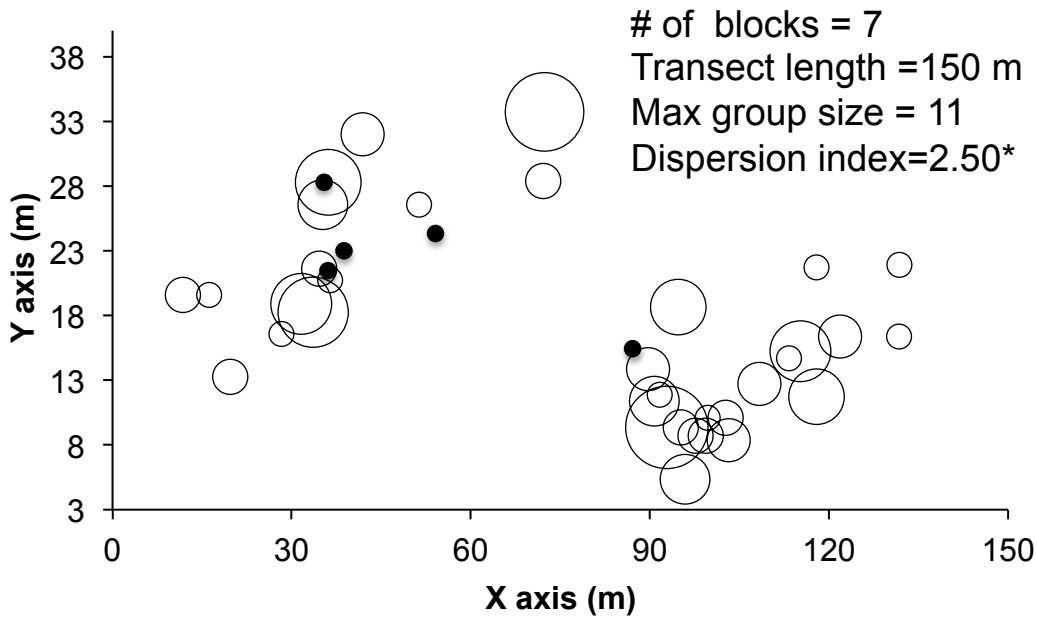

(a) 4 Argyrodes
(b) 5 Argyrodes
(c) 6 Argyrodes
(d) 7 Argyrodes
Number of Argyrodes searching at hub/mim
4
3
2
1
30
60
Time (min)

Figure 6. Spatial distribution of the host webs for seven species of Argyrodinae kleptoparasites. Figures 6(a) to 6(f) show the dispersions of the webs of spiders that serve as hosts for five different group-living Argyrodinae species. Figures 6(g) and 6(h) show the dispersions of the webs of spiders that serve as hosts for two solitary Argyrodinae species. The black dots in the figures indicate webs without kleptoparasites. The open circles indicate webs with kleptoparasites. The diameter of the circle indicates the size of the Argyrodinae group occupying the host web. The maximum group size in each population is labeled in each figure. The dispersion index of each population is also provided. * indicates clustered distribution of host webs.

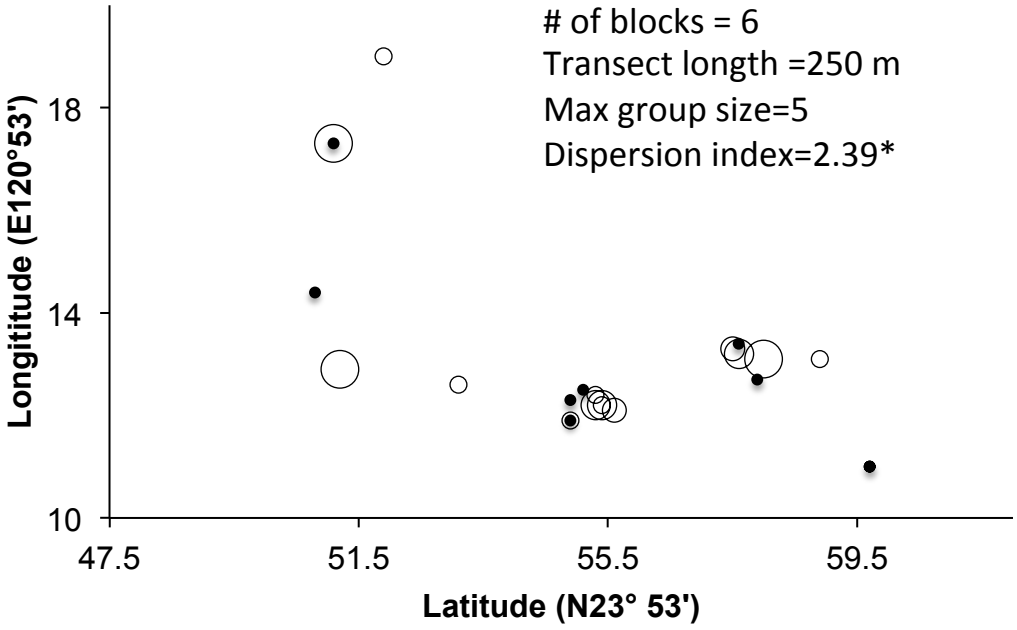
(a) *Argyrodes flavescens*



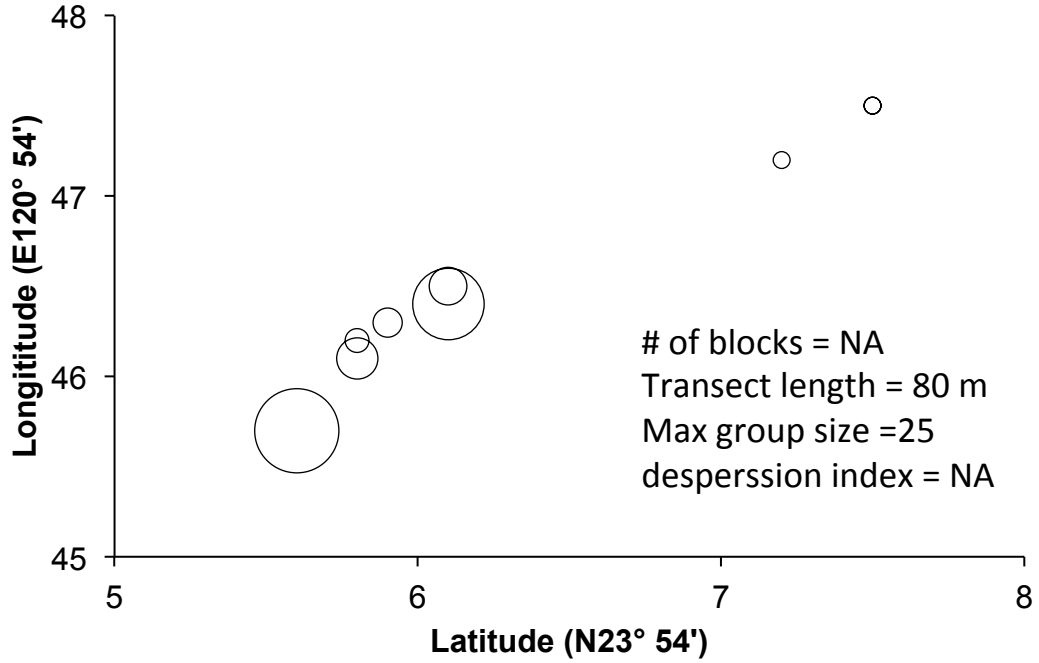
(b) *Argyrodes fissifrons*



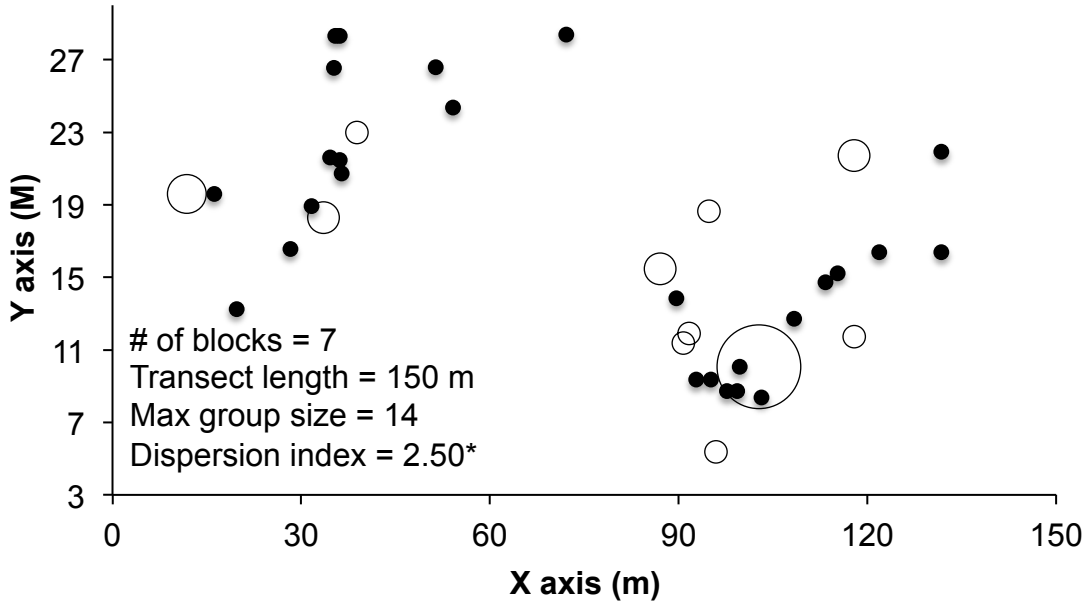
(c) *Argyrodes kumadai* – subpopulation 1



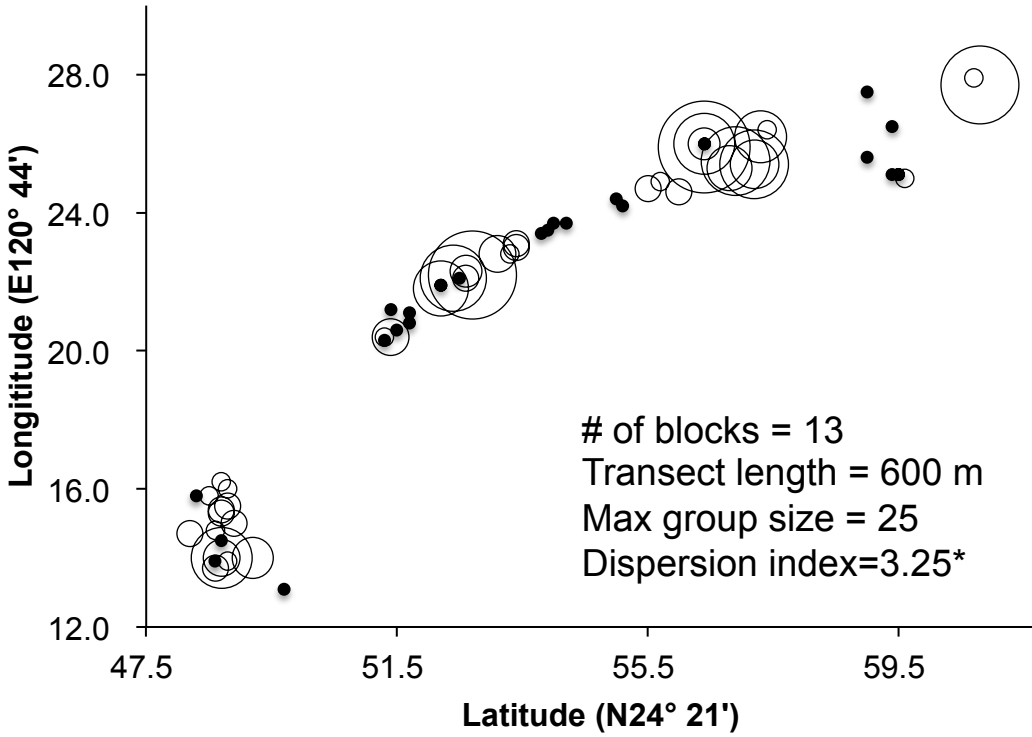
(d) *Argyrodes kumadai* – subpopulation 2



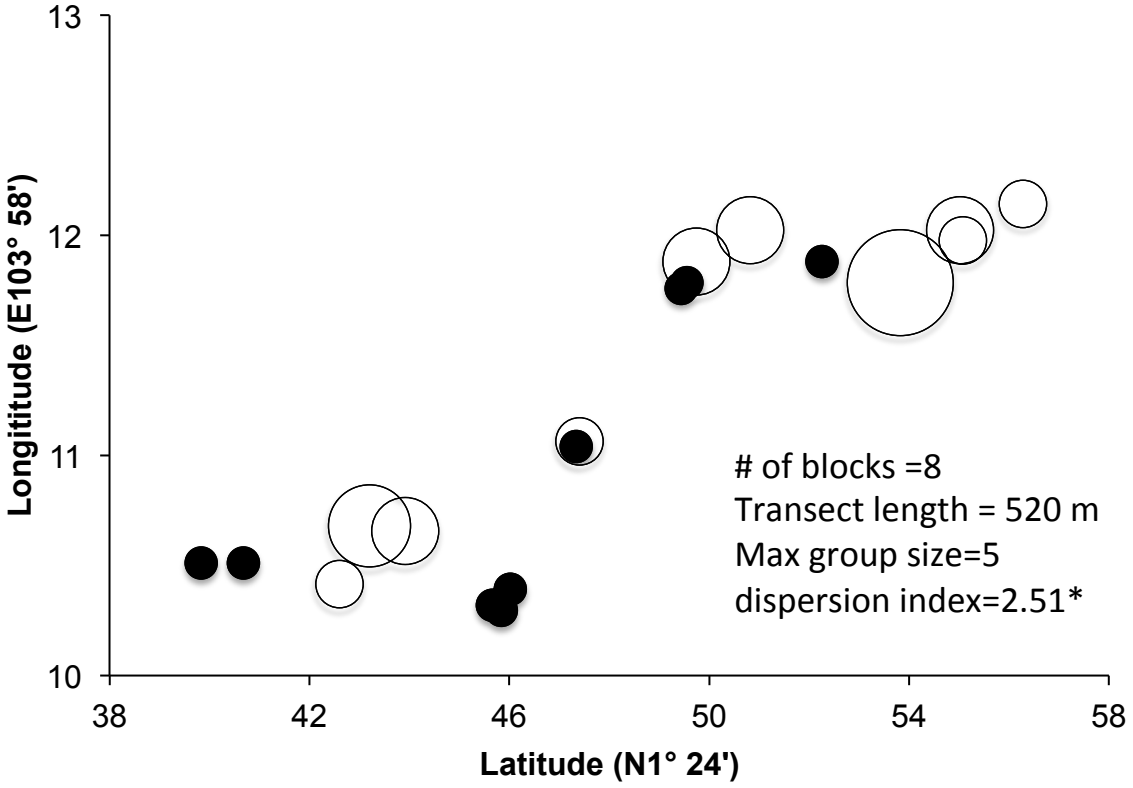
(e) *Argyrodes lanyuensis*



(f) *Argyrodes miniaceus*



(g) *Argyrodes fasciatus*



(h) *Neospintharus trigonum*

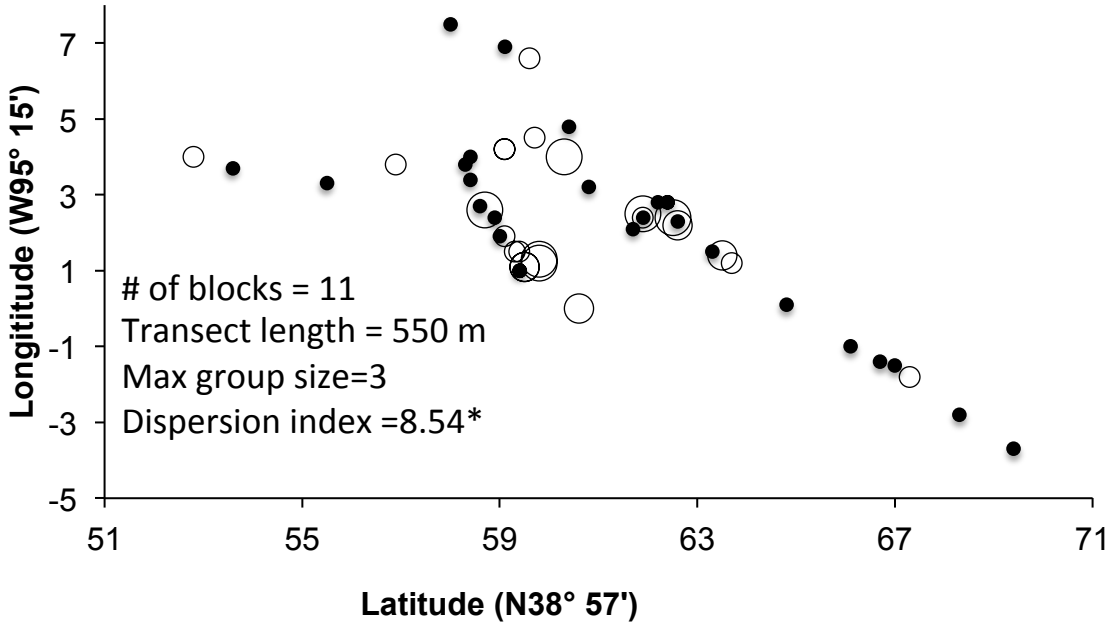
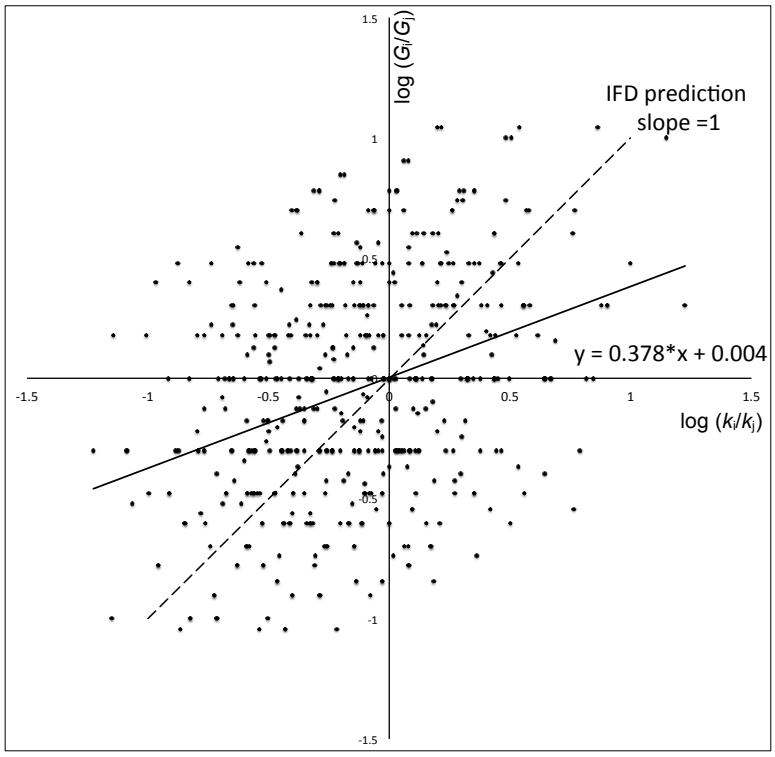
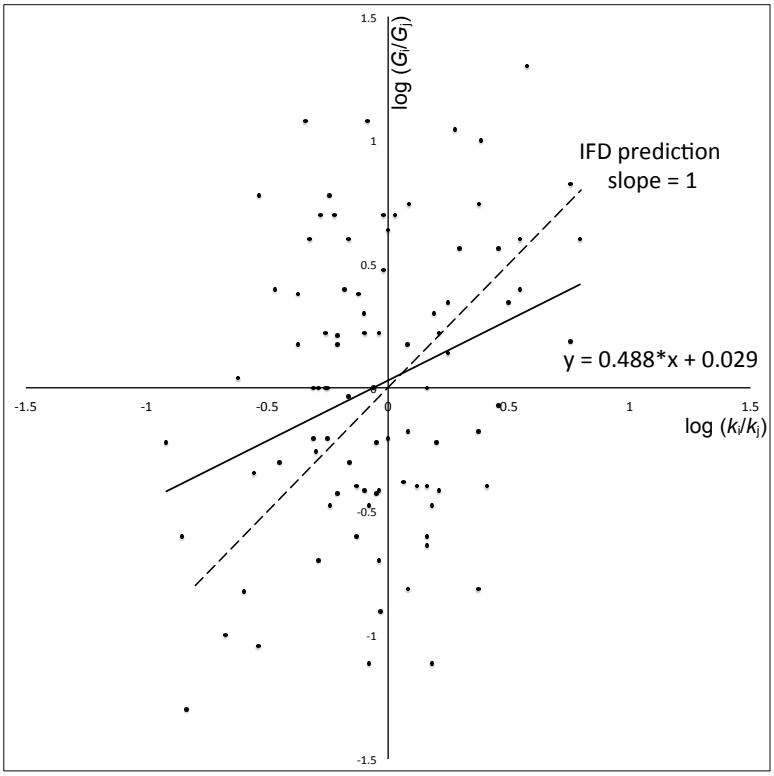


Figure 7. Results of ideal free distribution tests using matching rule. The logarithm of resource size, or web area, ratios (log k_i/k_j), and the logarithm of group size ratios (log A_i/A_j) were plotted for seven species. Figures 7 (a) to (d) show the test results of group-living species: *A. fissifrons*, *A. flavescens*, *A. kumadai*, and *A. lanyuensis*. Figure 7(f) and (g) show the test results of solitary species: *A. fasciatus* and *N. trigonum*. The solid line is the simple linear regression line. * near the slope of a simple linear regression equation indicates the significance of deviation from IFD predicted slope, which is the dashed line in each figure. The IFD predicted slope is one according to habitat matching rule (Fagen 1987). * near the intercept indicates the intercept is significantly different from zero.

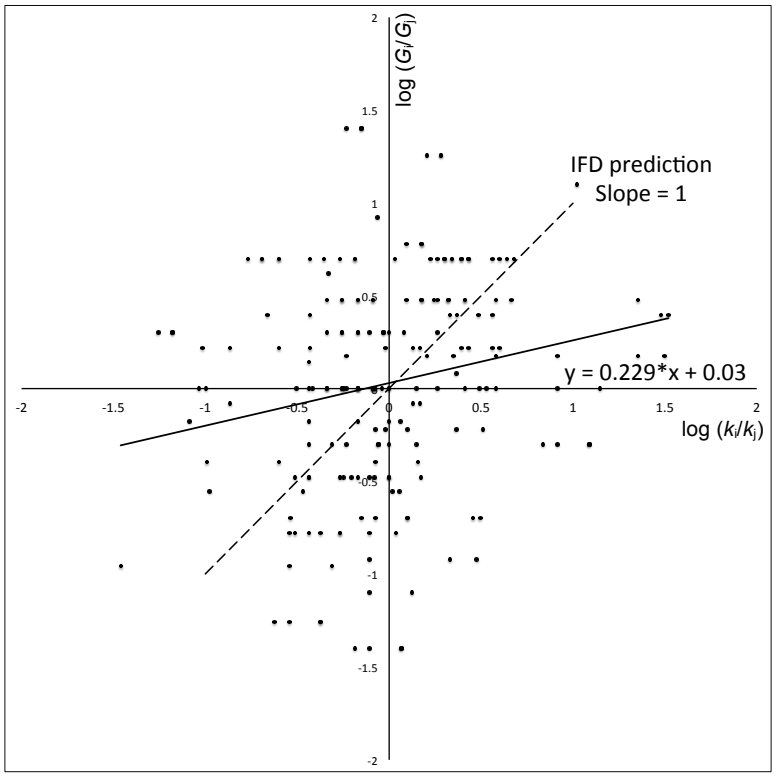
(a) *Argyrodes fissifrons*



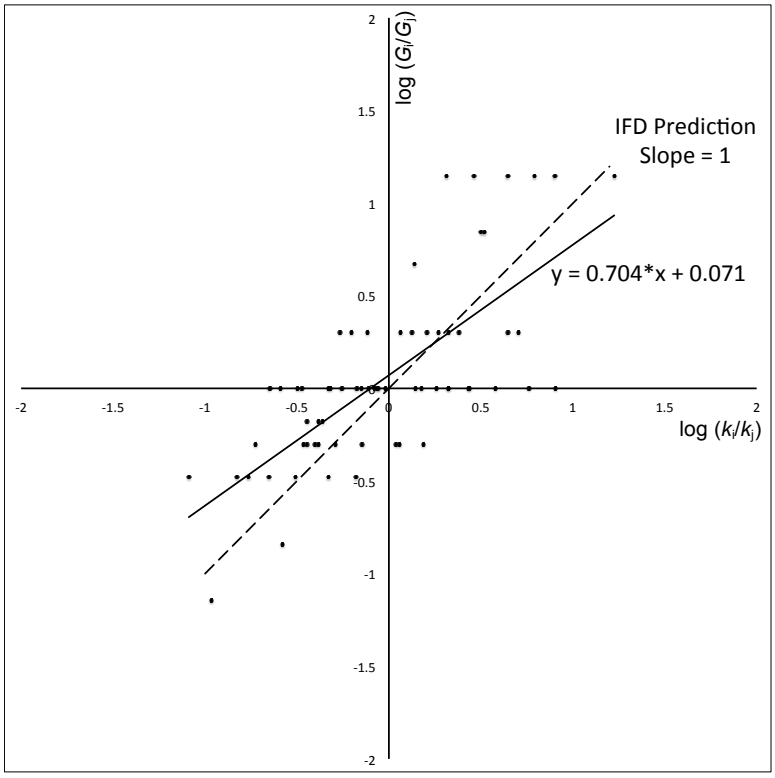
(b) *Argyrodes flavescens*



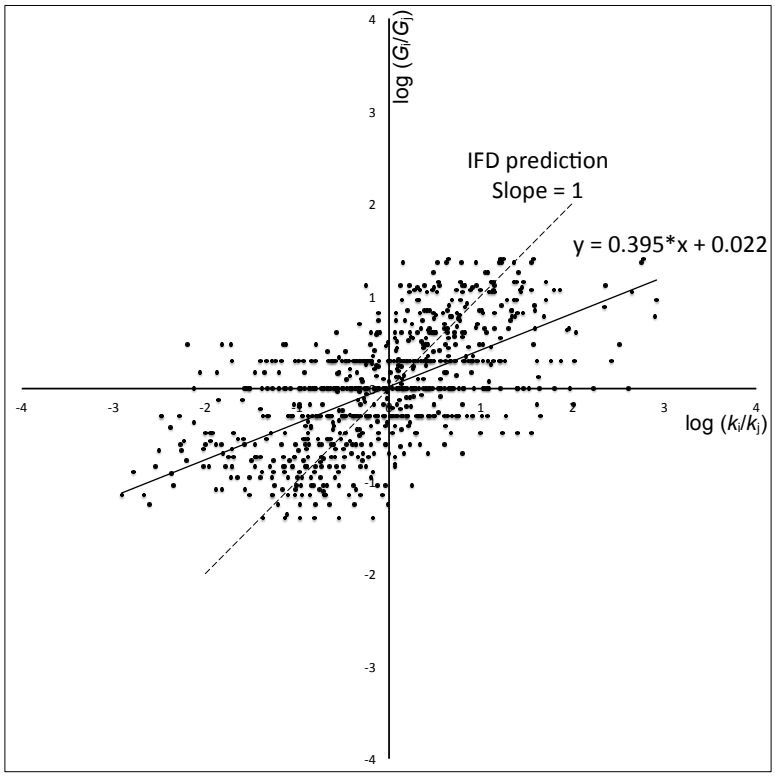
(C) *Argyrodes kumadai*



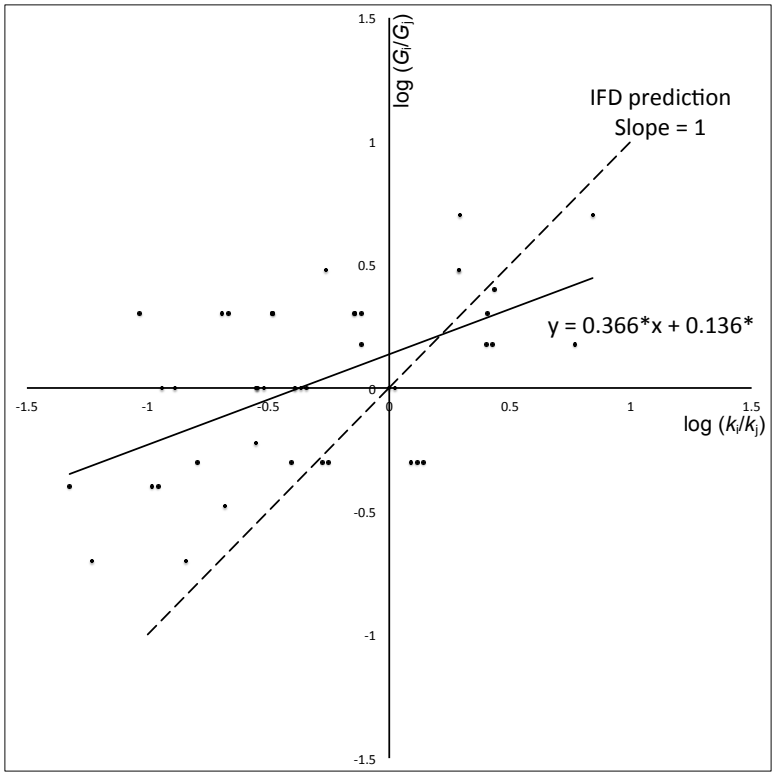
(d) *Argyrodes lanyuensis*



(e) *Argyrodes miniaceus*



(f) *Argyrodes fasciatus*



(g) *Neospintharus trigonum*

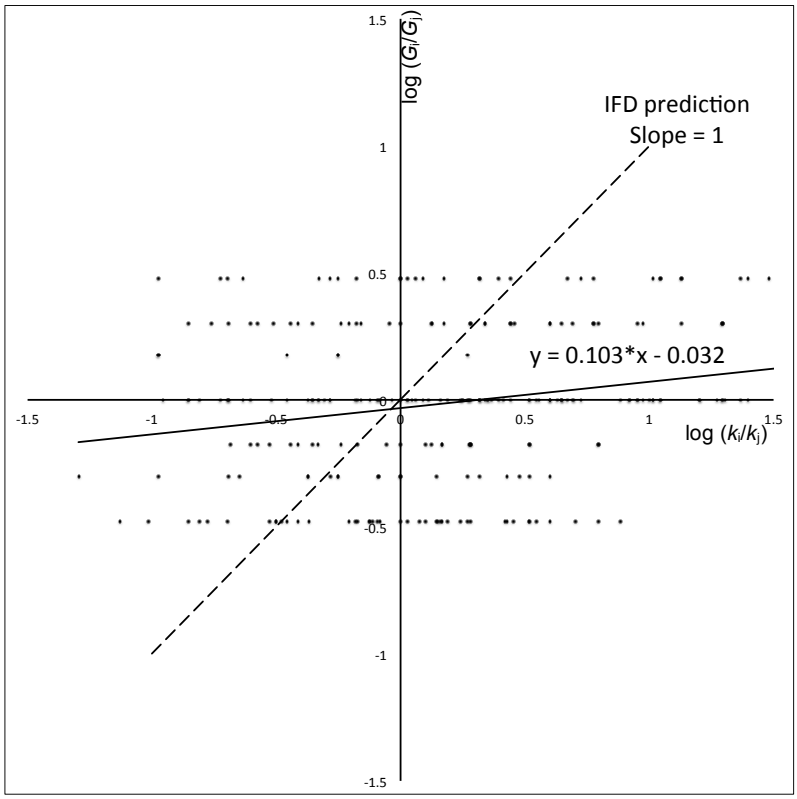
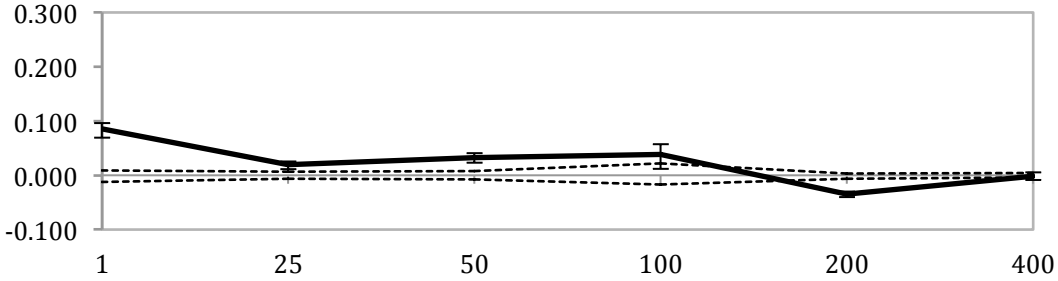
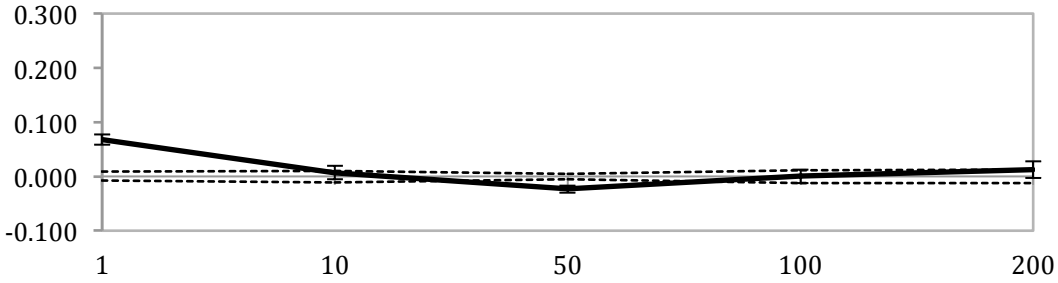


Figure 8. Results of spatial autocorrelation analyses of group-living species, *Argyrodes miniaceus* (a) and *A. kumadai* (b), and solitary species *A. fasciatus* (c) and *Neospintharus trigonum* (d). Dashed lines = upper and lower boundaries of 95% confidence interval for values of the autocorrelation coefficient, ***r***, under the null hypothesis of no geographic structure. Solid line connects ***r*** values for each distance class. Bars around computed ***r*** values are 95% bootstrap confidence intervals (Peakall et al. 2003).

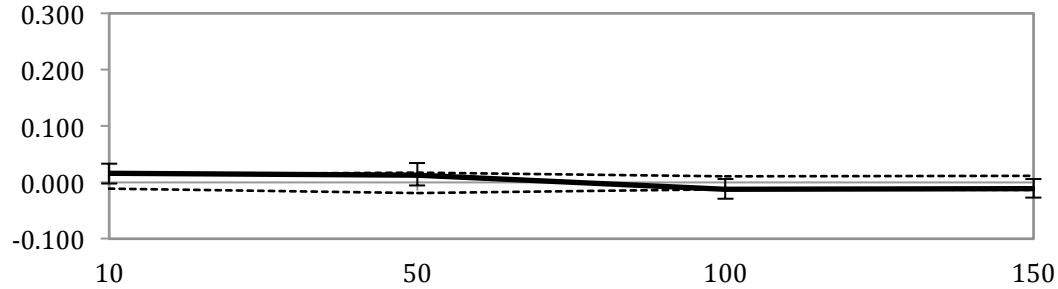
(a) *Argyrodes miniaceus*



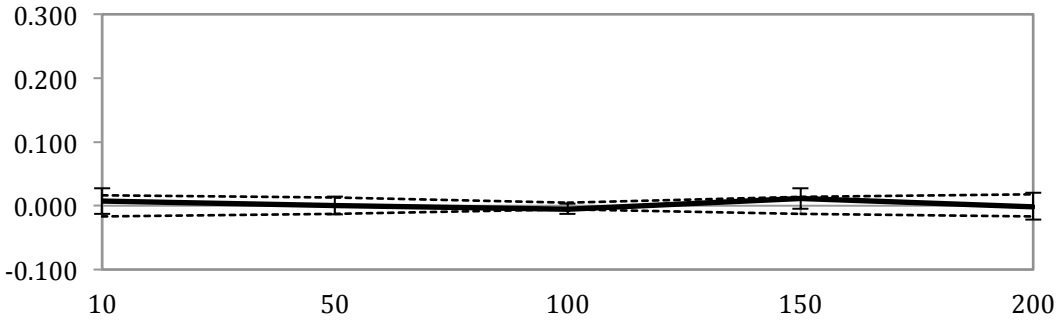
(b) *Argyrodes kumadai*



(c) *Argyrodes fasciatus*



(d) *Neospintharus trigonum*

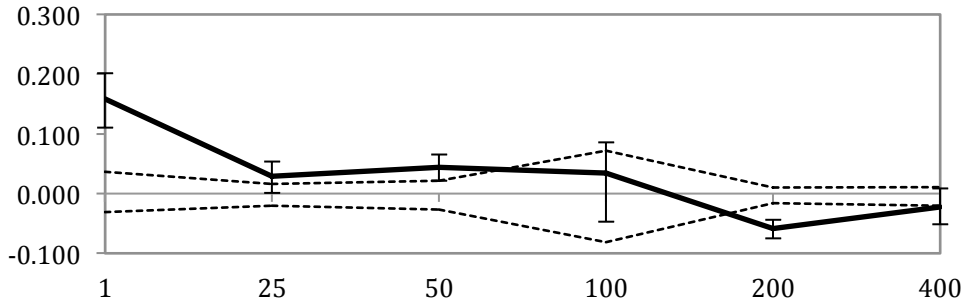


Autocorrelation coefficient (*r*)

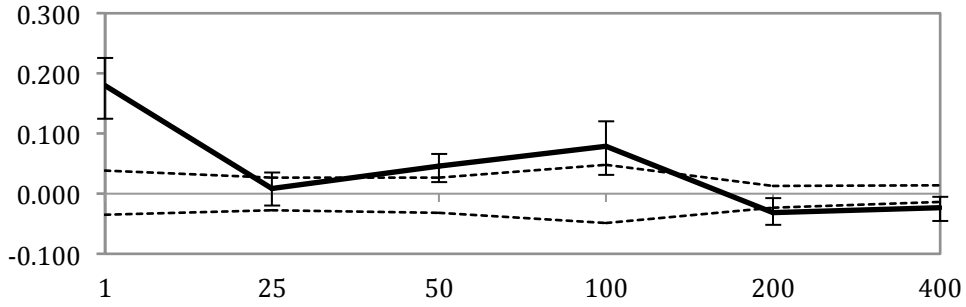
Distance class (m)

Figure 9. Results of spatial autocorrelation analyses for different age/sex classes of the group-living species, *Argyrodes miniaceus*: females and sub-adult females, males and sub-adult males, and juveniles (spiderling to instar three) were partitioned and the distance classes. Dashed lines = upper and lower boundaries of 95% confidence interval for values of the autocorrelation coefficient, ***r***, under the null hypothesis of no geographic structure. Solid line connects ***r*** values for each distance class. Bars around computed ***r*** values are 95% bootstrap confidence intervals (Peakall et al. 2003).

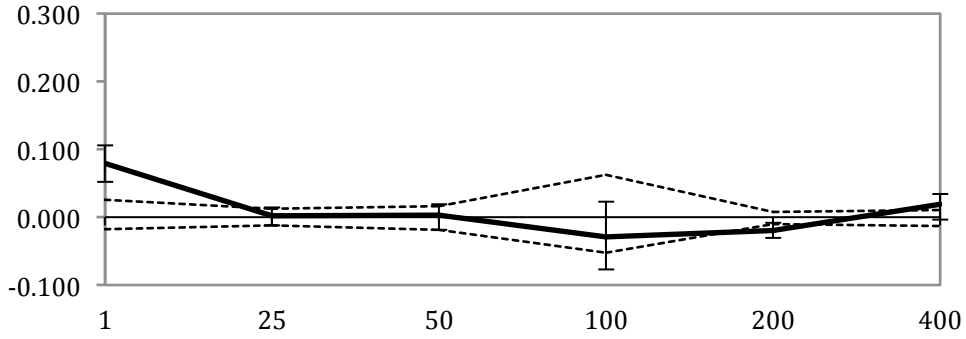
(a) *A. miniaceus* – Females and sub-adult females



(b) *A. miniaceus* – Males and sub-adult males



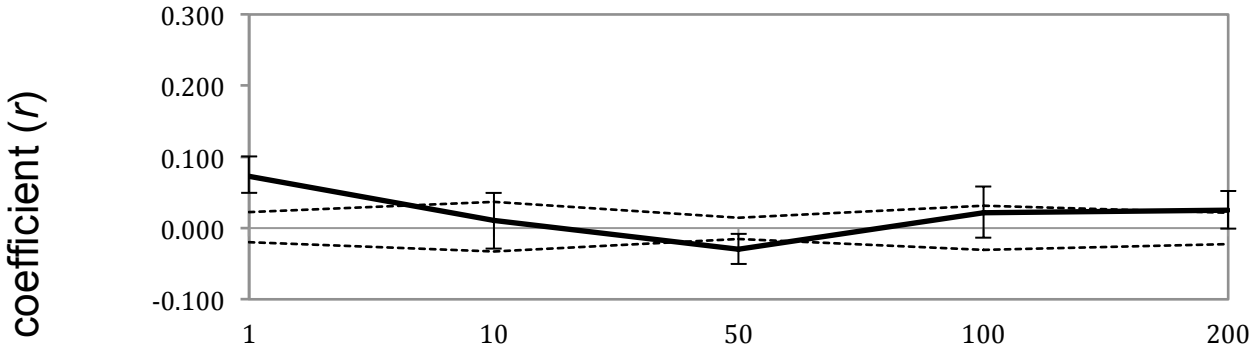
(c) *A. miniaceus* – Juveniles



Autocorrelation coefficient (*r*)

Figure 10. Results of spatial autocorrelation analyses for adults and juveniles of the group-living species, *A. kumadai.* Dashed lines = upper and lower boundaries of 95% confidence interval for values of the autocorrelation coefficient, ***r***, under the null hypothesis of no geographic structure. Solid line connects ***r*** values for each distance class. Bars around computed ***r*** values are 95% bootstrap confidence intervals (Peakall et al. 2003).

(a) *A. kumadai* - adults



(b) *A. kumadai* - juveniles

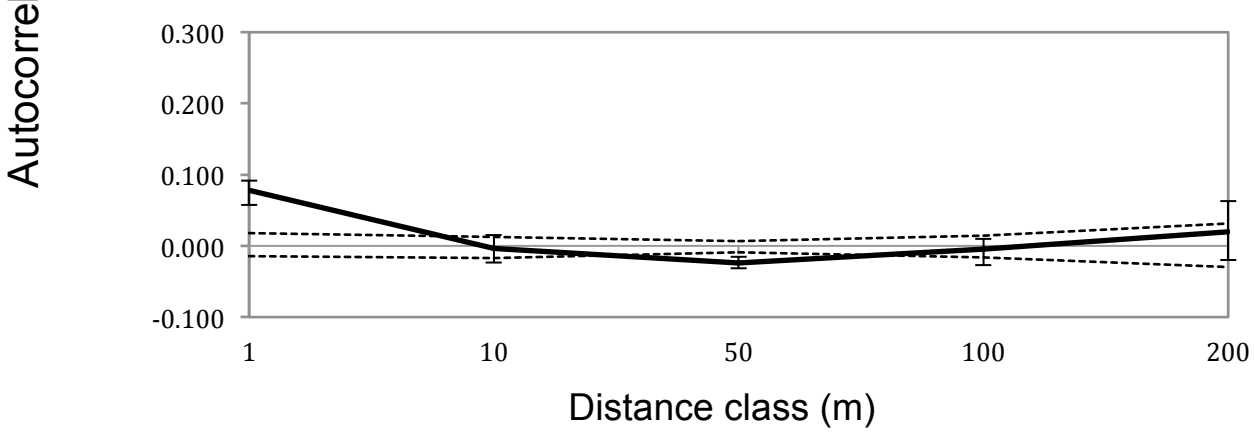
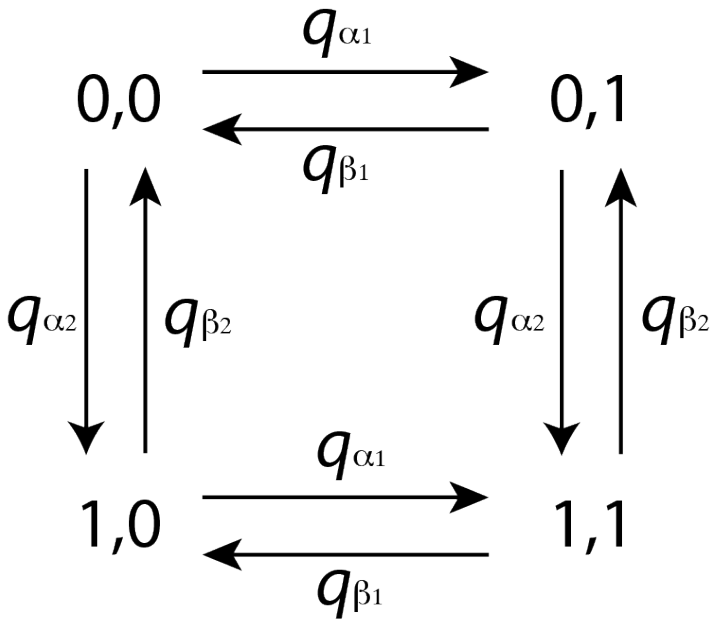


Figure 11. Parameters used in correlated character evolution tests. These describe the possible relationships between two characters (e.g., Character 1 and Character 2, or Type of social system and Type of host), each of which can exist in one of two states (e.g., 0 and 1, or non-group-living and group-living). The possible combinations of states of character 1 and character 2 are presented as {state of character 1, state of character 2}. The combinations of the character states of two characters are numbered as 1={0,0}, 2={0,1}, 3={1,0}, and 4={1,1}. (a) Independent model: there are four parameters, $q_{\alpha 1}$, $q_{\alpha 2}$, $q_{\beta 1}$, and $q_{\beta 1}$, which represent the transition rates between two states of one character, given that these transitions are independent of the states of the other character. (b) Dependent, or correlated, model: there are eight parameters because each transition from one combination of character states to another combination was assigned as a separate parameter, e.g., the transition rate parameter from {0,0} to {0,1}=q_{12}. The transition rate parameter would be {1,0} to {1,1}=q_{34}, which is different from q_{12}, event the second character state switching from 0 to 1 is the same as the earlier situation. Therefore, depending on the state of character 1, there would be two transition rate parameters when the state of character 2 switching from 0 to 1. This model allows us to estimate the likelihood of a character switching event in one character, given a character state of the other character, which is a test of the dependency of character switching events of two characters.

(a)


$q_{\alpha 1}$
0,0
0,1
$q_{\beta 1}$
$q_{\alpha 2}$
$q_{\beta 2}$
$q_{\alpha 2}$
$q_{\beta 2}$
$q_{\alpha 1}$
1,0
1,1
$q_{\beta 1}$

(b)

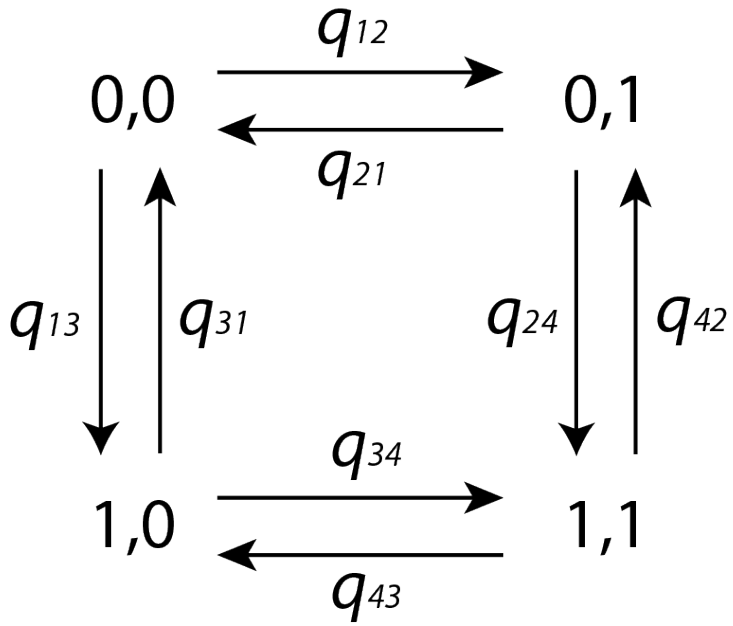

q_{12}
0,0
0,1
q_{21}
q_{13}
q_{31}
q_{24}
q_{42}
q_{34}
1,0
1,1
q_{43}

Figure 12. The preferred Bayesian phylogenetic tree based on the combined data from six genes. Nodal support values include posterior probability (≥0.9) from the Bayesian analysis. likelihood bootstraps (≥ 50%), and parsimony bootstraps (≥ 50%) respectively. If likelihood or parsimony methods did not support the Bayesian results, or formed a polytomy, this result is indicated with an "X".

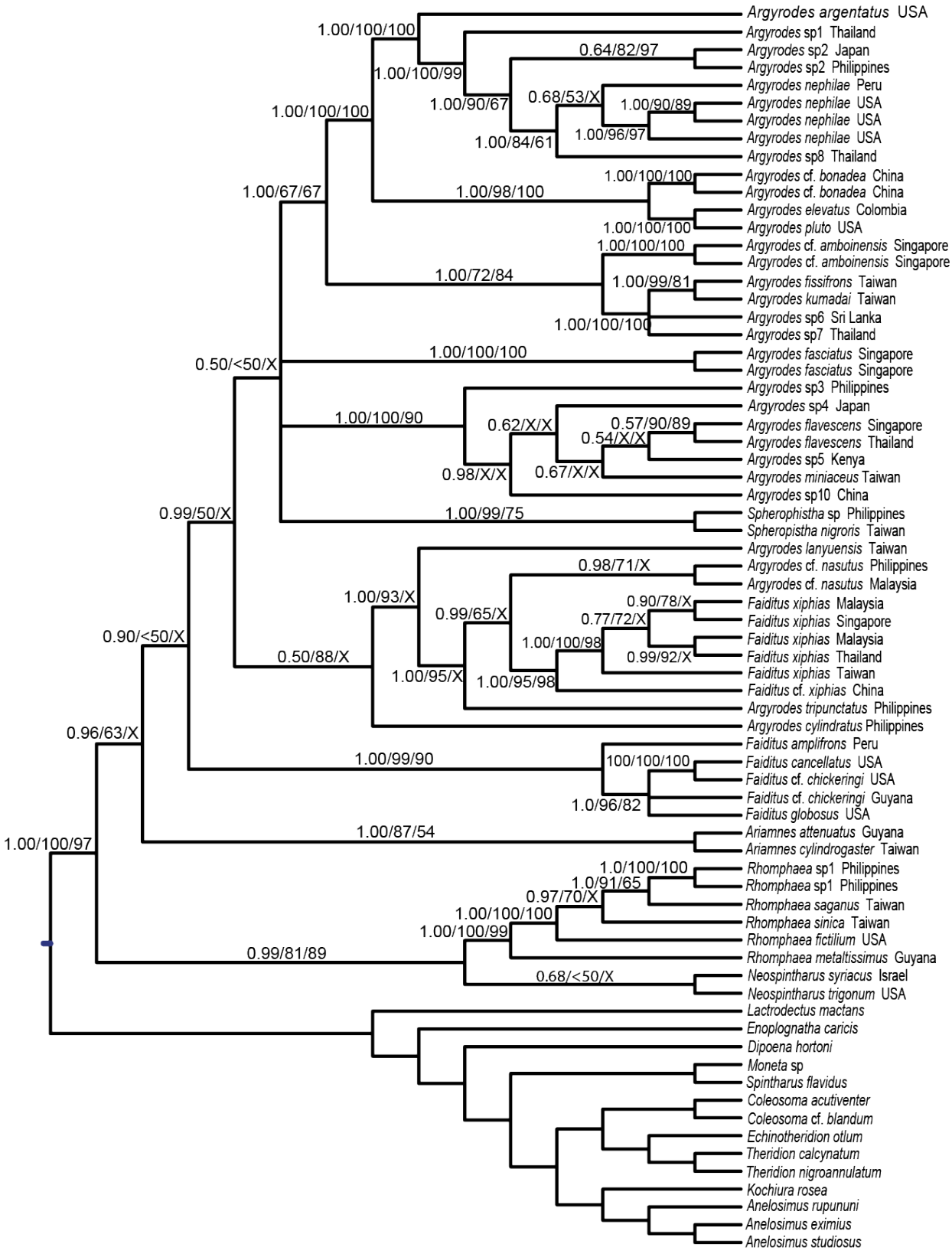

Argyrodes argentatus USA
Argyrodes sp1 Thailand
Argyrodes sp2 Japan
Argyrodes sp2 Philippines
Argyrodes nephilae Peru
Argyrodes nephilae USA
Argyrodes nephilae USA
Argyrodes nephilae USA
Argyrodes sp8 Thailand
Argyrodes cf. bonadea China
Argyrodes cf. bonadea China
Argyrodes elevatus Colombia
Argyrodes pluto USA
Argyrodes cf. amboinensis Singapore
Argyrodes cf. amboinensis Singapore
Argyrodes fissifrons Taiwan
Argyrodes kumadai Taiwan
Argyrodes sp6 Sri Lanka
Argyrodes sp7 Thailand
Argyrodes fasciatus Singapore
Argyrodes fasciatus Singapore
Argyrodes sp3 Philippines
Argyrodes sp4 Japan
Argyrodes flavescens Singapore
Argyrodes flavescens Thailand
Argyrodes sp5 Kenya
Argyrodes miniaceus Taiwan
Argyrodes sp10 China
Spherophistha sp Philippines
Spheropistha nigroris Taiwan
Argyrodes lanyuensis Taiwan
Argyrodes cf. nasutus Philippines
Argyrodes cf. nasutus Malaysia
Faiditus xiphias Malaysia
Faiditus xiphias Singapore
Faiditus xiphias Malaysia
Faiditus xiphias Thailand
Faiditus xiphias Taiwan
Faiditus cf. xiphias China
Argyrodes tripunctatus Philippines
Argyrodes cylindratus Philippines
Faiditus amplifrons Peru
Faiditus cancellatus USA
Faiditus cf. chickeringi USA
Faiditus cf. chickeringi Guyana
Faiditus globosus USA
Ariamnes attenuatus Guyana
Ariamnes cylindrogaster Taiwan
Rhomphaea sp1 Philippines
Rhomphaea sp1 Philippines
Rhomphaea saganus Taiwan
Rhomphaea sinica Taiwan
Rhomphaea fictilium USA
Rhomphaea metaltissimus Guyana
Neospintharus syriacus Israel
Neospintharus trigonum USA
Lactrodectus mactans
Enoplognatha caricis
Dipoena hortoni
Moneta sp
Spintharus flavidus
Coleosoma acutiventer
Coleosoma cf. blandum
Echinotheridion otlum
Theridion calcynatum
Theridion nigroannulatum
Kochiura rosea
Anelosimus rupununi
Anelosimus eximius
Anelosimus studiosus
1.00/100/100
1.00/100/99
0.64/82/97
1.00/90/67
0.68/53/X
1.00/90/89
1.00/84/61
1.00/96/97
1.00/100/100
1.00/67/67
1.00/98/100
1.00/100/100
1.00/100/100
1.00/72/84
1.00/99/81
1.00/100/100
0.50/<50/X
1.00/100/100
1.00/100/90
0.62/X/X
0.57/90/89
0.54/X/X
0.98/X/X
0.67/X/X
0.99/50/X
1.00/99/75
0.98/71/X
1.00/93/X
0.90/78/X
0.99/65/X
0.77/72/X
0.90/<50/X
1.00/100/98
0.50/88/X
0.99/92/X
1.00/95/X
1.00/95/98
0.96/63/X
1.00/99/90
100/100/100
1.0/96/82
1.00/87/54
1.00/100/97
1.0/100/100
1.0/91/65
0.97/70/X
1.00/100/100
1.00/100/99
0.99/81/89
0.68/<50/X

Figure 13. The summary consensus tree resulting from a partitioned Bayesian analysis of the concatenated dataset for six individual genes. I use the seven boxes to present the posterior probability supports of the nodes on the cladogram (see key). Black, shaded boxes indicate a nodal posterior probability support larger than 0.9 in the individual gene data or in combined data; unshaded boxes correspond to nodes with support lower than 0.9. The name of each species group was labeled.

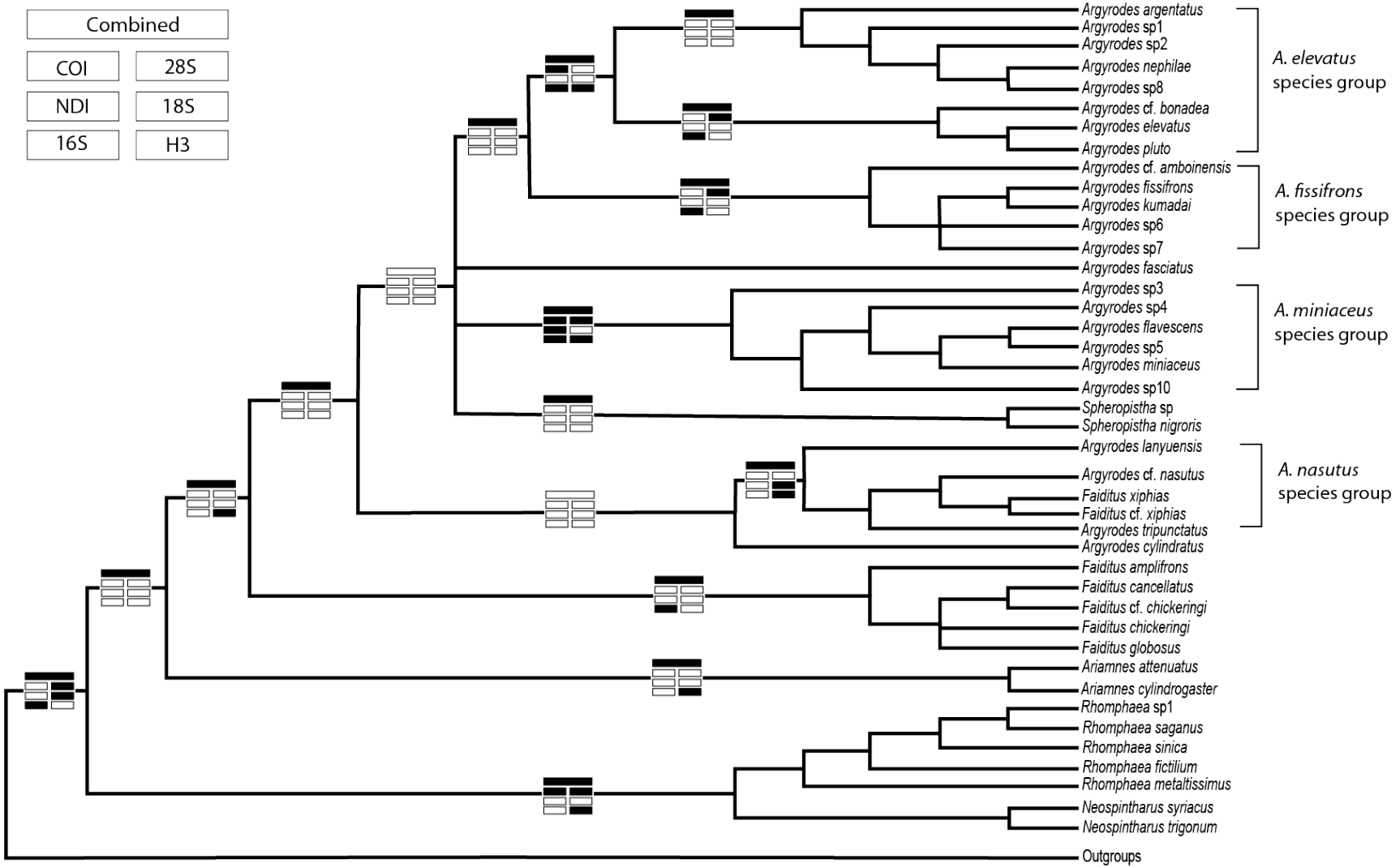
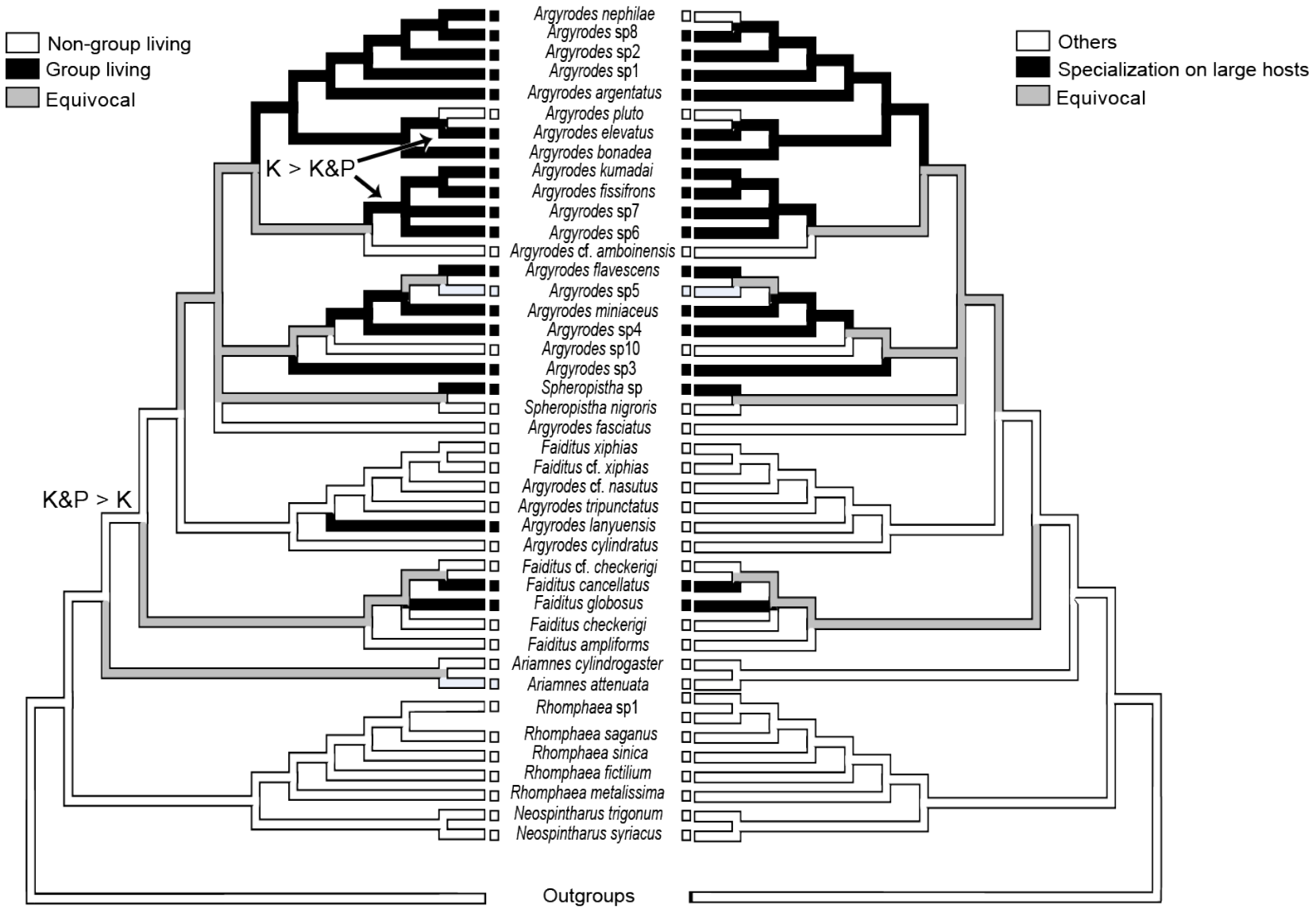

Combined
COI
28S
NDI
18S
16S
H3
Argyrodes argentatus
Argyrodes sp1
Argyrodes sp2
Argyrodes nephilae
Argyrodes sp8
Argyrodes cf. bonadea
Argyrodes elevatus
Argyrodes pluto
A. elevatus species group
Argyrodes cf. amboinensis
Argyrodes fissifrons
Argyrodes kumadai
Argyrodes sp6
Argyrodes sp7
A. fissifrons species group
Argyrodes fasciatus
Argyrodes sp3
Argyrodes sp4
Argyrodes flavescens
Argyrodes sp5
Argyrodes miniaceus
Argyrodes sp10
A. miniaceus species group
Spheropistha sp
Spheropistha nigroris
Argyrodes lanyuensis
Argyrodes cf. nasutus
Faiditus xiphias
Faiditus cf. xiphias
Argyrodes tripunctatus
Argyrodes cylindratus
A. nasutus species group
Faiditus amplifrons
Faiditus cancellatus
Faiditus cf. chickeringi
Faiditus chickeringi
Faiditus globosus
Ariamnes attenuatus
Ariamnes cylindrogaster
Rhomphaea sp1
Rhomphaea saganus
Rhomphaea sinica
Rhomphaea fictilium
Rhomphaea metaltissimus
Neospintharus syriacus
Neospintharus trigonum
Outgroups

Figure 14. Results of likelihood ancestral state reconstructions of group-living behavior, specialization on large hosts, and foraging strategy. The mirror tree format was used to present the character state switching events of social system and type of host. There are at least six character switching events in the tree on the right; black branches and boxes indicate group-living, white branches and boxes indicate non-group-living, grey branches show equivocal states; grey boxes indicate species for which data are missing. The tree on the left shows the character switching events for type of host. Black branches and boxes indicate specialization on large hosts, white branches and boxes show other behaviors, i.e., free-living, generalists, and specialists on small hosts. Grey branches indicate equivocal reconstructions, and grey boxes indicate species for which data are missing. The third behavioral character, foraging strategy, was also mapped on the left tree. There are three character state switching (K&P= the state in which a species uses both kleptoparasitism and predation, K=the state of using kleptoparasitism only). The first switching event from K&P to K represents the origin of "kleptoparasitism only" foraging strategy. This switching event is earlier than all events involving a switch from solitary to group-living behavior. After the origin of kleptoparasitism, there are two reversals (from K to K&P) in species *A. fissifrons* and *A. elevatus.*


Non-group living
Group living
Equivocal
Others
Specialization on large hosts
Equivocal
K > K&P
K&P > K
Argyrodes nephilae
Argyrodes sp8
Argyrodes sp2
Argyrodes sp1
Argyrodes argentatus
Argyrodes pluto
Argyrodes elevatus
Argyrodes bonadea
Argyrodes kumadai
Argyrodes fissifrons
Argyrodes sp7
Argyrodes sp6
Argyrodes cf. amboinensis
Argyrodes flavescens
Argyrodes sp5
Argyrodes miniaceus
Argyrodes sp4
Argyrodes sp10
Argyrodes sp3
Spheropistha sp
Spheropistha nigroris
Argyrodes fasciatus
Faiditus xiphias
Faiditus cf. xiphias
Argyrodes cf. nasutus
Argyrodes tripunctatus
Argyrodes lanyuensis
Argyrodes cylindratus
Faiditus cf. checkerigi
Faiditus cancellatus
Faiditus globosus
Faiditus checkerigi
Faiditus ampliforms
Ariamnes cylindrogaster
Ariamnes attenuata
Rhomphaea sp1
Rhomphaea saganus
Rhomphaea sinica
Rhomphaea fictilium
Rhomphaea metalissima
Neospintharus trigonum
Neospintharus syriacus
Outgroups