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Article

## Life-style classification of some Phytoseiidae (Acari: Mesostigmata) species based on gnathosoma morphometrics

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### Abstract

Life style of phytoseiid species (Acari: Mesostigmata) is an important feature in evaluating their potential as biological control agents. Previous studies suggested that some morphometrics of gnathosoma are related to the feeding habits of phytoseiids. In this study, we showed it is appropriate to estimate feeding habits and/or life styles of phytoseiids based on variations in gnathosoma morphology. Scanning electron microscope pictures of 10 phytoseiid species belonging to four major life styles were taken. For each species, 23 morphometrics of gnathosoma and the length of dorsal shield were measured. Principle Component Analysis (PCA) showed that the 10 species can be divided into three groups based on the first two principle components of these parameters. The 1<sup>st</sup> group includes one specialized predators of *Tetranychus* species (*Phytoseiulus persimilis*) and two selective predators of tetranychid mites (*Neoseiulus pseudolongispinosus*, *Neoseiulus californicus*), the 2<sup>nd</sup> group includes six generalist predators (*Amblyseius orientalis*, *Amblyseius swirskii*, *Amblyseius tsugawai*, *Neoseiulus barkeri*, *Neoseiulus bicaudus*, *Neoseiulus cucumeris*), and the 3<sup>rd</sup> group includes one specialized pollen feeder (*Euseius utilis*). This grouping is consistent with the life style division of McMurtry & Croft (1997), except that Type I and II were not separated. However, there is a trend that both Type II species were closer to each other. Overall, species in Group II have larger chelicerae and hypostome than those of Group I and III, while species in Group III have larger lobes and angle of fixed digit than those of the other two groups.

**Key words:** Phytoseiidae, feeding habit, life style, morphometrics of gnathosoma, Principle Component Analysis

### Introduction

Phytoseiid mites (Acari: Mesostigmata) are widely distributed arthropod predators. This family includes more than 90 genera and 2479 species (Moraes *et al.* 2004; Demite *et al.* 2017). Phytoseiid mites have received increasing attention worldwide due to their potential in biological control of small arthropod pests, such as spider mites, eriophyid mites, thrips and whiteflies and psyllids (Xu & Zhang 2015). More than 30 species are commercially produced as biological control agents, among which some became very successful and have been widely applied in many countries, including *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus cucumeris* (Oudemans), and *Amblyseius swirskii* Athias-Henriot, etc. (Van Lenteren 2012). However, these species account for a very small fraction of the big family. Due to increasing requirements for alternatives of chemical control worldwide, it will be valuable to investigate more biological control candidates among predatory mites. The first step to evaluate the biological control potential of a predator is to identify its feeding habits.

There are huge variations in feeding habits of phytoseiid mites, from very specialist species to quite generalist species. McMurtry & Croft (1997) divided predatory mites in this family into four

life styles primarily basing on their feeding habits, biological and morphological traits: Type I: specialized predators of *Tetranychus* spp., including only *Phytoseiulus* species, e.g. *P. persimilis*. Type II: selective predators of tetranychid mites, referring to species mainly feed on spider mite species that produces “webnest”, e.g. *Neoseiulus californicus* (McGregor), *Galendromus occidentalis* (Nesbitt). Type III: generalist predators, referring to species that consume a wide variety of prey or food such as different kinds of phytophagous mites, thrips, whiteflies, nematodes, pollen, fungus, plant excretates, and honeydew, etc. More than 70% of phytoseiid species belongs to this type. Some representative species are *Amblyseius swirskii*, *Neoseiulus barkeri* (Hughes), *Neoseiulus cucumeris*, etc. Type IV: specialized pollen feeders, referring to generalist predators belonging to the genus *Euseius*, which have higher reproductive potential when feeding on pollen than other types of food (McMurtry & Croft 1997; Croft *et al.* 1998a). McMurtry *et al.* (2013) further divided Type I and Type III into subgroups on the basis of prey preference, and living conditions, respectively. In addition, they added *Iphiseius* into Type IV.

This classification well grouped known species and directed their potentials in biological control, which is highly valuable in Phytoseiidae because some taxonomically similar species may have quite different prey range. The following question is whether it is possible to estimate the prey range of a species before its feeding habits have been well studied. In other words, are there common features of the species within the same life type group that can be achieved easier than trying to rear them on all possible prey?

Pratt *et al.* (1999) tried to estimate the life styles of five species (*P. persimilis*, *Neoseiulus fallacis* (Garman), *Typhlodromus pyri* Scheuten, *Euseius finlandicus* Oudemans and *Euseius hibisci* (Chant)) based on their oviposition rates. Their analyses separated life styles of Type I and Type II species successfully, but classifications of generalists were not consistent with that of life styles classified by McMurtry and Crofts (1997). Luh and Croft (1999) investigated 24 parameters, including mid-dorsal and margin-dorsal setal measurements, oviposition rates, development rate, and prey type, etc., of 37 species belonging to six genera. They declared that development rate is the key feature in determining life-styles. In their study, 32 out of 37 species were grouped into the same life styles as McMurtry and Croft (1997) (Luh and Croft 2001). Croft *et al.* (2004) suggested that life styles are related to multiple feature groups, including food types, morphology, reproduction and development, physiology and behavior, interspecies interactions, and dispersal, etc., which actually mean all aspects of phytoseiid biology. Overall, limited practical methods have been provided to estimate life style of phytoseiid mites without detailed biological experiments or wide range of rearing attempts, which will be very useful in preliminary evaluation for biological control potentials of species newly discovered or have not been well studied.

Predatory mites capture prey and feed using their gnathosoma, which includes tectum, a pair of chelicerae and pedipalpi, hypostome, and gnathobase. Kaneko (1988) and Buryn and Brandl (1992) measured multiple chelicerae morphometrics of oribatid mites and mesostigmatid mites, respectively. Differences among species with different prey types were detected in both studies. Flechtmann and McMurtry (1992a, b) described differences in chelicerae of phytoseiid species with different prey types qualitatively. It is valuable to further estimate whether these differences are sufficient in categorizing phytoseiid life styles quantitatively. Previous studies mainly focus on morphological comparisons among chelicerae, but it will be also valuable to compare other organs, such as tritosternum and hypostome, etc., which also play important roles in predating and feeding (Wernz and Krantz 1976).

In this study, scanning electron microscope (SEM) pictures of 10 phytoseiid species belonging to four major life styles were taken. For each species, 23 morphometrics of gnathosoma and the length of dorsal shield were measured. These measurements include lengths, angles, area, and counts

that described gnathosoma morphology comprehensively and quantitatively, in order to appropriately estimate phytoseiid life styles using a direct method.

## Materials and Methods

Ten species and their life styles used in this study according to McMurtry & Croft (1997) and Wu *et al.* (2008):

I: *P. persimilis*

II: *N. californicus*, *Neoseiulus pseudolongispinosus* Xin, Liang and Ke

III: *Amblyseius orientalis* (Ehara), *A. swirskii*, *Amblyseius tsugawai* Ehara, *N. barkeri*, *Neoseiulus bicaudus* (Wainstein), *N. cucumeris*

IV: *Euseius utilis* Liang & Ke.

Schicha (1975) considered *N. pseudolongispinosus* and *Neoseiulus womersleyi* synonymous, but Chant & McMurtry (2003) and Wu *et al.* (2010) listed *N. pseudolongispinosus* as a separate species. So we consider *N. pseudolongispinosus* as a separate species herein.

Individuals of nine species were collected from lab colonies maintained in the Lab of Predatory Mites for over five years and all species were reared by sufficient *Tetranychus urticae* (Koch) (Prostigmata: Tetranychidae) on *Phaseolus vulgaris* L., Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP-CAAS). *Euseius utilis* individuals were collected from *Lonicera maackii* (Caprifoliaceae) leaves in the yard of the Institute of Food Sciences and Technology, Chinese Academy of Agricultural Sciences (IFST-CAAs).

For each species, ca. 50 adult females were scalded in 100°C deionized water, to have their chelicerae extended. The individuals were then dehydrated in 70%, 80%, 90%, and 100% ethanol for two minutes successively, and dried in LEICA EM CPD030 drier for two hours. The dried specimens were glued on the stage, either dorsal side, ventral side, or lateral side up. Fifteen replicates of each position were prepared. For each of the lateral side up specimen, its pedipalpi and 1<sup>st</sup> pair of legs were removed with 00 insect pin to expose chelicerae under the stereomicroscope.

All samples were gold sputtered in MC1000 ion sputter for one minute, observed and imaged at magnifications of 1000x, 2000x and 3000x with a scanning electron microscope (FEI QUANTA 200F, National Center for Nanoscience and Technology).

For each species, 24 parameters were measured using ImageJ1.42I following the methods provided in Appendix (Table 4 and Figure 2). A minimum of 15 replicates of each parameter were achieved. Principle Components Analysis (PCA) was conducted for dimension reduction. The first two principle components of each replicate were scattered plotted for grouping.

One-way ANOVAs were conducted to compare inter-group differences of major principle components (PCs) and each of the 24 parameters. For each response variable, data were rank-transformed to satisfy normality test. Multiple comparisons were conducted with Turkey HSD test. All mean comparisons with  $p < 0.05$  were considered to have statistical significant differences. All analyses were processed with SPSS 19.0.

## Results

The first six principle components (PC) explained 83.76% of total variations, among which PC1 and PC2 explained 36.01% and 17.50% variations, respectively. For PC1, loadings of nine parameters were higher than 0.70, including length of chelicerae, length of fixed digit, width of fixed digit, length of teeth row of fixed digit, dorsal perimeter of fixed digit, width of movable digit, ventral

perimeter of movable digit, corniculi length, internal malae length. For PC2, only the angle of fixed digit and length of movable digit had high loading (0.76 and 0.80 respectively) (Table 1). When plotted with the two PCs as x and y values, respectively, as indicated in the Figure 1, the 10 species were assigned into three groups as follows:

Group I: *P. persimilis*, *N. californicus*, *N. pseudolongispinosus*

Group II: *N. barkeri*, *N. bicaudus*, *N. cucumeris*, *A. orientalis*, *A. swirskii*, *A. tsugawai*

Group III: *E. utilis*

Inter-group differences were detected for four of the first six PCs (Table 2) and 22 parameters (Table 3). Overall, species in Group I and Group III had smaller chelicerae, chelae and hypostome, with the lengths of these parameters ca. 80% as those of Group II. Group III had ca. 10 and 35 times larger lobe area than Group I and Group II, respectively. And its angle of fixed digit was ca. 2–3 times larger than those of the other two groups.

**TABLE 1.** Loadings of the first 6 principal components (PCs).

Parameter	PC1	PC2	PC3	PC4	PC5	PC6
Cheliceral length	0.83	0.01	0.26	0.21	0.07	-0.01
Cheliceral width	0.63	0.59	0.27	0.03	0.30	-0.02
Length of fixed digit	0.87	0.23	0.04	0.27	-0.12	-0.01
Width of fixed digit	0.76	-0.21	0.22	-0.12	0.41	-0.07
Length of teeth row of fixed digit	0.80	-0.25	0.09	-0.37	-0.12	0.24
Dorsal perimeter of fixed digit	0.90	0.24	0.06	0.22	-0.14	0.01
Angle of fixed digit	-0.17	0.76	0.47	0.01	-0.01	0.18
The number of denticles of fixed digit	0.31	-0.36	0.70	-0.22	-0.31	-0.20
Pilus dentilis length of fixed digit:	0.66	0.14	-0.40	-0.19	-0.28	0.31
Lobe area of fixed digit	-0.61	0.52	0.40	-0.02	0.17	0.13
Length of movable digit	0.16	0.80	-0.17	-0.20	0.41	-0.03
Width of movable digit	0.77	-0.16	0.05	-0.35	0.12	-0.04
Length of teeth row of movable digit	0.38	-0.32	-0.14	-0.68	0.27	-0.05
Ventral perimeter of movable digit (VP)	0.90	0.07	-0.27	0.21	0.08	-0.06
Angle of movable digit	-0.10	-0.38	0.42	-0.30	0.40	0.45
The number of denticles of movable digit	-0.05	-0.62	0.65	-0.14	-0.21	0.12
DP/VP	-0.39	0.33	0.61	-0.04	-0.28	0.10
Corniculi length	0.73	0.21	0.42	0.08	-0.06	0.01
Internal malae length	-0.72	0.41	0.27	0.17	0.22	0.16
Tritosternum length	0.27	-0.18	-0.27	0.44	-0.10	0.63
Subcapitular groove length	0.52	-0.36	0.06	0.37	0.35	0.17
Number of denticles scales in subcapitular groove	-0.48	-0.71	-0.02	0.31	0.16	0.11
Number of denticles of every scale in subcapitular groove	-0.34	-0.60	0.19	0.41	0.33	-0.21
Dorsal shield length	0.60	-0.03	0.36	0.49	-0.02	-0.21

**TABLE 2.** Means and standard deviations of diet categories on six principal components.

Group	N	n	PC1 (36.01%)	PC2 (17.50%)	PC3 (11.74%)	PC4 (8.52%)	PC5 (5.71%)	PC6 (4.28%)
1	3	45	-0.93±0.15	-0.91±0.81	-0.63±1.02	-0.12±1.20	0.22±0.69	0.01±0.87
2	6	90	0.74±0.44	0.14±0.53	0.10±0.81	0.08±0.96	-0.20±1.14	-0.04±1.12
3	1	15	-1.67±0.17	1.92±0.31	1.30±0.31	-0.13±0.36	0.51±0.49	0.20±0.41
P	df1=2,df2=147		<0.01	<0.01	<0.01	0.59	0.02	0.62

PC: principal component; N: number of species in a group; n: number of replicates in a group.

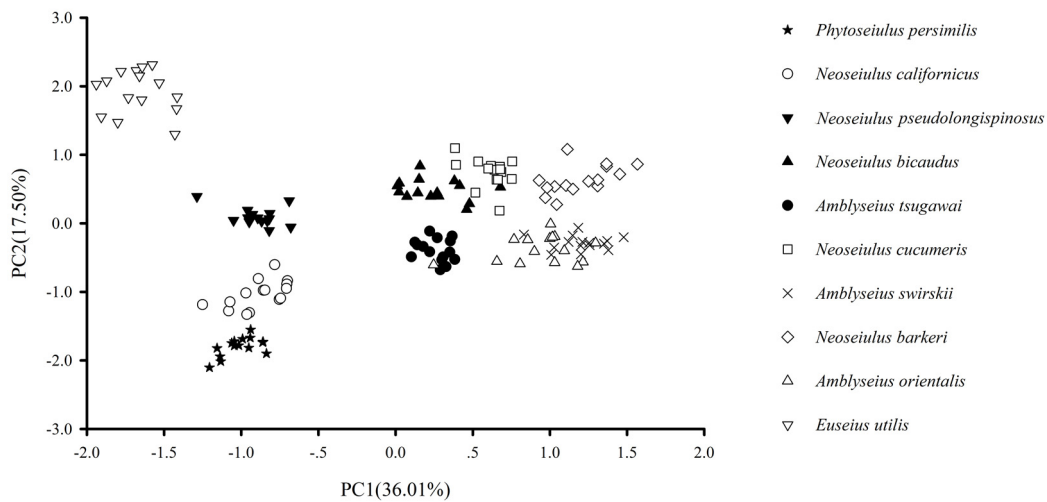
**TABLE 3.** Means and standard errors of three groups on the 24 morphometrics ( $\mu\text{m}$ ).

parameter	1	2	3	F	P
Cheliceral length	91.41±1.26b	113.20±0.89a	90.28±1.99b	102.05	<0.01
Cheliceral width	22.52±0.14b	28.16±0.22a	28.47±0.39a	106.53	<0.01
Length of fixed digit	22.56±0.16b	28.05±0.16a	22.64±0.22b	184.65	<0.01
Width of fixed digit	5.83±0.12b	7.07±0.12a	5.04±0.12c	46.89	<0.01
Length of teeth row of fixed digit	12.10±0.26b	16.26±0.33a	7.77±0.10c	88.79	<0.01
Dorsal perimeter of fixed digit	24.71±0.16b	31.60±0.18a	24.73±0.25b	188.54	<0.01
Angle of fixed digit	10.72±0.30c	14.77±0.22b	25.95±0.10a	103.39	<0.01
The number of denticles of fixed digit	6.00±0.21b	7.67±0.24a	6.00±0.00b	13.78	<0.01
Pilus dentilis length of fixed digit	3.08±0.22b	4.65±0.11a	1.68±0.08c	44.26	<0.01
Lobe area of fixed digit	7.26±0.68b	2.06±0.19c	62.96±5.88a	63.88	<0.01
Length of movable digit	23.94±0.63c	27.12±0.34b	32.28±0.71a	28.43	<0.01
Width of movable digit	4.18±0.06b	5.11±0.09a	3.38±0.06c	55.22	<0.01
Length of teeth row of movable digit	10.68±0.33a	10.22±0.33a	6.29±0.06b	16.13	<0.01
Ventral perimeter of movable digit (VP)	27.01±0.23b	34.18±0.33a	22.31±0.34c	198.18	<0.01
Angle of movable digit	31.68±0.53	29.83±0.53	31.71±1.00	2.86	0.06
The number of denticles of movable digit	2.33±0.14	2.17±0.10	2.00±0.00	1.93	1.49
DP/VP	0.92±0.01b	0.93±0.01b	1.11±0.02a	24.27	<0.01
Corniculi length	32.40±0.33c	39.30±0.25a	35.38±0.37b	128	<0.01
Internal malae length	12.03±0.15b	10.03±0.16c	18.16±0.31a	84.42	<0.01
Tritosternum length	63.97±1.31a	65.85±1.56a	48.94±2.54b	14.14	<0.01
Subcapitular groove length	50.06±0.72b	52.95±0.62a	42.89±0.75c	25.19	<0.01
Number of denticles scales in subcapitular groove	7.67±0.07a	7.00±0.00b	7.00±0.00b	102.9	<0.01
Number of denticles of every scale in subcapitular groove	2.67±0.14a	2.00±0.00b	2.00±0.00b	25.73	<0.01
Dorsal shield length	313.67±4.79b	352.01±1.81a	318.38±2.24b	61.48	<0.01

Means  $\pm$  SE followed by different lowercase letters in the same row were significantly different according to Tukey HSD tests at  $p = 0.05$ ,  $df=2$ . For teeth on fixed and movable digit, we used integer conversions of the mean values of each species.

## Discussion

In this study, we assigned 10 phytoseiid mite species into three groups based on morphometrics of their gnathosoma. The three groups: Group I, Group II and Group III represents the specialist, generalist and pollen feeder, respectively. This result is broadly consistent with the life styles classification provided by McMurtry and Croft (1997), suggesting it is appropriate to estimate the feeding habits of phytoseiid mites based on their gnathosoma morphology.



**FIGURE 1.** Scattered plot of the first two multifactorial axes of 10 Phytoseiidae species on their 24 morphometrics.

McMurtry and Croft (1997) assigned *P. persimilis* into Type I, and *N. californicus* into Type II. *N. pseudolongispinosus* is native species discovered in China. Wu *et al.* (2008) assigned it into Type II due to its high preference and consumption rates of *Tetranychus* spp. In general, these three species are all considered as specialist predators of spider mites. However, it has been a debate regarding whether *N. californicus* is a specialist? Although showing strong preferences for *Tetranychus* spp., this species also feeds on multiple species of mites and insects, and even on pollens (Pena and Osborne 1996; Easterbrook 2001; Rahmani *et al.* 2009; Saber 2012). It also shares some other features with generalists, such as being able to complete life cycle on immatures of other predatory mites (Walzer and Schausberger 1999a, b; Schausberger and Croft 1999, 2000), and having short mid-dorsal setae etc. (Croft *et al.* 1998b). Therefore, Croft *et al.* (1998b) preferred to assign it into Type III. However, McMurtry *et al.* (2013) insisted that it belongs to Type II, because it often coexists with spider mites that produces heavy webs and shows strong adaptation to such environments, which is quite different from generalists. In the present study, *N. californicus* is obviously separated from generalist predators. Among the three specialist species, data points of *N. californicus* and *N. pseudolongispinosus* overlapped more. This supported the viewpoint in McMurtry *et al.* (2013).

All the six species that constitute Group II are Type III generalists, while *E. utilis* is separated from all other species due to its specific features, such as huge lobe area that covers almost the whole chela and large angle of fixed digit. These features are consistent with descriptions in previous studies (Flechtmann and McMurtry 1992a; Adar *et al.* 2012). Our results also showed that *E. utilis* has relatively longer internal malae, equaling ca. 1/2 as long as its corniculi, while the internal malae of other species only are 1/5–1/3 of their corniculi in length. However, it will be necessary to investigate other Type IV species to confirm the pervasiveness of these characteristics in this group.

Overall, we are able to estimate the feeding habits of the 10 phytoseiid mites based on the 24 measurements. These parameters provided comprehensive descriptions of phytoseiid gnathosoma. All the parameters can be easily measured directly from field collected specimens, and showed limited intraspecific variation. Buryn and Brandl (1992) used PCA to estimate feeding habits of

gamasid mites based on 10 parameters of chelicerae. Our results showed that PCA is also an appropriate method to estimate feeding habits of phytoseiids.

Only 10 species were measured in this study, however, we expect that further life-style categorization of newly described species and/or some species with limited knowledge about their feeding habits would be possible based on their gnathosoma morphometrics. For example, to separate Type I and II specialists, or further divide Type III generalists into subgroups. Among the six generalists in this study, the three *Amblyseius* species have smaller values than the three *Neoseiulus* species in PC2. On the other axis, there are also trends of two subgroups, each containing 3 species whose data points are more overlapped. Although it is too early to discuss these trends in details due to limited number of species, they did suggest some possibilities for further classifications.

The purpose of our study is to develop a method that can be used to estimate feeding habits of phytoseiids directly and easily. Apparently, feeding habits and life styles of these species are also affected by many other factors as well as gnathosoma morphology, such as nutritional requirements, and digestive ability, etc. It is necessary to take measurements of more species in the future to test the reliability of this method and to discuss the possibilities for subgrouping. It will also be very valuable to compare different populations or strains belonging to same species, whether these parameters are variable or consistent at individual, population, and species levels.

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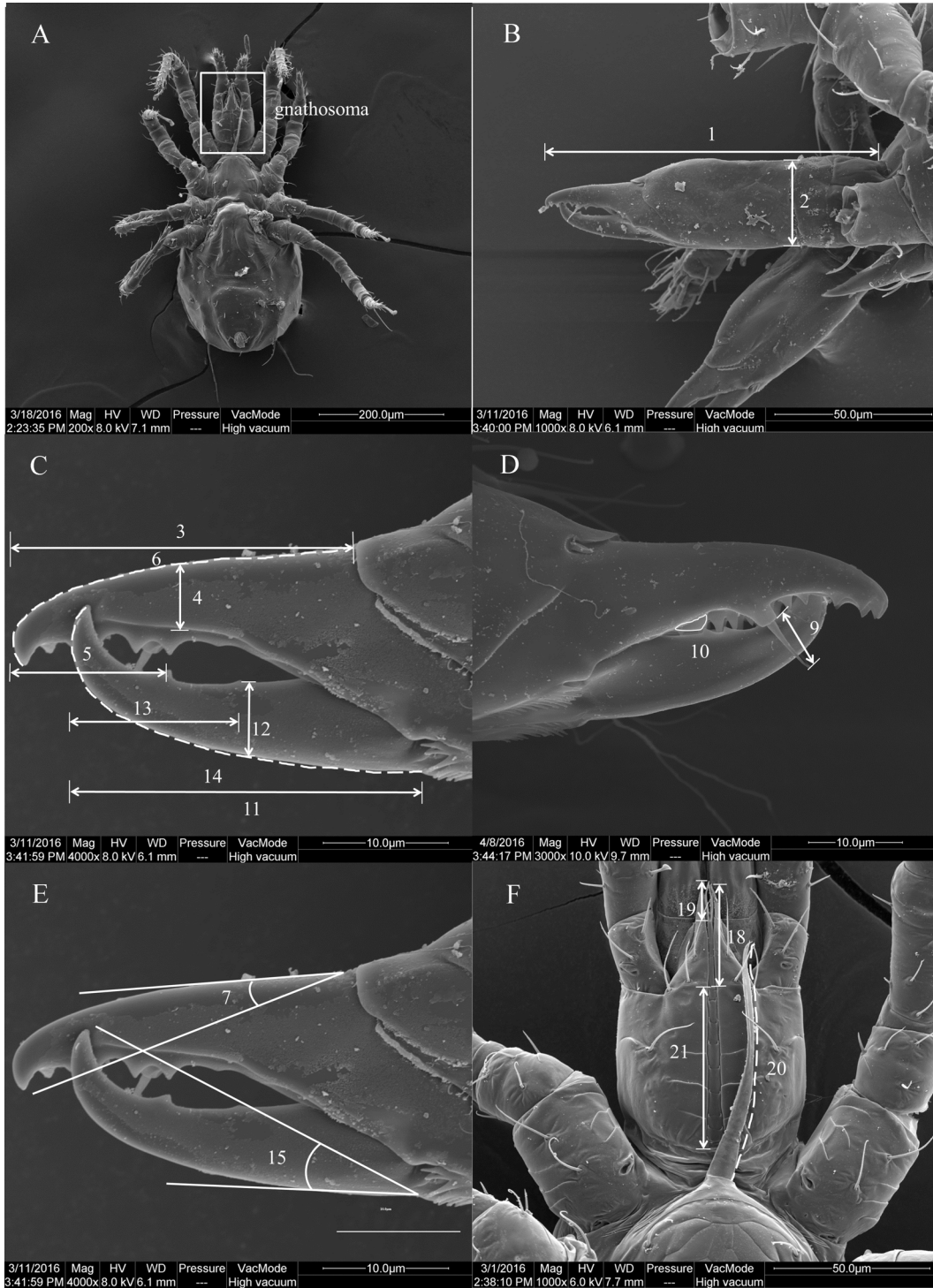
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## Appendix

**Table 4.** Twenty-four morphometrics and their descriptions.

Number	name	location	unit	description	figure
1	Cheliceral length	Chelicera	µm	The distance from the tip of fixed digit to the end of chelicera	B
2	Cheliceral width	Chelicera	µm	The width of chelicera at the boundary of chelicera and cheliceral sheath	B
3	Length of fixed digit	Chelicera	µm	The distance from the tip of fixed digit to the point of fixed digit sheath	C
4	Width of fixed digit	Chelicera	µm	The width at the middle point of fixed digit length	C
5	Length of teeth row of fixed digit	Chelicera	µm	The distance from the tip of fixed digit to the point of last tooth	C
6	Dorsal perimeter of fixed digit	Chelicera	µm	The curve length from the tip of fixed digit to the point of fixed digit sheath	C
7	Angle of fixed digit	Chelicera	degree	The angle between the tip of fixed digit to its apex and the tangent line of its dorsal perimeter	E
8	The number of denticles of fixed digit	Chelicera	µm	The number of denticles of fixed digit	-
9	Pilus dentilis length of fixed digit	Chelicera	µm	The length from the apex of pilus dentilis to its end	D
10	Lobe area of fixed digit	Chelicera	µm <sup>3</sup>	The lobe area of fixed digit	D
11	Length of movable digit	Chelicera	µm	The distance from the tip of movable digit to the end of movable digit	C
12	Width of movable digit	Chelicera	µm	The width at the middle point of movable digit length	C
13	Length of teeth row of movable digit	Chelicera	µm	The distance from the tip of movable digit to the point of last tooth	C
14	Ventral perimeter of movable digit (VP)	Chelicera	µm	The curve from the tip of movable digit to the end of movable digit	C
15	Angle of movable digit	Chelicera	degree	The angle between the tip of movable digit to its apex and the tangent line of its ventral perimeter	E
16	The number of denticles of movable digit	Chelicera	-	The number of denticles of movable digit	-
17	DP/VP	Chelicera	-	The rate of dorsal perimeter of fixed digit and ventral perimeter of movable digit	-
18	Corniculi length	Hypostome	µm	The length from the tip of corniculi to the boundary between hypostome and gnathobase	F
19	Internal malae length	Hypostome	µm	The distance from the tip of internal malae to the joint point of its two piece	F
20	Tritosternum length	Gnathobase	µm	The curve from the tip of tritosternum to its end	F
21	Subcapitular groove length	Gnathobase	µm	The length from the boundary between hypostome and gnathobase to the last scale of teeth in subcapitular groove	F
22	Number of denticles scales	Subcapitular groove	-	The number of denticles scales in subcapitular groove	-
23	Number of denticles of every scale	Subcapitular groove	-	The number of denticles of every scale in subcapitular groove	-
24	Dorsal shield length		µm	The distance from anterior margin to posterior margin of dorsal shield	-



**FIGURE 2.** Scanning electron microscope images of 24 morphometrics measured in this experiment (A–F).