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Original article

Next-generation sequencing analysis of *Pardosa pseudoannulata's* diet composition in different habitats

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ABSTRACT

Spiders are the most common and predominant predators in terrestrial ecosystems. The predatory behavior of spiders affects the energy flow across the food web within an ecosystem. Traditiaonal methods for analyzing spider diets such as field observation, anatomy and faeces analysis are not suitable for spider experiments due to spiders' special dietary behavior. The molecular method based on the specific primers of prey DNA seems to be inefficient either in spite of its wide application in diet analysis. As the next-generation sequencing (NGS) technology becomes prevalent in many different areas, several cases of the NGS-based analysis of mammal diets have been published. This study analyzed the diet differences of *Pardosa pseudoannulata* (Araneae: Lycosidae) in four habitats (a wetland, a tea plantation, an alpine meadow and a paddy field) by using the NGS technology, combined with the DNA barcode method. The results suggested that the *Pardosa pseudoannulata* feed on a broad range of prey, and 7 orders and 24 families of insects were detected in the four investigated habitats. Moreover, it is found that the diet diversity of *Pardosa pseudoannulata* is greatly influenced by their living environments and seasons. In a nutshell, this study established an NGS-based methodology for spider diets analysis, and the results provided some basic materials to inform the protection and utilization of the *Pardosa pseudoannulata* as a potential eco-friendly predator against pests.

1. Introduction

Spiders are the most common and predominant predators in terrestrial ecosystems (Nyffeler and Benz, 1987). Nyffeler and Birkhofer (2017) estimated that prey killed by the global spider community annually weighs about 400–800 million tons. Diet analysis is considered a core element in the study of animal nutrition needs, prey strategies, habitat utilization and interspecific relationships in ecosystems (Pompanon et al., 2012). As a generalist predator, spiders have predatory behavior that reportedly affects the energy flow across the ecosystem food web (Yang et al., 2017b, Suenaga and Hamamura, 2014). In the past, diet analysis was mainly based on field observation or residual fragments detection of prey from intestine or feces. These approaches are timing time-consuming, and require sophisticated expertise. Above all, they often fail to be accurate. Most spider species hunt nocturnally, and the body fluids of prey would be digested after being sucked in. The feeding characteristics, therefore, make it difficult to obtain the spider prey lists through the conventional methods mentioned above (Furlong, 2015). With the continuous development of molecular biology, more rapid, accurate and economical experimental methods have been available, and the predation mass of spiders can be exactly measured, marking the evolution of diet analysis from macroscope to microscope (Sheppard and Harwood, 2005). The single PCR (Sint et al., 2011) and multiplex PCR methods (Staudacher et al., 2016), temperature gradient gel electrophoresis (Harper et al., 2007), DNA barcodes and molecular cloning (Greenstone et al., 2014, Hamback et al., 2016), stable isotope analysis (Sanders et al., 2015, Speir et al., 2014), among others, have been applied to the study of diet analysis. However, these

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molecular biological methods remain inefficient when massive DNA information needs to be analyzed in diet experiments.

Global commercialization of the Next-Generation Sequencing (NGS) technology enabled us to obtain large amounts of genomic data quickly at a low cost. The NGS has been applied to several fields in ecological and biodiversity analysis, including taxon identification (Valentini et al., 2009, Shendure and Ji, 2008). The metabarcoding method in combination with DNA taxonomy and the NGS technology, which has become more popular, offers an ideal approach for food analysis (Ji et al., 2013, Coissac et al., 2012, Taberlet et al., 2012). Soininen et al. (2009) analyzed the gastric contents of *Microtus oeconomus* and *Myodes rufocanus* using both the traditional micro-histology and metabarcoding methods, and came to the conclusion that the metabarcoding method is more accurate since human-induced deviations could be avoided. Shehzad et al. (2012) identified 18 prey taxa of leopard cats (*Prionailurus bengalensis*) from 38 faeces using the NGS technology. Meredith et al. (2016) studied the feeding habits of *Chrysaora quinquecirrha* in Barnegat Bay and indicated that the "shotgun" NGS approach could supplement visual identification methods.

Pardosa pseudoannulata (Araneae: Lycosidae) is a perennial dominant species in China's farmland (J.J. et al., 2001), and could be seen throughout China. Its impressive predatory ability and the broad ecological niche endow *Pardosa pseudoannulata* an important role in pest control (Wang, 2007). In this study, the NGS technology was used in combination with the DNA barcode method to analyze the diet components of *Pardosa pseudoannulata*. The results presented the prey taxa of *Pardosa pseudoannulata* in four different habitats (a wetland, a tea plantation, an alpine meadow and a paddy field) and provided significant evidence that the diet diversity of spiders can be influenced by their living environments and seasons.

2. Materials and methods

2.1. Sample collection

A total number of 150 *Pardosa pseudoannulata* were collected with clean 50 mL centrifuge tubes between June to September 2014 (Table 1). In order to prevent the further digestion of the prey, the samples were immediately frozen in ice boxes, taken back to the laboratory and stored at −80 °C.

2.2. Prime design

Mitochondrial cytochrome oxidase subunit I (COI) has been generally recognized as a standard "taxon barcode" for most animal groups, and has a wide application (Hebert et al., 2003, Meyer, 2015, Hoareau and Boissin, 2010). The hypervariable region across the COI gene can categorize incognizable animal samples into different taxa by sequencing and comparing the results with reference databases that contain millions of species gene information. However, the amplification products of classical versatile primers (LCO1490 and HCO2198) have a length of 658 bp, which is likely to cause degradation or breakage (Jo et al., 2016, Huber et al., 2009). In addition, the longer the distance between forward and reverse primers, the better the required integrity of the prey target gene sequence and the lower the identification efficiency. Therefore, ideal primers used for diet analysis should have high classification coverage and resolution, and the target DNA fragments should to be short (Casiraghi et al., 2010). In order to increase the efficiency and accuracy of the taxon detection and identification, we modified metabarcoding primer sequences proposed by Leray et al. (2013) to enhance the adaptability of the degenerate bases (Table 2).

2.3. DNA extraction and library preparation

The gut contents of all sample spiders were taken, mixed and homogenized by groups. All the samples were digested with 360 μL of ATL Buffer (Qiagen) and 40 μL of Protease (Takara) overnight. The DNA extraction was performed according to the kit instruction (DNeasy Blood & Tissue Kit, Qiagen), and ultrapure water was used to substitute for the sample DNA as a negative control throughout the extraction process. The concentration and quality of the extracted DNA were tested with Nanodrop spectrophotometers (Thermo Scientific).

Library preparation consisted of two PCR steps. The first round of PCR was performed in a final volume of 20 μL. Each tube contained: 13.0 μL of H_2O, 2 μL of 10 × PCR Buffer, 1.6 μL of dNTP (10 mmol/L), 0.1 μL of Ex Taq enzyme (Takara), 0.4 μL of each primer (10 μmol/L), 1 μL of bovine albumin serum (Roche Diagnostic) and 1.5 μL of the sample DNA. The PCR protocol was comprised of an initial step of 4 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 50 °C, 90 s at 72 °C, and a final cycle of 10 min at 72 °C. Each library was repetitively amplified for three times.

The PCR products of the first round of amplification were mixed and subject to the second round of PCR, which was also performed in a final volume of 20 μL. The amplified protocol and program of the second round of PCR was consistent with those of the first round of PCR, except that 1.5 μL of the preceding PCR products were used as DNA templates, 0.4 μL of the second-round primes (10 μmol/L) were added to replace the previous primers. Each library was repetitively amplified for three times. About 60 μL of the final product was mixed, electrophoresed by 2% agarose, and ~500 bp DNA bands were purified and recovered using QIAquick Gel Extraction Kit (Qiagen).

2.4. DNA sequencing

The purified PCR products were sent to the Kunming Institute of Zoology, Chinese Academy of Sciences (CAS) for sequencing. The sequence dates were carried out on the Illumina Miseq platform, the Paired-end method was uesd and the target fragment size was 319 bp.

3. Results

3.1. Sequencing data analysis

As more and more amplicon sequencing studies were published, it was found that slight contamination in the sequencing

Table 1
Collection of *Pardosa pseudoannulata* from four different habitats.

Sampling location	Sampling time	Sample size	Habitat	Longitude and latitude
Jindian mountain	2014.9	60	Wetland	102°47′E, 25°5′N
Gaoligong mountain	2014.7	30	Tea plantation	98°49′E, 25°59′N
Middle reaches of N'Mai river	2014.7	30	Alpine meadow	98°21′E, 27°54′N
Paddy field of Hunan Academy of Agricultural Sciences	2014.6	30	Paddy field	113°5′E, 28°11′N

Table 2
Prime sequences for NGS based on COI.

PCR round	Group	Primer sequences（5'-3'）
First PCR	Wetland	F-primer：CCTAAACTACGGGGTCAACAAATCATAAAGATATTGG
		R-primer：CCTAAACTACGGGGNGGRTANANNGTYCANCCNGYNCC
	Tea plantation	F-primer：GTGGTATGGGAGTGGTCAACAAATCATAAAGATATTGG
		R-primer：GTGGTATGGGAGTGGNGGRTANANNGTYCANCCNGYNCC
	Alpine meadow	F-primer：TGTTGCGTTTCTGTGGTCAACAAATCATAAAGATATTGG
		R-primer：TGTTGCGTTTCTGTGGNGGRTANANNGTYCANCCNGYNCC
	Paddy field	F-primer：GTTACGTGGTTGATGAGGTCAACAAATCATAAAGATATTGG
		R-primer：GTTACGTGGTTGATGAGGNGGRTANANNGTYCANCCNGYNCC
Second PCR	Wetland	F-primer：CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTAAACTACGG
		R-primer：AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTAAACTACGG
	Tea plantation	F-primer：CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGTATGGGAGT
		R-primer：AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGGTATGGGAGT
	Alpine meadow	F-primer：CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTTGCGTTTCTGT
		R-primer：AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTTGCGTTTCTGT
	Paddy field	F-primer：CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTACGTGGTTGATGA
		R-primer：AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTACGTGGTTGATGA

Note: The box sequence is the index sequence, the underline sequence is the Linker sequence.

process was unavoidable, therefore sequences with reads below a certain threshold should be excluded in the final data analysis (Deagle et al., 2009, Binladen et al., 2007). In this study, sequences with reads below 10 were excluded from the final results. After removing remove chimeras with Uchime, we obtained a total of 14,262 valid reads after eliminating the predator and problematic sequences. Sequences whose similarity is greater than 97% were defined as an Operational taxonomic units (OTU), and a total of 63 OTUs were clustered. The exact information of species was searched adopting the Statistical Assignment Package (SAP) (Munch et al., 2008) approach in GenBank.

3.2. Diet analysis of Pardosa pseudoannulata

Due to the absence of comprehensive gene information from public databases, it is challeging to identify more accurate classification based on the sequencing results. In this study, 7 out of 63 OTUs were identified to Insecta only and the remaining 56 were identified to order, of which 40 were identified to family, 25 to genus, and only 15 to species (Table 3). The results showed that the predation efficiency of *Pardosa pseudoannulata* on *Coleoptera* and *Diptera* was 52% and 28% respectively, significantly higher than that of other insects. This could be resulted from higher sensitivities of ocelli to the change of ambient light, and highly mobile insects (*Coleoptera* and *Diptera*) that would cause more detectable light changes around *Pardosa pseudoannulata* were more likely to be preyed on. This coud also be attributed to the long-term evolution of the predatory behavior of spiders, which resulted in the predatory preference in a certain ecological environment.

3.3. The diet diversity of Pardosa pseudoannulata in different habitats

Habitats affect the diet diversity of spiders (Sanders et al., 2015). By sorting the OTUs using the index sequence, the prey taxa of *Pardosa pseudoannulata* in different habitats were then obtained (Table 4).

The sampled wetland is located at the foot of the Jindian mountain in the eastern outskirts of Kunming city. It has a subtropical plateau monsoon climate with a wide range of vegetation types and diverse biocenoses. Its geographic characteristics coupled with random predatory behavior of spiders determined the most abundant prey taxa of *Pardosa pseudoannulata* in this location. The sampled alpine tea plantation and meadow are both located in dry lands with altitudes of about 3500 m. These two places have a cool and dry climate with a single vegetation, and the prey taxa of *Pardosa pseudoannulata* are relatively simple. The biocenosis of paddy fields are accepted to be flourishing (Wang et al., 2017). During the outbreak of pests, the population densities of prey (such as planthoppers and leafhoppers) will balloon, and spiders can feed themselves easily. However, in this study, the results indicated that the prey taxa of *Pardosa pseudoannulata* were not as abundant as expectated. The possible explaination is as follows: the sampling took place at the end of June, when early rice had been harvested and late rice has not yet been sown. During this period, the most common pests in southern Chinese paddy fields (such as *Nilaparvata lugens*, *Laodelphax striatellus* and *Cnaphalocrocis medinalis*) migrated to other surrounding habitats for foraging. The frequent use of pesticides during the rice growing period could also lead to a significant decline of the pests population during the alternate husbandry period. Therefore, the gene fragments of those common pests were not detected in the guts of spiders, and the prey taxa of *Pardosa pseudoannulata* appeared to be relatively scarce under this seasonal condition. The histograms present a more visualized comparison of the prey taxa in four habitats (Figs. 1–3).

The diversity of spider prey taxa and the capacity of spider predation are largely affected by the environmental conditions such as vegetation types, biocenosis diversity, geographical locations and seasonal conditions, etc. Generally speaking, the diet of *Pardosa*

Table 3
List of diet items (OTUs) sequenced from gut contents of *Pardosa pseudoannulata*.

OTUs	order	Ident	family	Ident	genus	Ident	species	Ident
OTU 133	*Coleoptera*	100%	*Coccinellidae*	100%	*Azoria*	100%	*Azoria bayeri*	100%
OTU 420	*Coleoptera*	100%	*Carabidae*	100%	*Bembidion*	96%	*Bembidion parviceps*	84%
OTU 421	*Coleoptera*	97%	*Carabidae*	97%	*Bembidion*	95%	*Bembidion salinarium*	95%
OTU 322	*Coleoptera*	98%	*Throscidae*	98%	*Pactopus*	98%	*Pactopus hornii*	98%
OTU 2230	*Coleoptera*	100%	*Melandryidae*	86%	*Rushia*	83%	*Rushia parreyssi*	83%
OTU 192	*Coleoptera*	95%	*Carabidae*	95%	*Bembidion*	92%		
OTU 925	*Coleoptera*	100%	*Curculionidae*	100%	*Curculio*	100%		
OTU 215	*Coleoptera*	100%	*Chrysomelidae*	100%	*Gonioctena*	94%		
OTU 898	*Coleoptera*	100%	*Curculionidae*	100%	*Scolytoplatypus*	100%		
OTU 946	*Coleoptera*	100%	*Cleridae*	100%	*Stigmatium*	82%		
OTU 433	*Coleoptera*	100%	*Throscidae*	100%	*Trixagus*	100%		
OTU 246	*Coleoptera*	100%	*Chrysomelidae*	100%				
OTU 251	*Coleoptera*	100%	*Chrysomelidae*	100%				
OTU 4105	*Coleoptera*	100%	*Chrysomelidae*	100%				
OTU 422	*Coleoptera*	100%	*Coccinellidae*	100%				
OTU 741	*Coleoptera*	100%	*Oedemeridae*	100%				
OTU 20	*Coleoptera*	100%	*Scarabaeidae*	100%				
OTU 398	*Coleoptera*	100%	*Scarabaeidae*	100%				
OTU 78	*Coleoptera*	100%	*Scarabaeidae*	100%				
OTU 110	*Coleoptera*	100%						
OTU 112	*Coleoptera*	100%						
OTU 115	*Coleoptera*	100%						
OTU 3565	*Coleoptera*	100%						
OTU 37	*Coleoptera*	100%						
OTU 3842	*Coleoptera*	89%						
OTU 390	*Coleoptera*	95%						
OTU 4351	*Coleoptera*	100%						
OTU 694	*Coleoptera*	96%						
OTU 88	*Coleoptera*	100%						
OTU 1203	*Diptera*	100%	*Tachinidae*	99%	*Chrysoexorista*	99%	*Chrysoexorista dawsoni*	99%
OTU 419	*Diptera*	100%	*Stratiomyidae*	100%	*Hermetia*	100%	*Hermetia illucens*	100%
OTU 294	*Diptera*	100%	*Mycetophilidae*	100%	*Mycomya*	100%	*Mycomya circumdata*	100%
OTU 2746	*Diptera*	100%	*Stratiomyidae*	100%	*Odontomyia*	100%	*Odontomyia garatas*	100%
OTU 4895	*Diptera*	100%	*Dolichopodidae*	99%	*Dolichopus*	98%		
OTU 375	*Diptera*	100%	*Mycetophilidae*	100%	*Exechia*	86%		
OTU 2172	*Diptera*	100%	*Phoridae*	99%	*Megaselia*	99%		
OTU 4777	*Diptera*	100%	*Phoridae*	98%	*Megaselia*	98%		
OTU 2219	*Diptera*	100%	*Chironomidae*	100%				
OTU 54	*Diptera*	92%	*Chironomidae*	89%				
OTU 2254	*Diptera*	100%	*Dolichopodidae*	100%				
OTU 4635	*Diptera*	100%	*Sciaridae*	100%				
OTU 1925	*Diptera*	96%						
OTU 3672	*Diptera*	100%						
OTU 4683	*Diptera*	82%						
OTU 326	*Hemiptera*	100%	*Reduviidae*	100%	*Triatoma*	100%	*Triatoma dimidiata*	100%
OTU 4682	*Hemiptera*	100%	*Plataspidae*	90%				
OTU 2493	*Hemiptera*	100%						
OTU 3262	*Hymenoptera*	100%	*Formicidae*	100%				
OTU 810	*Hymenoptera*	100%	*Tenthredinidae*	100%				
OTU 129	*Hymenoptera*	100%						
OTU 3461	*Lepidoptera*	100%	*Lycaenidae*	100%	*Luthrodes*	100%	*Luthrodes pandava*	98%
OTU 272	*Lepidoptera*	100%	*Pieridae*	100%	*Pieris*	100%	*Pieris canidia*	100%
OTU 1708	*Lepidoptera*	100%	*Lycaenidae*	100%	*Pseudozizeeria*	97%	*Pseudozizeeria maha*	97%
OTU 1877	*Lepidoptera*	100%						
OTU 311	*Psocoptera*	100%	*Amphipsocidae*	100%	*Polypsocus*	100%	*Polypsocus corruptus*	100%
OTU 2353	*Trichoptera*	100%	*Lepidostomatidae*	100%	*Lepidostoma*	100%	*Lepidostoma flavum*	100%

pseudoannulata is more diverse in low altitude areas than in high altitude areas; more diverse in diversiform biocenoses than in simplex ones; more diverse during the crop growth period than during the alternate husbandry period, and more diverse in fields where no pesticides are used.

4. Disscusion

It has been more than 50 years since people first used pesticides to control farmland pests (Soloneski and Larramendy, 2013). Although pesticides clearly have advantages for they are highly efficient, simple to use and easy to promote, but they also increase the cost of farmland insect control. More importantly, the use of pesticides have posed significant negative effects, including the generally acknowledged pesticide residues in vegetables, ecological environment destruction and in vivo pest resistance gene generation (Yang et al., 2017a, Riaz et al., 2009, Turgut et al., 2011, Hou et al., 2001). In the modern society, people pay more attention to improving their quality of life, and focus on food safety and environmental protection. As a novel and eco-friendly approach, biological control of pests has been widely discussed in recent years (Rutledge et al., 2004, Cullen et al., 2008, Orr, 2009). Up to now, 47,061 species of spiders have been found worldwide (Laws and Joern, 2017). As one of the predominant predators in terrestrial ecosystems (Norma-Rashid et al., 2014), spiders have varied hunting behavior, habitat preferences and activity periods. Moreover,

Table 4
Diet diversity of *Pardosa pseudoannulata* in different habitats.

	Prey taxa			
	Wetland	Tea plantation	Alpine meadow	Paddy field
Species				
Azoria bayeri	1	0	0	0
Bembidion parviceps	1	0	1	0
Bembidion salinarium	1	0	0	0
Chrysoexorista dawsoni	1	0	0	0
Hermetia illucens	1	0	0	0
Lepidostoma flavum	1	0	0	0
Luthrodes pandava	1	0	0	0
Mycomya circumdata	0	0	0	1
Odontomyia garatas	1	0	0	0
Pactopus hornii	1	0	0	0
Pieris canidia	0	0	0	1
Polypsocus corruptus	1	0	0	0
Pseudozizeeria maha	0	0	0	1
Rushia parreyssi	1	0	0	0
Triatoma dimidiata	0	0	0	2
Genus				
Azoria	1	0	0	0
Bembidion	3	0	2	1
Chrysoexorista	1	0	0	0
Curculio	1	0	0	0
Dolichopus	1	0	0	0
Exechia	0	1	0	0
Gonioctena	1	0	0	0
Hermetia	1	0	0	0
Lepidostoma	1	0	0	0
Luthrodes	1	0	0	0
Megaselia	2	0	0	0
Mycomya	0	0	0	1
Odontomyia	1	0	0	0
Pactopus	1	0	0	0
Pieris	0	0	0	1
Polypsocus	1	0	0	0
Pseudozizeeria	0	0	0	1
Rushia	1	0	0	0
Scolytoplatypus	1	0	0	0
Stigmatium	1	0	0	0
Triatoma	0	0	0	2
Trixagus	1	0	0	0
Family				
Amphipsocidae	1	0	0	0
Carabidae	3	0	2	1
Chironomidae	1	0	0	0
Chrysomelidae	2	0	0	2
Cleridae	1	0	0	0
Coccinellidae	2	0	0	0
Curculionidae	2	0	0	0
Dolichopodidae	2	0	0	0
Formicidae	0	0	0	1
Lepidostomatidae	1	0	0	0
Lycaenidae	1	0	0	1
Melandryidae	1	0	0	0
Mycetophilidae	0	1	0	1
Oedemeridae	1	0	0	0
Phoridae	2	0	0	0
Pieridae	0	0	0	1
Plataspidae	1	0	0	0
Reduviidae	0	0	0	2
Scarabaeidae	3	0	0	1
Sciaridae	1	0	0	0
Stratiomyidae	2	0	0	0
Tachinidae	1	0	0	0
Tenthredinidae	0	0	0	1
Throscidae	2	0	0	0

spiders have long life cycles and will not cause crops or fruits damage. All of these characteristics make spiders a potential ideal hunter in efficient pest control (Afzal et al., 2013, Clausen, 1986).

Diet analysis plays an important role in ecological research, and it is a prerequisite to understand the energy flow within ecosystems. However, it is difficult to obtain the detailed prey lists of predators in a given habitat, especially those of generalist predators. In order to compare what spiders feed on in different habitats, we need a more economical and efficient method. The power of the NGS technology and its association with the DNA barcode could provide an ideal solution (De Barba et al., 2014). In this solution, a short piece of the standard DNA barcode is amplified using

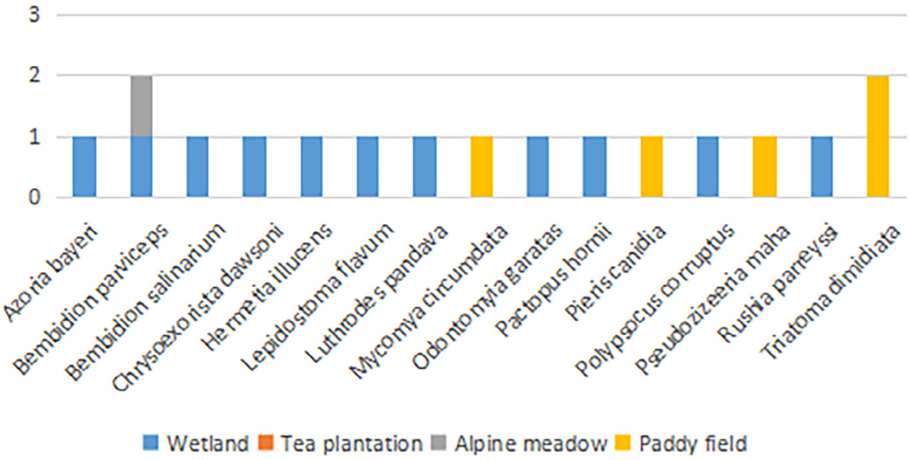

Fig. 1. Diet diversity of *Pardosa pseudoannulata* at species level.

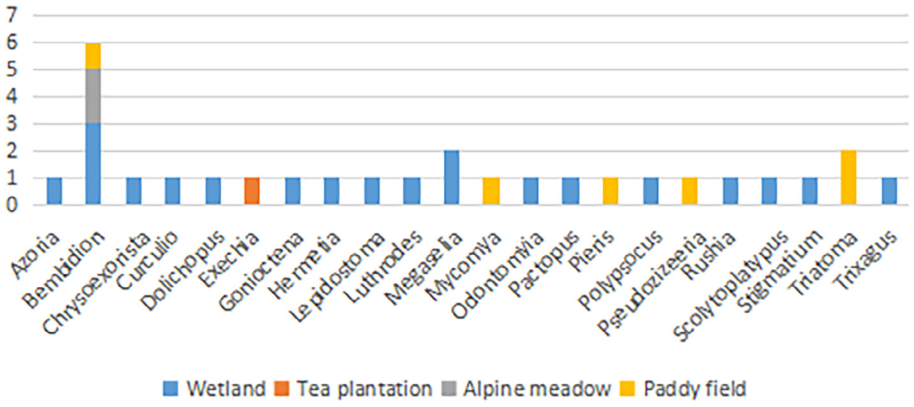

Fig. 2. Diet diversity of *Pardosa pseudoannulata* at genus level.

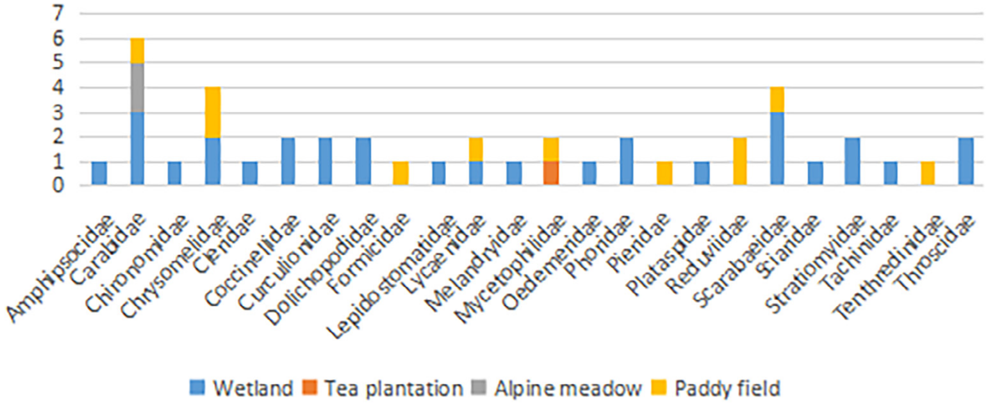

Fig. 3. Diet diversity of *Pardosa pseudoannulata* at family level.

universal primers, under the premise of accuracy, numerous amplicons are sequenced simultaneously via the NGS system to obtain species classification information, which greatly reduce the experimental costs and improve the efficiency (Hajibabaei, 2012). In addition, the original data could be obtained directly with no previous understanding of the target habitats required, and the results could be accepted as important evidence for predator diet analysis.

However, no technology is impeccable. The NGS also has several defects that are worth noting in the process of the experimental design, implementation and datas analysis: 1. During the sequencing process, the reliability of sequencing data gradually decreases because of the declining activity of the Taq enzyme, which is an important factor that limits the read-lengths. In addition, the digestion effect of spiders and the shearing force in the DNA extraction process can fragment the nucleic acid chains, reducing PCR amplification productivity (Leray et al., 2013, Symondson and Harwood, 2014). Therefore, the length of the amplicon needs to be taken into consideration to ensure that the data obtained could be used for later database comparison. 2. While the NGS technology does an excellent job in qualitative analysis of the prey taxa (Amend et al., 2010), it could hardly quantify the amount of the prey DNA compared with qPCR. 3. Barcode information in public resource databases (such as Genebank, BOLD, etc.) is patchy and needs constant update. In diet analysis, many prey OTUs sequences can be only identified to genus, family or even order, and the identification at species level remains chanllenging. As a result, it is difficult to know exactly what predators feed on (Casiraghi et al., 2010, Burgar et al., 2014). That said, the advantages of NGS in diet analysis are irreplaceable.

This study used the NGS method for the first time to analyze the diet of *Pardosa pseudoannulata* in four habitats (a wetland, a tea plantation, an alpine meadow and a paddy field). Under the conditions of the study, the results suggested that the prey taxa of *Pardosa pseudoannulata* was very wide, and in the sample wetland, *Pardosa pseudoannulata* predominatly preyed on *Coleoptera* and *Diptera* insects. In all of the four habitats of this study, 7 orders and 24 families of insects were detected. The living environment and season determined the abundance of insects, which had a great impact on *Pardosa pseudoannulata* diet diversity, the rules of which could be concluded as follows: low altitude areas > high altitude areas; diversiform biocenoses > simplex ones; crop growth period > alternate husbandry period; fields with no pesticide use > fields with pesticide use. The results would provide theoretical support and scientific information for the effective protection and utilization of *Pardosa pseudoannulata*.

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Conflict of interest

The authors declare that they have no competing interests.

Data accessibility

Sequences used for designing primers were provided by the Moorea Biocode Project and sequenced datas performed BLAST searches in GENBANK.

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