Experimental and Applied Acarology (2019) 79:323–344
https://doi.org/10.1007/s10493-019-00443-8

Unravelling the phylogeny, cryptic diversity and morphological evolution of *Diptilomiopus* mites (Acari: Eriophyoidea)

Qing Liu[1] · Yan-Mei Yuan[1] · Yan Lai[2] · Guo-Quan Wang[2] · Xiao-Feng Xue[1]

Received: 22 July 2019 / Accepted: 20 November 2019 / Published online: 30 November 2019

Abstract
The Eriophyoidea, notable for specific morphological characters (four-legged mites) and gall-formation in host plants (gall mites), is one of the most species-rich superfamilies of Acari. Monophyly of the superfamily Eriophyoidea is accepted by all acarologists; however, monophyly of most genera has not been evaluated in a molecular phylogenetic network. Furthermore, most eriophyoid mites, especially species in the genus *Diptilomiopus*, are morphologically similar, challenging their identification. Here we test the phylogeny and cryptic diversity of *Diptilomiopus* species using fragments of two mitochondrial (COI and 12S) and two nuclear (18S and 28S) genes. Our results revealed the monophyly of *Diptilomiopus*. Sequence distance, barcode gap, and species delimitation analyses of the COI gene allowed us to resolve cryptic diversity of *Diptilomiopus* species. Additionally, we supposed that characteristics of genu fused with femur on both legs and seta *ft′* absent on leg II evolved only once within *Diptilomiopus*, which are potential morphological synapomorphies. In contrast, characteristics of both setae *ft′* and *ft″* divided into a short branch and a long branch were supposed evolving multiple times independently. Our findings contribute to the understanding of phylogeny and morphological evolution of *Diptilomiopus* species and provide a DNA-based approach for species delimitation of *Diptilomiopus* mites.

Keywords Eriophyoid mites · Phylogeny · Cryptic diversity · Morphological synapomorphies

Electronic supplementary material The online version of this article (doi:https://doi.org/10.1007/s10493-019-00443-8) contains supplementary material, which is available to authorized users.

✉ Xiao-Feng Xue
xfxue@njau.edu.cn

[1] Department of Entomology, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China

[2] Department of Plant Protection, Guangxi University, Nanning 530005, Guangxi, China

Introduction

The Eriophyoidea is one of the most species-rich superfamilies of Acari, consisting of more than 4400 described species (Zhang 2011). Eriophyoid mites are notable for their specific and distinct morphological characters, e.g., two pairs of legs (four-legged mites), very small body size (200 μm long on average), fusiform or vermiform body shape, and ringed opisthosoma (Amrine et al. 2003). They are totally phytophagous, having high host-plant specialization and specificity (Skoracka et al. 2010). Some are of economic importance, e.g., *Aceria tosichella* Keifer (wheat curl mite), a major pest of the world's grain industry (Navia et al. 2013). Most eriophyoid mites are vagrant on their host plant, whereas some induce galls (gall mites), erinea, large buds, curved leaves, or dried branches (Petanovic and Kielkiewicz 2010).

The Eriophyoidea comprises three families: Phytoptidae, Eriophyidae and Diptilomiopidae. The genus *Diptilomiopus*, established by Nalepa (1916), belongs to the Diptilomiopidae (Amrine et al. 2003). Based on morphological characters, 116 *Diptilomiopus* species have been described worldwide (Table S1). The majority of these species have been recorded in the Oriental realm (Capinera 2008), whereas only a few have been reported from the remaining realms, i.e., three in the Palearctic realm, two in the Nearctic realm, three in the Afrotropical realm, and two in the Australasian realm (Fig. 1).

Current eriophyoid mite taxonomy relies mostly on a few external morphological characters (Amrine et al. 2003), of which the pattern of prodorsal shield is widely used for species delimitation. The prodorsal shield of eriophyoid mites may have one median line, two admedian lines and several submedian lines (Fig. 2); however, *Diptilomiopus* species typically have a network-like prodorsal shield pattern (median, admedian and submedian lines are connected by short transverse lines; Fig. 2), which provides limited information to differentiate one species from another, challenging the species delimitation in *Diptilomiopus*.

Previous molecular studies of eriophyoid mites were performed on the mitochondrial genomes (Xue et al. 2016), high-level (superfamily) phylogenetic positions of Eriophyoidea (Xue et al. 2017; Klimov et al. 2018), low-level (subfamily, tribe or genus) phylogeny of Eriophyoidea (Li et al. 2014a; Chetverikov et al. 2015), and genetic diversity of *Ac. tosichella* (Eriophyidae) (Skoracka et al. 2018) and *Tetra pinnatifidae* Xue et al. (Eriophyidae) (Li et al. 2014b). By using the mitochondrial cytochrome oxidase subunit I (COI) gene and nuclear D2 region of 28S (28S) rDNA, Lewandowski et al. (2014) revealed the genetic and morphological diversity of *Trisetacus* species (Phytoptidae), Cvrković et al. (2016) revealed the cryptic speciation of *Phytoptus avellanae* s.l. Nalepa (Phytoptidae), Duarte et al. (2019) revealed *Abacarus* species on sugarcane plants, Skoracka and Dabert (2010) revealed the *Abacarus hystrix* (Nalepa) complex (Eriophyidae), and Skoracka et al. (2013) revealed the *Ac. tosichella* complex (Eriophyidae). By using the COI, 18S and 28S genes, Guo et al. (2015) revealed the protogyne and deutogyne of *Tegolophus celtis* Guo et al. (Eriophyidae). These studies show that the COI gene can well resolve the identification and classification of species within Phytoptidae and Eriophyidae. However, no molecular studies have been performed on the genera in Diptilomiopidae, and the phylogeny and genetic diversity of *Diptilomiopus* species are largely unknown.

In this study, we sequenced two mitochondrial (COI and 12S) and two nuclear (18S and 28S) gene fragments of representative species (25 terminals). By constructing phylogenetic trees and using integrative taxonomy approaches, we attempted to (1) test the

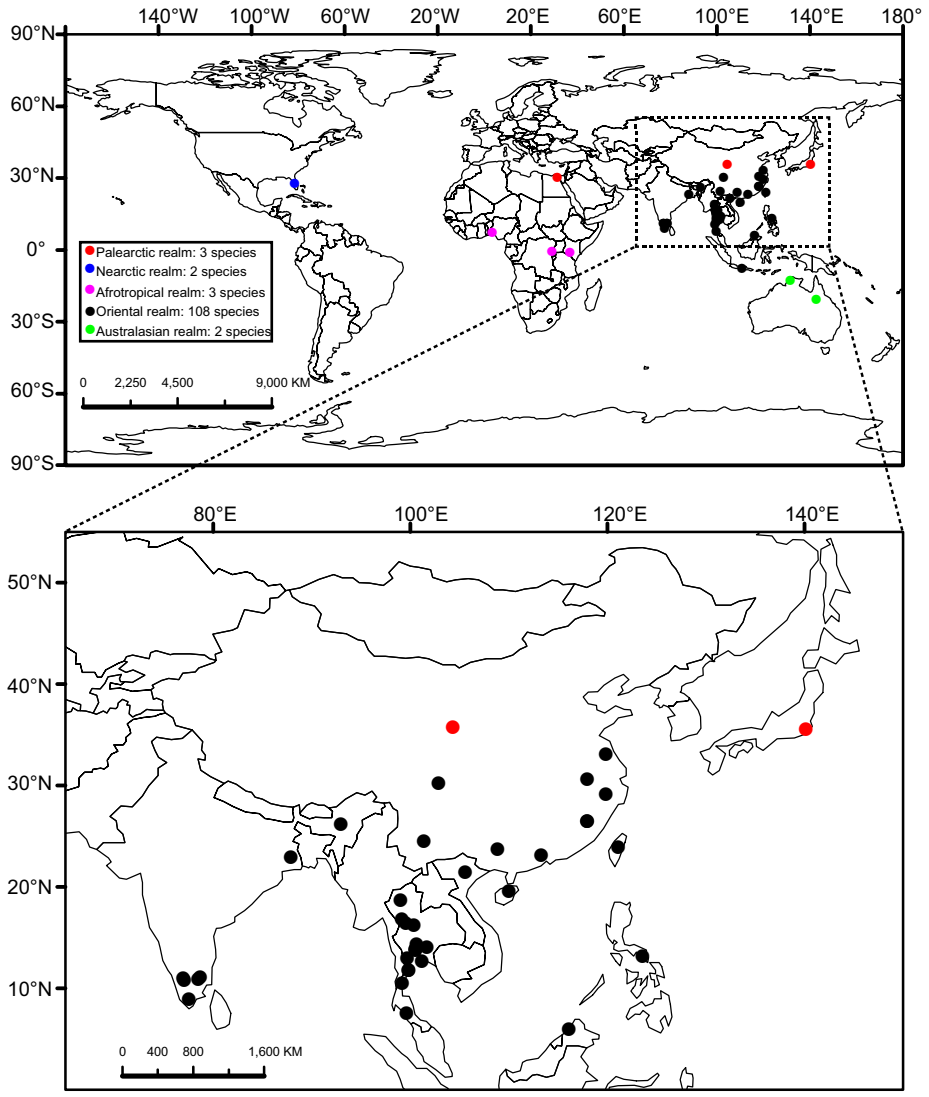

Fig. 1 Global records/localities map of *Diptilomiopus* species. The red, blue, pink, black, and green dots represent the records/localities where *Diptilomiopus* species have been found in the Palearctic, Nearctic, Afrotropical, Oriental, and Australasian realms, respectively. (Color figure online)

monophyly of *Diptilomiopus*, (2) resolve the delimitation of *Diptilomiopus* species and cryptic diversity, and (3) reveal the morphological evolution of legs and demonstrate synapomorphies of *Diptilomiopus*.

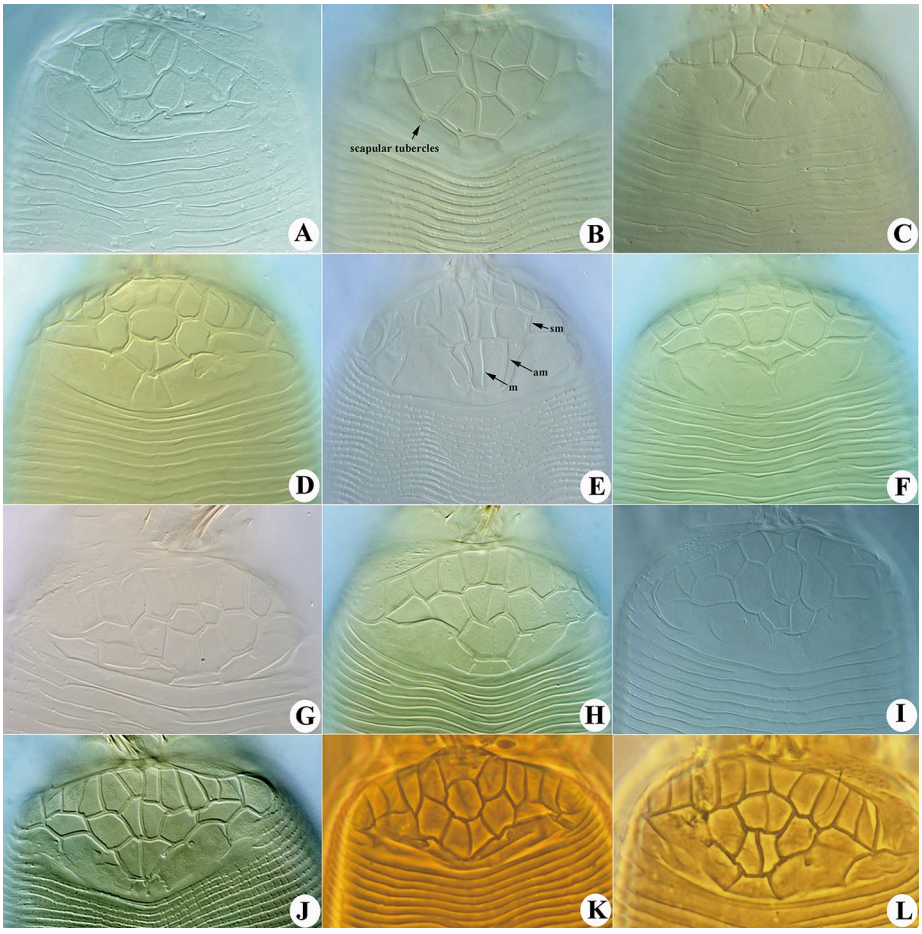

Fig. 2 Patterns of prodorsal shield in *Diptilomiopus* species. **a** *D. milletus*, **b** *D. rotundus*, **c** *D. octandrus*, **d** *D. nobilus*, **e** *D. broussonetus*, **f** *D. fortunus,* **g** *D. sabahus*, **h** *D. callicarpus*, **i** *D. melastomae*, **j** *D. keningaus*, **k** *D. ligustri*, **l** *D. bischofiae*; m, median line; am, admedian line; sm, submedian line

Materials and methods

Specimen collection and morphological identification

Twenty-five *Diptilomiopus* samples were collected randomly on host plants with the help of a hand-lens (30×) in China and Malaysia in 2017 and 2018 (Table 1). Some mite specimens were used immediately for DNA extraction, whereas the remaining were preserved in 100% ethanol at − 20 °C prior to DNA extraction. Specimens of each species were also slide mounted as vouchers, using modified Berlese medium (Amrine and Manson 1996) for morphological checking with a Zeiss A2 microscope equipped with the AxioCam MRc camera. Microphotographs were taken with a Zeiss A2 research microscope with phase contrast or differential interference, using × 100 oil magnification; the microscope was connected to a computer using Axiovision image analysis software. The morphological terminology used herein follows that of Lindquist (1996); the generic classification is made

Table 1 Species used in this analysis

Family	Species	Voucher	Host plant	Locality	GenBank number					References
					18S	28S D2-5	28S D9-10	COI	12S	
Nematalycidae	*Cunliffea* sp.	UMMZ BMOC 08-1012-004 AD1387	–	USA	KY922118	KY921986	KY921986	KY922363	–	Klimov et al. (2018)
	Gordialycus sp.	UMMZ BMOC 08-1012-004	–	USA	KY922131	KY921999	KY921999	KY922376	–	Klimov et al. (2018)
	Osperalycus tenerphagus	UMMZ BMOC 12-0128-005 AD1457	–	USA	KY922130	KY921998	KY921998	KY922375	–	Klimov et al. (2018)
	Psammolycus sp.	UMMZ BMOC 12-0128-003 AD1454	–	USA	KY92212	–	–	KY922374	–	Klimov et al. (2018)
Phytoptidae	*Loboquintus subsquamatus*	UMMZ BMOC 15-0717-006 AD1918	*Cupressus sempervirens* (Cupressaceae)	Israel	KY922123	KY921992	KY921992	KY922368	–	Klimov et al. (2018)
	Novophytoptus rostratae	UMMZ BMOC 15-0717-010 AD1922	*Scirpus* sp. (Cyperaceae)	Russia	KY922120	KY921989	KY921989	KY922365	–	Klimov et al. (2018)
	Oziella atherodes	UMMZ BMOC 15-0717-005 AD1917	*Carex atherodes* (Cyperaceae)	Russia	KY922119	KY921988	KY921988	KY922364	–	Klimov et al. (2018)
	Trisetacus piceae	UMMZ BMOC 15-0717-011 AD1923	*Picea abies* (Pinaceae)	Russia	KY922121	KY921990	KY921990	KY922366	–	Klimov et al. (2018)
	Trisetacus pini	UMMZ BMOC 15-0717-009 AD1921	*Pinus sylvestris* (Pinaceae)	Estonia	KY922122	KY921991	KY921991	KY922367	–	Klimov et al. (2018)
Eriophyidae	*Cecidophyopsis ribis*	UMMZ BMOC 15-0717-008 AD1920	*Ribes alpinum* (Grossulariaceae)	Russia	KY922128	KY921997	KY921997	KY922373	–	Klimov et al. (2018)

Table 1 (continued)

Family	Species	Voucher	Host plant	Locality	GenBank number					References
					18S	28S D2-5	28S D9-10	COI	12S	
	Eriophyes sp.	UMMZ BMOC 07-0628-001 AD1122	*Tilia cordata* (Malvaceae)	USA	KY922124	KY921993	KY921993	KY922369	–	Klimov et al. (2018)
	Eriophyidae sp.	UMMZ BMOC 07-1015-008 AD1015	*Salix* sp. (Salicaceae)	Kazakhstan	KY922126	KY921995	KY921995	KY922371	–	Klimov et al. (2018)
	Tergilatus sparsus	UMMZ BMOC 15-0717-007 AD1919	*Portulacaria afra* (Didiereaceae)	South Africa	KY922125	KY921994	KY921994	KY922370	–	Klimov et al. (2018)
Diptilomiopidae	*Diptilomiopus bischofiae*	NJAUAcariS11	*Bischofia javanica* (Phyllanthaceae)	China	MK440001	MK440024	–	–	–	This study
		GUBY	*B. javanica*	China	MK440002	MK440025	MK440046	MK516826	MK516816	This study
		GUWM	*B. javanica*	China	MK440003	MK440026	MK440047	MK516827	MK516817	This study
	Diptilomiopus broussonetus	NJAUAcariKK18	*Broussonetia* sp. (Moraceae)	Malaysia	MK440019	MK440041	MK440060	MK516838	MK516822	This study
	Diptilomiopus buxusis	NJAUAcari493	*Buxus* sp. (Buxaceae)	China	MK440004	MK440027	–	–	–	This study
	Diptilomiopus callicarpus	NJAUAcariKK23b	*Callicarpa bodinieri* (Lamiaceae)	Malaysia	MK440022	MK440044	MK440063	MK516841	–	This study
	Diptilomiopus cayratus	NJAUAcariQ84	*Cayratia japonica* (Vitaceae)	China	MK440005	MK440028	MK440048	–	–	This study
	Diptilomiopus engelhardter	NJAUAcariQ210	*Engelhardtia roxburghiana* (Juglandaceae)	China	MK440006	MK440029	–	–	–	This study

Table 1 (continued)

Family	Species	Voucher	Host plant	Locality	GenBank number					References
					18S	28S D2-5	28S D9-10	COI	12S	
	Diptilomiopus fortunus	NJAUA-cariKK19	*Alniphyllum fortune* (Styracaceae)	Malaysia	MK440020	MK440042	MK440061	MK516839	MK516823	This study
		NJAUA-cariKK21a	*A. fortune*	Malaysia	MK440021	MK440043	MK440062	MK516840	MK516824	This study
	Diptilomiopus keningaus	NJAUA-cariKK16	*Stephania* sp. (Menispermaceae)	Malaysia	MK440017	MK440039	MK440058	MK516836	MK516821	This study
	Diptilomiopus ligustri	NJAUA-cariYN253	*Ligustrum quihoui* (Oleaceae)	China	KM111031	KM111065	–	KM111097	–	This study
		GUML	*L. quihoui*	China	MK440008	MK440030	MK440049	MK516828	–	This study
		NJAUAcariS6	*L. quihoui*	China	MK440009	MK440031	MK440050	–	–	This study
		GUYN	*L. quihoui*	China	MK440010	MK440032	MK440051	MK516829	–	This study
		GUGX	*L. quihoui*	China	KJ841968	KF134815	–	–	–	This study
	Diptilomiopus melastomae	NJAUA-cariKK27	*Melastoma malabathricum* (Melastomaceae)	Malaysia	MK440023	MK440045	MK440064	MK516842	MK516825	This study
	Diptilomiopus meliae	GUSZ	*Melia azedarach* (Meliaceae)	China	MK440011	MK440033	MK440052	MK516830	–	This study
	Diptilomiopus milletus	NJAUAcariFJ20	*Adinandra milletii* (Pentaphylacaceae)	China	MK440013	MK440035	MK440054	MK516832	MK516819	This study
	Diptilomiopus nobilus	NJAUA-cariGD137	*Sterculia nobilis* (Malvaceae)	China	MK440016	MK440038	MK440057	MK516835	–	This study

Table 1 (continued)

Family	Species	Voucher	Host plant	Locality	GenBank number					References
					18S	28S D2-5	28S D9-10	COI	12S	
	Diptilomiopus octandrus	NJAUA-cariGD120	*Aporosa octandra* (Phyllanthaceae)	China	MK440015	MK440037	MK440056	MK516834	MK516820	This study
	Diptilomiopus pamithus	GUMM	*Mangifera indica* (Anacardiaceae)	China	MK440012	MK440034	MK440053	MK516831	MK516818	This study
	Diptilomiopus retusus	NJAUAcariQ89	*Fissistigma retusum* (Annonaceae)	China	MK440007	–	–	–	–	This study
	Diptilomiopus rotundus	NJAUA-cariGD112	*Ilex rotunda* (Aquifoliaceae)	China	MK440014	MK440036	MK440055	MK516833	–	This study
	Diptilomiopus sabahus	NJAUA-cariKK17	*Morus* sp. (Moraceae)	Malaysia	MK440018	MK440040	MK440059	MK516837	–	This study
	Quadracus urticarius	UMMZ BMOC 15-0717-004 AD1916	*Urtica dioica* (Urticaceae)	Russia	KY922127	KY921996	KY921996	KY922372	–	Klimov et al. (2018)

according to Amrine et al. (2003). All of the specimens and vouchers were deposited in the Arthropod/Mite Collection of the Department of Entomology, Nanjing Agricultural University (NJAU), Jiangsu Province, China (Zhang 2018).

DNA extraction and PCR amplification

Genomic DNA was extracted from one specimen for each sample of eriophyoid mites, using a DNeasy Blood and Tissue Kit (Qiagen), following a previously reported modified protocol (Dabert et al. 2008). We amplified the fragments of two mitochondrial genes (COI and 12S) and two nuclear genes (18S and 28S) using published or modified PCR primer pairs for each fragment (Table 2). The PCR cycling conditions were as follows: 3 min of denaturation at 94 °C; 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 42–55 °C (depending on the primers) and 1 min of extension at 72 °C; 5 min final extension at 72 °C; holding at 4 °C. Each PCR contained 12.5 µl of PCR SuperMix (Transgen Biotech, Beijing, China), 2 µl of template DNA, and 0.4 µM of each primer, in a total volume of 25 µl. PCR products were visualized on a 1% agarose gel. Products were purified and sequenced in both directions at General Biosystems (Anhui, China) on an ABI 3730XL DNA Analyzer (Applied Biosystems).

Data matrices and sequence alignments

Sequences of four gene (COI, 12S, 18S and 28S) fragments of 25 *Diptilomiopus* samples representing 17 morphospecies were blasted in GenBank and checked for possible contaminants. All the sequences were deposited in GenBank under accession numbers: MK440001–MK440064, and MK516816–MK516842 (Table 1). The sequences of four outgroups (Nematalycidae), five phytoptid mites, four eriophyid mites and one diptilomiopid species were retrieved from GenBank (Table 1). Three rRNA genes were aligned individually using MAFFT v.7.423 web server (Katoh and Standley 2013) (http://mafft.cbrc.jp/alignment/server/) with G-INS-i strategy for global homology and manually inspected before concatenation. For COI, a preliminary alignment was generated using ClustalW in MEGA 6.0 (Tamura et al. 2013). Large gaps and ambiguous sites were deleted manually. Alignments of individual genes were concatenated in Geneious v.8.1.9 (Kearse et al. 2012). The final concatenated DNA dataset consisted of 4989 bp: 18S rRNA = 2297 bp, 28S rRNA D2–D5 = 916 bp, 28S rRNA D9–D10 = 752 bp, COI = 658 bp, and 12S rRNA = 366 bp. We analyzed the dataset as nucleotide sequences. Dataset partitioning was performed by PartitionFinder 2 (Lanfear et al. 2017), based on an initial total of five data blocks (18S, 28S D2–D5, 28S D9–D10, COI, and 12S). Models were predicted by PartitionFinder 2 using the Bayesian information criterion (BIC). PartitionFinder used unlinked branch lengths, the greedy search algorithm for nucleotide sequences and the MrBayes model. The GTR + G substitution model was chosen by PartitionFinder as the best for two partitions (18S + 28S D2–D5 + 28S D9–D10 and COI + 12S).

To test the monophyly and its phylogenetic position of *Diptilomiopus* within Eriophyoidea, we constructed an additional data matrix including four species of Nematalycidae, eight species of Phytoptidae, 21 species of Eriophyidae, and 30 species of Diptilomiopidae (Table S2). The nucleotide sequences of 18S rRNA of these species were retrieved from GenBank. Alignments were performed by MAFFT, and the substitution model (GTR + G) was predicted by PartitionFinder.

Table 2 PCR primers used in this research

Fragment	Primer	Sequence (5′–3′)	Annealing temperature (°C)	Source
18S (a)	18Sfw	CTTGTCTCAAAGATTAAGCCATGCA	50	Dabert et al. (2010)
	rev480R	GTTATTTTTCTTCACTACAT		This study
18S (b)	fw390R	AGTTAGGGCTCGACTCCGGAGA	52	This study
	rev960R	ATCGGTCTAAGAATTTCAC		This study
18S (b1)	fw390R	AGTTAGGGCTCGACTCCGGAGA	55	This study
	rev771	AGCACTCTAATTTTCTCAAGG		This study
18S (b2)	fw770R	ACCTTGAGAAAATTAGAGTGC	47	This study
	rev960R	ATCGGTCTAAGAATTTCAC		This study
18S (c)	fw961	TGAAATTCTTAGACCGATGC	50	This study
	rev1460	CATCACAGACCTGTTATTGC		Dabert et al. (2010)
18S (d)	fw1462	AATAACAGGTCTGTGATGC	55	This study
	rev18S	TGATCCTTCCGCAGGTTCACCT		Dabert et al. (2010)
28S D2–5 (1)	ND2f	AGTACCGTGAGGGAAAGTTG	55	Campbell et al. (1994)
	D2R	TTGGTCCGTGTTTCAAGACGGG		Campbell et al. (1994)
28S D2–5 (2)	28S3F	GACCCGTCTTGAAACACGGA	55	Whiting et al. (1997)
	28S3R	TCGGAAGGAACCAGCTACTA		Whiting et al. (1997)
28S D9–10	28S9F	GTAGCCAAATGCCTCGTCA	52	Hillis and Dixon (1991)
	28S9R	CACAATGATAGGAAGAGCC		Hillis and Dixon (1991)
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	46	Folmer et al. (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. (1994)
12S	SR1-14199	TACTATGTTACGACTTAT	42	Kambhampati and Smith (1995)
	SR2-14594	AAACTAGGATTAGATACCC		Kambhampati and Smith (1995)

Phylogenetic analyses

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods. ML analyses were performed using the GTRGAMMAI model in RAxML-HPC-PTHREADS (Stamatakis 2006) implemented in raxmlGUI 1.3 (Silvestro and Michalak 2011). Clade support was assessed using a nonparametric bootstrapping with 1000 replicates. Nodes supported by bootstrap values (BSP) ≥ 70% were considered strongly supported (Hillis and Bull 1993). BI analyses were performed with MrBayes v.3.2.2 (Ronquist et al. 2012). For MrBayes v.3.2.2, we used separate data partitions plus mixed models and conducted two independent runs each with four Markov Chains Monte Carlo (one cold chain and three heated chains). The combined dataset was run for 20 million generations, with trees sampled every 1000 generations. The convergence of the parameter estimates was performed with Tracer v.1.6. A conservative burn-in of 25% was then applied. The consensus tree was edited with FigTree1.4.0. Nodes supported by posterior probabilities (BPP) ≥ 95% were considered strongly supported (Alfaro 2003).

Genetic distance, barcode gap discovery, and species delimitation

Sequence genetic distances were calculated for COI, 18S and 28S (Table 1) using MEGA 6.0 (Tamura et al. 2013) under the Kimura two-parameter (K2P) model (Kimura 1980). The substitution model was chosen by d: transitions + transversions. Heatmaps were drawn by R v.3.5.2 (R Core Team 2018). Pairwise distances of 12S gene were not measured because only a few *Diptilomiopus* species were successfully sequenced.

Barcode gap was analyzed by Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) web server https://bioinfo.mnhn.fr/abi/public/abgd/ using *X* value 0.9 and K2P distance. We applied ABGD to each of the three genes (COI, 18S, 28S) with the following P_{max} settings: 0.002–0.130 in COI, 0.001–0.033 in 18S, 0.001–0.023 in 28S, which were consistent with the range of intraspecific distances of each gene dataset (Table S3).

All phylogenetic trees constructed from the concatenated dataset showed that the *Diptilomiopus* was a monophyletic clade. We therefore used *Diptilomiopus* species as a reduced dataset (excluding outgroups and other eriophyoid mite species) for species delimitation analysis. The General Mixed Yule Coalescent (GMYC) model identifies the transition points between inter-and intraspecific processes on an ultrametric tree (Pons et al. 2006). Ultrametric trees were constructed in BEAST v.1.8.0 (Drummond and Rambaut 2007) through the CIPRES Science Gateway (Miller et al. 2010), using GTR + G model, a Yule speciation prior and a lognormal uncorrelated relaxed clock. As COI is one of protein coding genes in the mitochondrial genome, the 1st, 2nd and 3rd codon positions have different evolutionary rates. The COI data were either not partitioned or partitioned into 1st, 2nd and 3rd codon positions. Two independent runs of 100 million generations were executed with sampling every 1000 generations. Post burn-in trees were merged and re-sampled at a lower frequency (every 10,000 generations) using the LogCombiner of BEAST. The final ultrametric trees were entered into R (R Core Team 2018) package splits v.1.0–19 (Ezard et al. 2014) with the single threshold option as recommended by Fujisawa and Barraclough (2013). bPTP analyses were performed in the bPTP server (http://species.h-its.org/) (Zhang et al. 2013) with default values. We used MrBayes v.3.2.2 to reconstruct input trees of COI, 18S and 28S to bPTP. The reduced datasets were run for 5 million generations, with trees sampled every 5000 generations.

Results

Molecular phylogeny of *Diptilomiopus* species

The ML and BI analyses showed very similar topologies (Figs. 3, 4, S1–S4). Our results demonstrate that the *Diptilomiopus* is monophyletic with strong support (BSP > 95, BPP = 1) based on the dataset of nucleotide sequences of a single gene (18S) or multiple genes (18S, 28S, COI, and 12S) (Figs. 3, 4, S1–S4). Monophyly of the *Diptilomiopus ligustri* Wang et al. group was recovered with strong support (BSP > 87, BPP = 1) based on the dataset of a single gene (18S) (Figs. 3, S2) and multiple genes (Figs. 4, S4). The *Diptilomiopus bischofiae* Li et al. group is monophyletic with strong support (BSP = 100, BPP = 1) based on the dataset of multiple genes (Figs. 4, S4) or a single gene (Figs. 3, S2). Two representatives of *Diptilomiopus fortunus* Liu et al. were grouped with strong support (BSP = 100, BPP = 1) (Figs. S1–S4). Except for these species groups, some clades within *Diptilomiopus* was observed with low or very low support; species from the same regions (China or Malaysia) or having characteristics of divided setae *ft′* and *ft″* were not grouped (Figs. 3, 4).

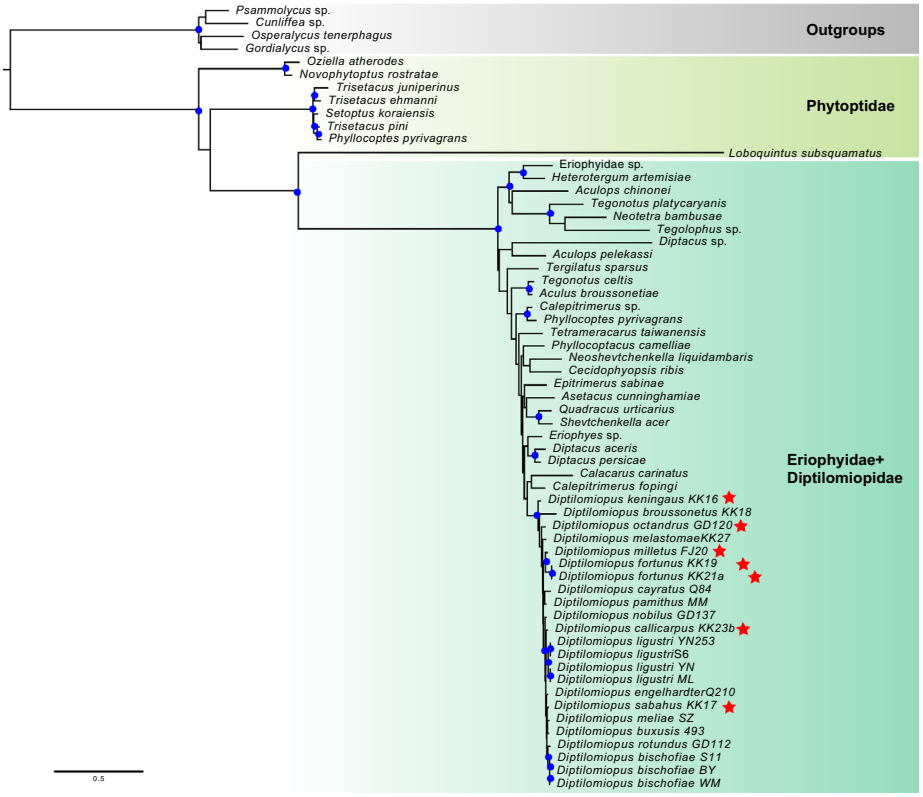

Fig. 3 Phylogenetic trees inferred from nucleotide sequences of 18S gene using maximum likelihood method. Branch lengths presented here follow the maximum likelihood analysis using the best partition found by PartitionFinder. Nodes, marked with a blue dot, indicate bootstrap values (BSP) ≥ 70%. Red stars indicate the species having divided seta *ft′* and *ft″*. (Color figure online)

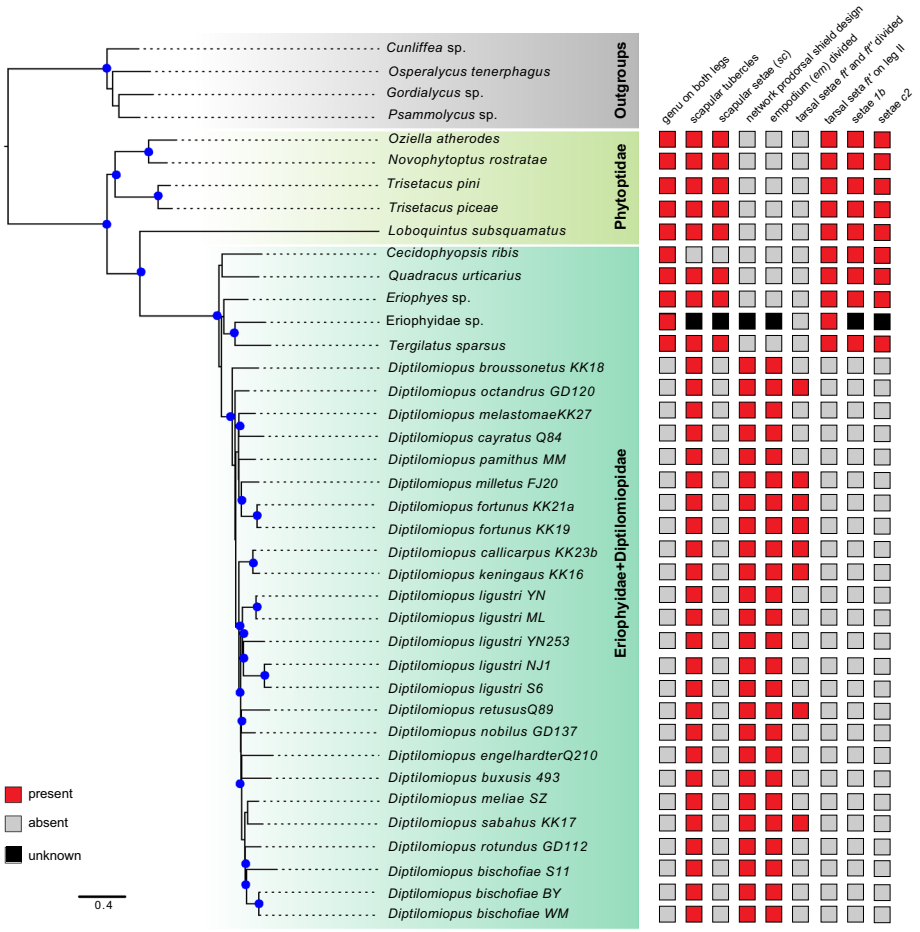

Fig. 4 Phylogenetic trees inferred from nucleotide sequences of two mitochondrial (COI and 12S) and two nuclear (18S and 28S) gene fragments using maximum likelihood method. Nodes, marked with a blue disc, indicate bootstrap values (BSP) ≥ 70%. Characters of potential synapomorphies were traced: genu on both legs, scapular tubercles, scapular setae (*sc*), network prodorsal shield design, shape of the empodium (*em*), tarsal setae *ft′* and *ft″*, setae *1b* and setae *c2*. (Color figure online)

Genetic distance and molecular delimitation of *Diptilomiopus* species

The pairwise K2P interspecies distances of COI gene fragment ranged from 25.3 to 41.3%, and the intraspecies variation ranged from 0.2 to 13% (Fig. 5a, Table S3). The greatest genetic intraspecies distances occurred in *D. fortunus*, possibly indicating cryptic species. For 18S, the interspecies distances ranged from 1.6 to 14%, and the intraspecies variation ranged from 0.0 to 3.3% (Fig. 5b, Table S3). The greatest genetic intraspecies and interspecies distances occurred in *D. ligustri* (YN253). For 28S, the interspecies distances ranged from 2.4 to 30.4%, and the intraspecies variation ranged from 0.0 to 2.3% (Fig. 5c, Table S3). The greatest genetic intraspecies variation occurred in *D. ligustri* (YN253).

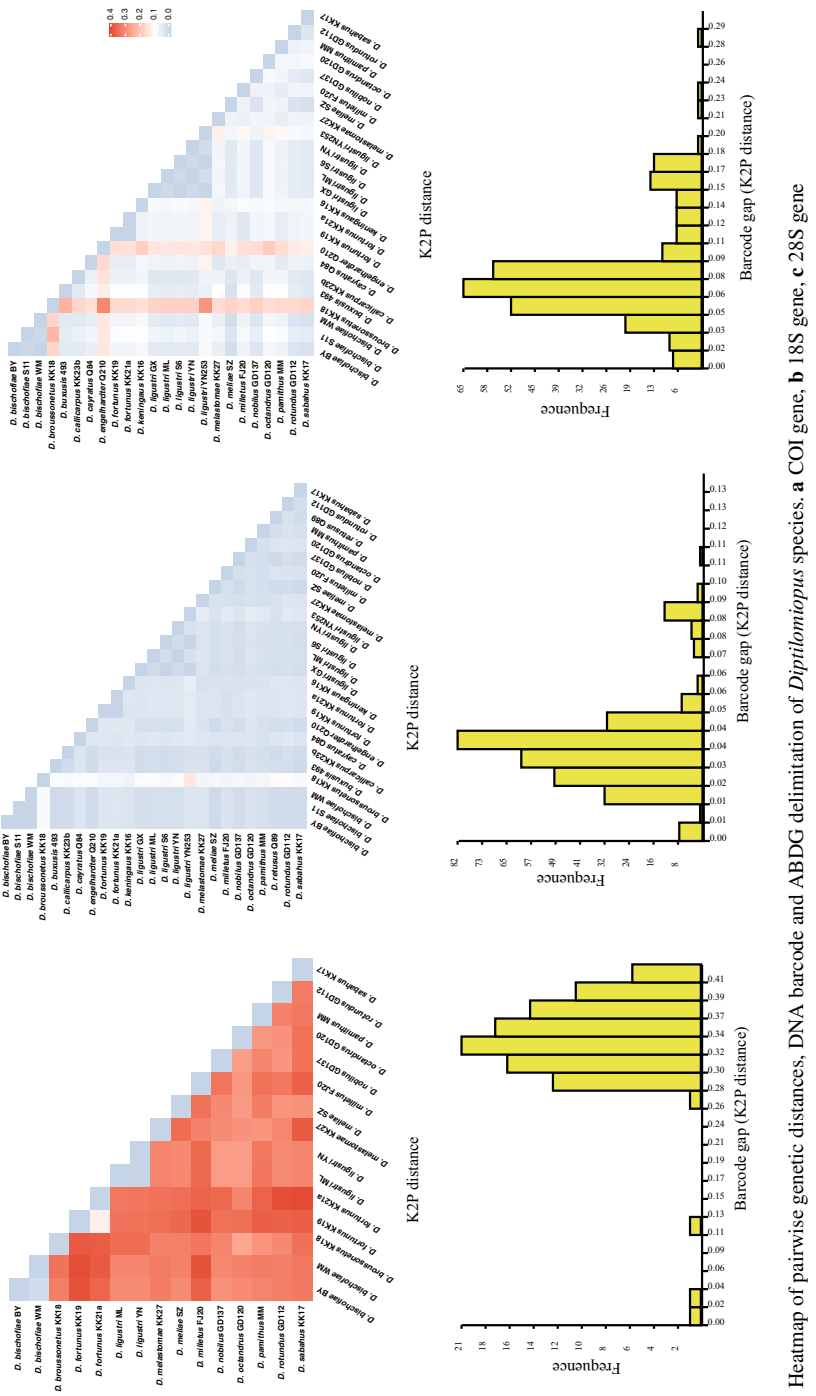

Fig. 5 Heatmap of pairwise genetic distances, DNA barcode and ABDG delimitation of *Diplotomiopus* species. **a** COI gene, **b** 18S gene, **c** 28S gene

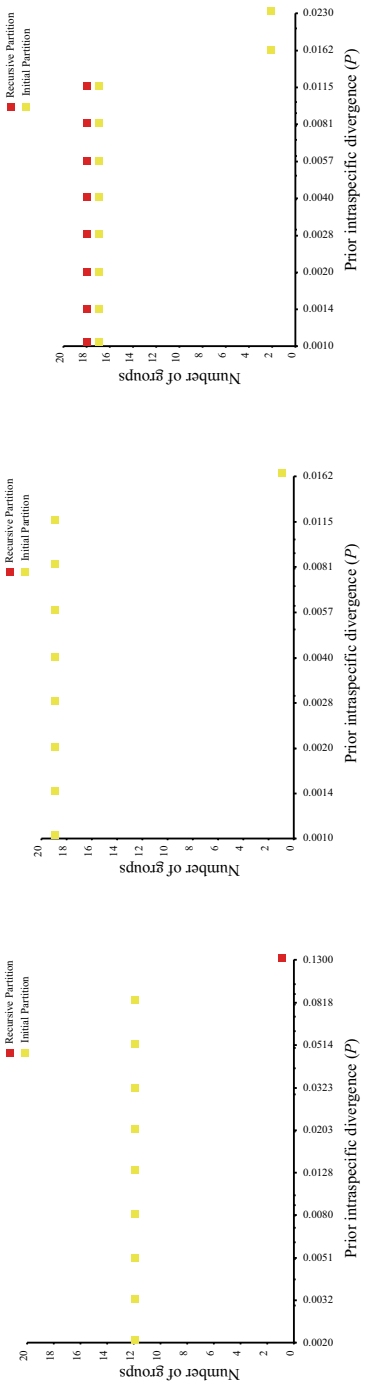

Fig. 5 (continued)

ABGD of COI delimited 12 initial partitions with prior intraspecific divergences (*P*) varying from 0.2 to 13% (Fig. 5a). Barcode gaps were observed at K2P distances of 4–11% and 13–26% (Fig. 5a). Initial partitions were identical at 12 molecular operational taxonomic units (MOTUs), corresponding to our morphologically identified 12 species. For 18S, ABGD delimited initial partitions of 19 MOTUs with *P* varying from 0.1 to 1.03% (Fig. 5b), which was consistent with our 18 a prior morphospecies, except populations of *D. ligustri* were split into two groups, ML + YN, GX + S6 + YN253. No barcode gap was observed. For 28S, ABGD delimited 17 MOTUs with *P* varying from 0.1 to 1.15% (Fig. 5c), corresponding to our morphologically identified 17 species. No barcode gap was observed.

The single-threshold GMYC analyses of COI gene identified 13 entities with significant support (not partitioned, p = 0.02; partitioned by codon, p = 0.004), which were consistent with our morphologically identified 12 species, except *D. fortunus* KK19 and *D. fortunus* KK21a which were inferred as distinct species (Figs. S5A, S5B). For 18S, the single-threshold GMYC analyses gave the same results as the ABGD analysis (see above) (Fig. S5C). For 28S, the single-threshold GMYC analyses identified 19 entities with significant support (p = 0.002), which were inconsistent with our morphologically identified 17 species, as populations of *D. ligustri* were split into two entities (ML + YN, GX + S6 + YN253) and also populations of *D. bischofiae* were split into two entities (S11, BY + WM) (Fig. S5D). bPTP analyses of COI, 18S and 28S genes resulted in similar species delimitation as the GMYC analyses (Fig. S5).

Morphological evolution of legs in *Diptilomiopus* species

We mapped some morphological characters (at the generic or species level) on the phylogenetic tree (Fig. 4), i.e., genu on both legs, scapular tubercles, scapular setae (*sc*), network prodorsal shield design, shape of the empodium (*em*), tarsal setae *ft′* and *ft″*, setae *1b* and setae *c2*, and found that *Diptilomiopus* species were united by some genetic morphological characters (genu fused with femur on both legs, scapular tubercles and setae absent, empodium divided, setae *1b* and setae *c2* absent). Intriguingly, the species sharing divided tarsal setae *ft′* and *ft″* (*D. octandrus*, *D. milletus*, *D. fortunus*, *D. callicarpus*, *D. keningaus*, *D. retusus*, and *D. sabahus*) were not grouped (Fig. 4), indicating these characteristics may have evolved multiple times independently. However, characteristics of genu fused with femur on both legs and tarsal setae *ft″* absent from leg II were suggested evolving only once, indicating they are morphological synapomorphies of *Diptilomiopus* (Fig. 4).

Discussion

The Eriophyoidea comprises more than 357 genera (Zhang 2011); however, the monophyly of these genera has seldom been tested by morphological characters or molecular approaches (Lewandowski et al. 2014). Herein, we inferred the phylogeny of *Diptilomiopus* by nucleotide sequences of multiple genes for the first time. Our phylogenetic results demonstrated the monophyly of *Diptilomiopus* with strong support (BSP > 95, BPP = 1) (Figs. 3, 4, S2, S4). All *Diptilomiopus* species in our molecular analyses were collected from the Oriental realm. Therefore, more *Diptilomiopus* species, especially from the remaining realms, should be explored and included in future analyses.

In addition to molecular evidence, the monophyly of *Diptilomiopus* might be supported by some morphological synapomorphies: genu fused with femur on leg I and leg II, seta *ft′* absent on leg II, scapular setae *sc* absent, empodium divided, coxal setae *1b* absent, and setae *c2* absent from the opisthosoma (Fig. 4). The combination of character states – genu fused with femur on both legs and seta *ft′* absent on legs II – are specific to *Diptilomiopus* species and were not found in other extant species of Eriophyoidea (Amrine et al. 2003) or fossil species of Triasacaroidea (Sidorchuk et al. 2015). We propose that these characters have evolved and occurred only once within *Diptilomiopus*, indicating they are synapomorphies (Fig. 4). However, new genera of eriophyoid mites were consistently erected in recent years, and more new genera, having those characters, cannot be ruled out in future studies. The remaining potential synapomorphies were found in the species of more than one genus of Eriophyoidea or Triasacaroidea. For instance, scapular setae *sc* absent is characteristic for Cecidophyini tribe (Eriophyidae), which includes 11 genera (Amrine et al. 2003), and for Calacarini tribe (Eriophyidae), which includes 15 genera (Amrine et al. 2003; Huang and Wang 2004; Xue et al. 2007; Chandrapatya et al. 2016); and it is characteristic for four genera (*Pseudocalepitrimerus*, *Knorella*, *Schizacea*, and *Namengia*) of Acaricalini (Eriophyidae) (Amrine et al. 2003; Zhao et al. 2018), 14 genera of Diptilomiopinae (Diptilomiopidae) (Amrine et al. 2003), and two genera (*Asetacus* and *Sakthirhynchus*) of Rhyncaphytoptinae (Diptilomiopidae) (Amrine et al. 2003). A deeply divided empodium is the main character of the subfamily Diptilomiopinae that differentiates it from the other subfamily Rhyncaphytoptinae (Amrine et al. 2003). However, this is also characteristic of Acaricalini tribe (Eriophyidae), which comprises 23 extant genera (Amrine et al. 2003; Flechtmann 2004; Chandrapatya et al. 2016; Chetverikov et al. 2018; Zhao et al. 2018), and of two fossil genera (*Triasacarus* and *Minyacarus*) (Sidorchuk et al. 2015). The character of coxal setae *1b* absent or setae *c2* absent was consistently found in many genera of Eriophyidae and Diptilomiopidae.

Most eriophyoid mites have normal tarsal seta *ft′* and seta *ft″* on legs I and II (Fig. 6). Intriguingly, seta *ft′* and seta *ft″*, divided into a short branch and a long branch, respectively (Fig. 6), has been found in a few *Diptilomiopus* species (Craemer et al. 2017). These divided setae were not observed in four fossil species (Schmidt et al. 2012; Sidorchuk et al. 2015). Moreover, the *Diptilomiopus* species, sharing these characters, were not grouped in our phylogenetic topologies (Figs. 3, 4), indicating that divided seta *ft′* and seta *ft″* were evolved at the species level.

The genus *Diptilomiopus* comprises 116 currently described species (Table S1). Morphological similarity challenges the delimitation of *Diptilomiopus* species. Our ABGD, GMYC and bPTP results showed that most of our tested morphospecies of *Diptilomiopus*, except three (*D. fortunus*, *D. ligustri* and *D. bischofiae*), were resolved by fragments of COI, 18S or 28S genes. However, those resolved morphospecies were based on single sequences from one population. More *Diptilomiopus* species, especially species from different populations, are needed in future collections. Inferred by different methods (ABGD, GMYC or bPTP) or genes (COI, 18S or 28S), three species (*D. fortunus*, *D. ligustri* and *D. bischofiae*) showed inconsistency between morphospecies and MOTUs, indicating the presence of cryptic species. However, monophyly of each of these species was always recovered in our ML and BI trees. Further, most eriophyoid mites were reported having high host-plant specificity (Skoracka et al. 2010). The various population of each of our tested species of *D. fortunus*, *D. ligustri* or *D. bischofiae* were all collected from the same corresponding host plant (i.e., one host plant species per mite species; Table 1). We therefore suggest that the high sequence genetic diversity (distance) within populations of those three mite species may be an effect of host-constrained isolation, which leads to

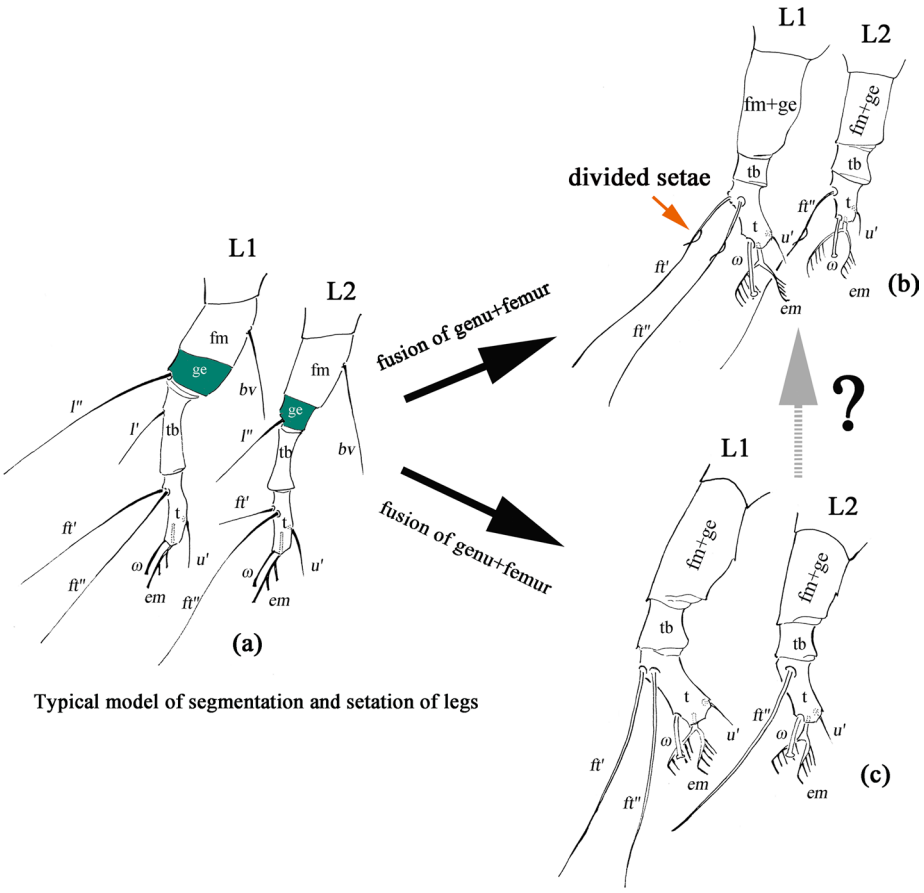

Fig. 6 Hypothesized schematic evolutionary route of legs. **a** Legs of *Epitrimerus gaotainensus,* **b** legs of *D. fortunus*, **c** legs of *D. rotundus*; L1, leg I; L2, leg II; fm, femur; ge, genu; tb, tibia; t, tarsus; *bv*, femural seta; *l''*, genual seta; *l'*, tibial seta; *ft'*, tarsal seta *ft'*; *ft''*, tarsal seta *ft''*; *u'*, seta *u'*; *em*, empodium; *ω*, solenidion.

incomplete lineage sorting (Toews and Brelsford 2012). It is widely accepted that simply relying on one approach to delimitate species, especially when they are highly morphologically similar or have two forms (protogyne an deutogyne in eriophyoid mites), is problematic (Cvrković et al. 2016; Guo et al. 2015; Skoracka et al. 2013). Herein, we underline the integrative taxonomy approach, combining morphological characters and molecular approaches, in resolving species delimitation of eriophyoid mites.

Eriophyoid mites are distributed worldwide; however, most genera are distributed regionally (Amrine and Stasny 1994) due to low dispersal ability (Michalska et al. 2010), high host-plant specificity (Skoracka et al. 2010), or possibly uneven regional field surveys. Similarly, most *Diptilomiopus* species have been reported from the Oriental realm, only a few have been recorded in the remaining realms (Fig. 1). Consistent with a previous hypothesis of Craemer et al. (2017), the most parsimonious explanation for this uneven distribution could be that *Diptilomiopus* species originated in the Oriental realm, and some dispersed to the remaining realms. Eriophyoid mites have a low

positive dispersal ability (Sabelis and Bruin 1996). Long-distance dispersal is achieved by aerial dispersal (Zhao and Amrine 1997), phoresy on host-specific insects (Sabelis and Bruin 1996; Liu et al. 2016), or probable transportation of host plants (Craemer et al. 2017). If the Oriental origin of *Diptilomiopus* is true, then *Diptilomiopus* species should occasionally occur in the Palearctic realm, Nearctic realm, Ethiopian realm, and Australasian realm due to such dispersal modes. Additional field surveys of *Diptilomiopus* spp. worldwide are obviously necessary to decipher the biogeographical distribution and dispersal routes of *Diptilomiopus* species.

Conclusions

In this study, we demonstrated that the genus *Diptilomiopus* is monophyletic by multiple lines of evidence from molecular approaches and morphological synapomorphies. Most *Diptilomiopus* species are highly similar in morphology, what may hinder their correct indentification and induce species complexes. We provide an integrative taxonomic approach to the resolution of cryptic *Diptilomiopus* species or other eriophyoid mite complexes. These findings highlight the cryptic species diversity within *Diptilomiopus*; more descriptions of new *Diptilomiopus* species and new findings related to their biogeographical distributions are expected.

Acknowledgements We would like to thank Mr. Jimmy Chew (Borneo Jungle Girl Camp, Keningau, Sabah, Malaysia) for kindly collecting mite specimens in Sabah, Malaysia. This research was funded by the National Natural Science Foundation of China (31970437).

References

Alfaro ME (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol Biol Evol 20:255–266. https://doi.org/10.1093/molbev/msg028

Amrine JW Jr, Manson DCM (1996) Preparation, mounting and descriptive study of eriophyoid mites. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites: their biology, natural enemies and control. World Crop Pests, vol 6. Elsevier, Amsterdam, pp 383–396

Amrine JW Jr, Stasny TA, Flechtmann CHW (2003) Revised keys to world genera of Eriophyoidea (Acari: Prostigmata). Indira Publishing House, Michigan

Amrine JW Jr, Stasny TA (1994) Catalog of the Eriophyoidea (Acarina: Prostigmata) of the world. Indira Publishing House, Michigan

Campbell CL, Tanaka N, White KH, Thorsness PE (1994) Mitochondrial morphological and functional defects in yeast caused by yme1 are suppressed by mutation of a 26S protease subunit homologue. Mol Biol Cell 5:899–905. https://doi.org/10.1091/mbc.5.8.899

Capinera JL (2008) Encyclopedia of entomology. Springer, Dordrecht

Chandrapatya A, Konvipasruang P, Amrine JW Jr (2016) New eriophyoid mites from Thailand. Syst Appl Acarol 21:55–78. https://doi.org/10.11158/saa.21.1.5

Chetverikov PE, Craemer C, Neser S (2018) New pseudotagmic genus of acaricaline mites (Eriophyidae, Acaricalini) from a South African palm *Hyphaene coriacea* and remarks on lateral opisthosomal spines and morphology of deutogynes in Eriophyoidea. Syst Appl Acarol 23:1073–1101. https://doi.org/10.11158/saa.23.6.6

Chetverikov PE, Cvrkovic T, Makunin A, Sukhareva S, Vidovic B, Petanovic R (2015) Basal divergence of Eriophyoidea (Acariformes, Eupodina) inferred from combined partial COI and 28S gene sequences and CLSM genital anatomy. Exp Appl Acarol 67:219–245. https://doi.org/10.1007/s10493-015-9945-9

Craemer C, Amrine JW Jr, Childers CC, Rogers ME, Achor DS (2017) A new eriophyoid mite species, *Diptilomiopus floridanus* (Acari: Eriophyoidea: Diptilomiopidae), from citrus in Florida, USA. Syst Appl Acarol 22:386–402. https://doi.org/10.11158/saa.22.3.5

Cvrković T, Chetverikov P, Vidović B, Petanović R (2016) Cryptic speciation within *Phytoptus avellanae* s.l. (Eriophyoidea: Phytoptidae) revealed by molecular data and observations on molting *Tegonotus*-like nymphs. Exp Appl Acarol 68:83–96. https://doi.org/10.1007/s10493-015-9981-5

Dabert J, Ehrnsberger R, Dabert M (2008) *Glaucalges tytonis* sp. n. (Analgoidea, Xolalgidae) from the barn owl *Tyto alba* (Strigiformes, Tytonidae): compiling morphology with DNA barcode data for taxon descriptions in mites (Acari). Zootaxa 1719:41–52

Dabert M, Witalinski W, Kazmierski A, Olszanowski Z, Dabert J (2010) Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts. Mol Phylogenet Evol 56:222–241. https://doi.org/10.1016/j.ympev.2009.12.020

Duarte ME, de Mendonca RS, Skoracka A, Silva ES, Navia D (2019) Integrative taxonomy of *Abacarus* mites (Eriophyidae) associated with hybrid sugarcane plants, including description of a new species. Exp Appl Acarol 78: 373–401. https://doi.org/10.1007/s10493-019-00388-y

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214. https://doi.org/10.1186/1471-2148-7-214

Ezard THG, Fujisawa TE, Barraclough TG (2014) Splits: species' limits by threshold statistics. R package version 1.0–19/r51. http://RForge.Rproject.org/projects/splits/

Flechtmann CHW (2004) Eriophyid mites (AcariL Eriophyidae) from brazilian sedges (Cyperaceae). Int J Acarol 30:157–164. https://doi.org/10.1080/01647950408684385

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Fujisawa T, Barraclough TG (2013) Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. Syst Biol 62:707–724. https://doi.org/10.1093/sysbio/syt033

Guo J-F, Li H-S, Wang B, Xue X-F, Hong X-Y (2015) DNA barcoding reveals the protogyne and deutogyne of *Tegolophus celtis* sp. nov. (Acari: Eriophyidae). Exp Appl Acarol 67:393–410. https://doi.org/10.1007/s10493-015-9953-9

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411–453. https://doi.org/10.1086/417338

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42:182–192. https://doi.org/10.1093/sysbio/42.2.182

Huang K-W, Wang C-F (2004) Eriophyoid mites of Taiwan: descriptin of three species of Calacarini from Hueysuen (Acari: Eriophyoidea: Phyllocoptinae). Zootaxa 527:1–8

Kambhampati S, Smith PT (1995) PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Mol Biol 4:233–236. https://doi.org/10.1111/j.1365-2583.1995.tb00028.x

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/BF01731581

Klimov PB, OConnor BM, Chetverikov PE, Bolton SJ, Pepato AR, Mortazavi AL, Tolstikov AV, Bauchan GR, Ochoa R (2018) Comprehensive phylogeny of acariform mites (Acariformes) provides insights on the origin of the four-legged mites (Eriophyoidea), a long branch. Mol Phylogenet Evol 119:105–117. https://doi.org/10.1016/j.ympev.2017.10.017

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol 34:772–773. https://doi.org/10.1093/molbev/msw260

Lewandowski M, Skoracka A, Szydlo W, Kozak M, Druciarek T, Griffiths DA (2014) Genetic and morphological diversity of *Trisetacus* species (Eriophyoidea: Phytoptidae) associated with coniferous trees in Poland: phylogeny, barcoding, host and habitat specialization. Exp Appl Acarol 63:497–520. https://doi.org/10.1007/s10493-014-9805-z

Li H-S, Xue X-F, Hong X-Y (2014a) Homoplastic evolution and host association of Eriophyoidea (Acari, Prostigmata) conflict with the morphological-based taxonomic system. Mol Phylogenet Evol 78:185–198. https://doi.org/10.1016/j.ympev.2014.05.014

Li H-S, Xue X-F, Hong X-Y (2014b) Cryptic diversity in host-associated populations of *Tetra pinnatifidae* (Acari: Eriophyoidea): What do morphometric, mitochondrial and nuclear data reveal and conceal? Bull Entomol Res 104:221–232. https://doi.org/10.1017/S0007485313000746

Lindquist EE (1996) External anatomy and notation of structures. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites: their biology, natural enemies and control. World Crop Pests, vol 6. Elsevier, Amsterdam, pp 3–31

Liu S, Li J, Guo K, Qiao H, Xu R, Chen J, Xu C, Chen J (2016) Seasonal phoresy as an overwintering strategy of a phytophagous mite. Sci Rep 6:25483. https://doi.org/10.1038/srep25483

Michalska K, Skoracka A, Navia D, Amrine JW Jr (2010) Behavioural studies on eriophyoid mites: an overview. Exp Appl Acarol 51:31–59. https://doi.org/10.1007/s10493-009-9319-2

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov 2010, New Orleans, pp 1–8

Nalepa A (1916) Neue Gallmilben (32. Fortsetzung). Anzeiger der kaiserlichen Akademie Wissenschaften. Mathematische-naturwissenschaftliche Klasse Wien 53:283–284

Navia D, de Mendonca RS, Skoracka A, Szydlo W, Knihinicki D, Hein GL, da Silva Pereira PR, Truol G, Lau D (2013) Wheat curl mite, *Aceria tosichella*, and transmitted viruses: an expanding pest complex affecting cereal crops. Exp Appl Acarol 59:95–143. https://doi.org/10.1007/s10493-012-9633-y

Petanovic R, Kielkiewicz M (2010) Plant-eriophyoid mite interactions: specific and unspecific morphological alterations. Part II. Exp Appl Acarol 51:81–91. https://doi.org/10.1007/s10493-009-9328-1

Pons J, Barraclough T, Gomez-Zurita J, Cardoso A, Duran D, Hazell S, Kamoun S, Sumlin W, Vogler A (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol 55:595–609. https://doi.org/10.1080/10635150600852011

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol Ecol 21:1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Sabelis MW, Bruin J (1996) Evolutionary ecology: Life history patterns, food plant choice and dispersal. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites: their biology, natural enemies and control. World Crop Pests, vol 6. Elsevier, Amsterdam, pp 329–366

Schmidt AR, Jancke S, Lindquist EE, Ragazzi E, Roghi G, Nascimbene PC, Schmidt K, Wappler T, Grimaldi DA (2012) Arthropods in amber from the Triassic Period. Proc Natl Acad Sci USA 109:14796–14801. https://doi.org/10.1073/pnas.1208464109

Sidorchuk EA, Schmidt AR, Ragazzi E, Roghi G, Lindquist EE (2015) Plant-feeding mite diversity in Triassic amber (Acari: Tetrapodili). J Syst Palaeontol 13:129–151. https://doi.org/10.1080/14772019.2013.867373

Silvestro D, Michalak I (2011) raxmlGUI: a graphical front-end for RAxML. Org Divers Evol 12:335–337. https://doi.org/10.1007/s13127-011-0056-0

Skoracka A, Dabert M (2010) The cereal rust mite *Abacarus hystrix* (Acari: Eriophyoidea) is a complex of species: evidence from mitochondrial and nuclear DNA sequences. Bull Entomol Res 100:263–272. https://doi.org/10.1017/S0007485309990216

Skoracka A, Kuczyński L, Szydło W, Rector B (2013) The wheat curl mite *Aceria tosichella* (Acari: Eriophyoidea) is a complex of cryptic lineages with divergent host ranges: evidence from molecular and plant bioassay data. Biol J Linnean Soc 109:165–180. https://doi.org/10.1111/bij.12024

Skoracka A, Lopes LF, Alves MJ, Miller A, Lewandowski M, Szydlo W, Majer A, Rozanska E, Kuczynski L (2018) Genetics of lineage diversification and the evolution of host usage in the economically important wheat curl mite, *Aceria tosichella* Keifer, 1969. BMC Evol Biol 18:122. https://doi.org/10.1186/s12862-018-1234-x

Skoracka A, Smith L, Oldfield G, Cristofaro M, Amrine JW Jr (2010) Host-plant specificity and specialization in eriophyoid mites and their importance for the use of eriophyoid mites as biocontrol agents of weeds. Exp Appl Acarol 51:93–113. https://doi.org/10.1007/s10493-009-9323-6

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Toews DP, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol 21:3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997) The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst Biol 46:1–68. https://doi.org/10.1093/sysbio/46.1.1

Xue X-F, Song Z-W, Amrine JW Jr, Hong X-Y (2007) Eriophyoid mites on coniferous plants in China with descriptions of a new genus and five new species (Acari: Eriophyoidea). Int J Acarol 33:333–345. https://doi.org/10.1080/01647950708683695

Xue X-F, Dong Y, Deng W, Hong X-Y, Shao R (2017) The phylogenetic position of eriophyoid mites (superfamily Eriophyoidea) in Acariformes inferred from the sequences of mitochondrial genomes and nuclear small subunit (*18S*) rRNA gene. Mol Phylogenet Evol 109:271–282. https://doi.org/10.1016/j.ympev.2017.01.009

Xue X-F, Guo J-F, Dong Y, Hong X-Y, Shao R (2016) Mitochondrial genome evolution and tRNA truncation in Acariformes mites: new evidence from eriophyoid mites. Sci Rep 6:18920. https://doi.org/10.1038/srep18920

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29:2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Zhang Z-Q (2011) Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Magnolia Press, Auckland

Zhang Z-Q (2018) Repositories for mite and tick specimens: acronyms and their nomenclature. Syst Appl Acarol 23:2432–2466. https://doi.org/10.11158/saa.23.12.12

Zhao S, Amrine JW Jr (1997) Investigation of snowborne mites (Acari) and relevancy to dispersal. Int J Acarol 23:209–213. https://doi.org/10.1080/01647959708683565

Zhao Y, Li W, Wang G-Q (2018) New asian phyllocoptines (Eriophyidae, Phyllocoptinae): descriptions of *Namengia latifloris* gen. nov. & sp. nov. (Acaricalini) and *Petanovicia cathartica* sp. nov. (Phyllocoptini) from northwest Laos. Syst Appl Acarol 23:2022–2032. https://doi.org/10.11158/saa.23.10.11