Inference about Monophyly of the Family Oedipodidae and the Classification of Subfamilies Based on 16S rDNA Sequences

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Abstract: Most of grasshoppers in the family Oedipodidae are the famous agriculture pests in China. However monophyly and the relationships among the subfamilies within this family are unclear up to now. Here the phylogeny of the Oedipodidae was reconstructed based on 16S rDNA sequence fragments by using *Mekongiella kingdoni* and *Atractomorpha sinensis* as outgroups under weighted MP, NJ and Bayesian criteria. The 408 bp fragments of mitochondrial 16S rRNA gene were sequenced for 15 species from 4 subfamilies of the family Oedipodidae, and the homologous sequences of other 15 species of grasshoppers were downloaded from the GenBank data library. The numbers of transitions and transversions among pairwise comparisons of the 16S fragments were respectively plotted against percentage sequence differences. Saturation of transitions was discovered, and transversions were not saturated with the increase of percentage sequence difference in the plots. All the individuals of the Oedipodidae excluding *Trilophidia annulata* were gathered together in the three trees. Our results are very different from the traditionary taxonomy of the Oedipodidae including 4 subfamilies. The Bryodemellinae is not supported as a subfamily, and neither Locustinae nor Oedipodinae are supported as a monophyletic group in this study.

Keywords: Oedipodidae, 16S rDNA, monophyly, subfamily.

1. INTRODUCTION

The family Oedipodidae is one of the largest families in the super family Acridoidea, including 137 described species of 38 genera of 4 subfamilies in China. The Oedipodidae species are largely distributed in the Palaeoacrtic regionwith a few species distributed in the Oriental region [1,2]. Historically *Locusta migratoria manilensis* of the Oedipodidae was a famous agriculture pest in China. Because the Oedipodidae is a larger group in the Acridoidea, and the phylogenetic study on them is crucial for understanding the phylogeny of Acridoidea, even to the Caelifera.

The taxonomic position of the Oedipodidae has been revised several times. It is usually recognised as a family of the Acridoidea by Chinese specialists [2,3], while classified as a subfamily by other specialists [4-7]. Phylogenetic analysis did not support the monophyly of Catantopidae, Arcypterdae, Gomphoceridae and Acrididae in our previous study based on 18S rDNA [8], but unfortunately it did not resolve the monophyly of the Oedipodidae.

In order to identify the monophyly of the Oedipodidae and to obtain a clear understanding of the relationships among the four subfamlies of the Oedipodidae, we utilized partial sequences of the mitochondrial 16S rDNA to reconstruct the phylogeny of the Oedipodidae. The 16S rDNA is one of the most comprehensively studied genes in insects [9], and generally considered well to effectively research organism divergences before 50 millions of years [10]. Fifteen species from the Oedipodidae were sequenced in this study. The 16S rDNA sequences of other fifteen grasshoppers were download from GenBank for comparisons, in which six species belongs to the Oedipodidae. In the thirty grasshoppers studied, *Mekongiella kingdoni* of the Chrotogonidae and *Atractomorpha sinensis* of the Pyrgormorphidae were chosen as the outgroups.

2. MATERIALS AND METHODS

2.1. Samples and DNA Extraction

The 15 species of grasshoppers used in this study were collected from China (Table **1**). The individuals of alive grasshoppers were stored in absolute ethanol. The species was identified by using Xia's taxonomic system [11]. Total genomic DNA was extracted from legs of single grasshopper by using a simple proteinase K/SDS method.Tissue was ground and incubated in 0.02 M Tris HCl (pH 8), 0.01 M EDTA, 0.5% SDS, and 50 mg/mL of Proteinase K overnight at 50°C. This mixture was extracted with phenol/chloroform, and finally the DNA samples were precipitated with ethanol as described by [12].

2.2. PCR Amplification

The primers, which were used for amplification in this study, were according to Simon *et al.* [13]. For the

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Table 1: Original of the Researched Sanples

Subfamily	Species	Collection site	Collector	Voucher	Dates	Accession of GenBank
Locustinae	*Locusta migratoria manilensis*	Laibin, Guangxi	JGF	H5042	2000. 7	
	Pternoscirta sauteri	Tiane, Guangxi	JGF	H5011	2002. 4	
	Pternoscirta pulchripes	Fangcheng, Guangxi	JGF	H5012	2002. 4	
Oedipodinae	*Trilophidia annulata*	longzhou, Guangxi	LJW	H5281	2002. 7	
	Epacromius coerulies	Ningxian, Gansu	LDF	H5311	2004. 8	
	Oedaleus decorus asiaticus	Diebu, Gansu	LDF	H5192	2004. 8	
	Celes akitanus	Jilin	RBZ	H5231	2003. 8	
	Parapheurus alliaceus	Jilin	RBZ	H5091	2003. 8	
	Sphingonotus salinus	Diebu, Gansu	LDF	H5332	2004. 8	
	Sphingonotus ningsianus	Wuwei, Gansu	LDF	H5331	2004. 8	
Bryodemellinae	*Bryodemilla diamesum*	Lasa, Xizang	JGF	H5051	2002. 7	
	Bryodemella xizangensis	Lasa, Xizang	JGF	H5071	2002. 7	
Bryodeminae	*Bryodema luctuosum indum*	Qingtongxia, Ningxia	LDF	H5072	2004. 8	
	Bryodema gansuensis	Diebu, Gansu	LDF	H5074	2004. 8	
	Angaracris rhodopa	Xining, Qinghai	JGF	H5082	2003. 7	

Note. Collector abbreviations: JGF, Jiang GuoFang; LDF, Liu DianFeng; LJW, Liu JianWen; RBZ, Ren BingZhong.

LSU rRNA fragment, primers are LR-N-13398 5'-CGCCTGTTTAACAAAAACAT-3' and LR-J-12887 5'-CCGGTCTGAACTCAGATCACGT-3'. The primers were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd (Shanghai, China).

PCRs were 30μL in volume and contained 10m moL/L Tris (pH8.3), 50m moL/L KCL, 0.01% TritonX-100, 1.5m moL/L $MgCl_2$, 0.2m moL/L dNTP, 0.4m moL/L primers, 1.0 unit of Taq-polymerase and 1μL template DNA (10-25ng). Amplifications were performed under the following conditions: an initial denaturation step of 5 min at 94°C; 30 cycles of 30 s 94°C, 40 s 48°C, 30S 72°C, and a final extension step at 72°C for 10 min.

Products of successful PCR amplifications were purified using a GeneClean III kit (Anachem, USA), following the protocol in the manual. Purified product were sequenced by Shanghai United Gene Company (Shanghai, China). Both strands of the 16S rDNA sequences were sequenced for each sample.

2.3. Data Analysis

The 16S rDNA sequences of other 15 grasshoppers were download from GenBank for comparison, among them six species belongs to the Oedipodidae (Table **2**). All sequences were aligned using Clustal X [14] with parameters set to default. Alignments were improved by comparison to secondary structures and regions of uncertain alignment were omitted from subsequent analyses. In order to examine our 16S rDNA sequences for saturation, we plotted the uncorrected pairwise genetic distance (p-distance) versus the absolute number oftransitions (TS) and absolute number of transversions (TV) among all taxa. Nucleotide variation and substitution patterns were examined using the software package MEGA6.0 [15] based on the Tamura-Nei model. We used three different types of phylogenetic analyses, neighbour-joining (NJ), maximum- parsimony (MP) and Bayesian inference. NJ and MP analyses were conducted using PAUP4.0b10 [16], Bayesian inference was used MrBayes3_0b4 [17]. Trees saved below the burn-in generation were discarded from the set of saved trees, and a majority rule consensus of the remaining trees were calculated in Mrbayes3_0b4, providing posterior probabilities for clades.

Mekongiella kingdoni and *Atractomorpha sinensis* were chosen as the outgroups in our analyses. For the NJ analysis, we selected the Tamura-Nei model. Branch support was assessed for all topologies using 1000 bootstrap replications. For the parsimony analyses, we performed a heuristic search using

Table 2: GenBank Sequences Data Used in this Study

Families	Subfamilies	Species	Number	Reference	Accession of GenBank
Oedipodidae	Locustinae	*locusta migrator*	H5f	Flook *et al.*, 1994	NC_001712
		Gastrimargus marmoratus	h5031	Jiang and Liu, 2004	AY566264
	Oedipodinae	*Oedipoda coerulescens*	H5h	Flook and Rowell, 1997	Z93293
		Aiolopus thalassinus	H5l	Rowell and Flook, 2003	AY352428
		Sphingonotus haitensis	H5s1	Rowell and Flook, 2003	AY352436
		Sphingonotus fuscoirroratus	H5s2	Rowell and Flook, 2003	AY352434
Catantopidae	Catantopinae	*Xenocatantops humilis*	H4822	Jiang and Liu, 2004	AY566258
	Cytacanthacridinae	*Chondracris rosea rosea*	H4651	Jiang and Liu, 2004	AY566262
Arcypteridae	Arcypterinae	*Chorthippus intermedius*	H6d	Yin, *et al.*, 2003	AY379750
		Arcyptera fusca	H6w	Flook and Rowell, 1997	Z93286
Gomphoceridae	Gomphoceriae	*Dasyhippus peipingensis*	H7b	Yin, *et al.*, 2003	AY379751
Acrididae	Acridinae	*Acrida cinerea*	H8z	Yin, *et al.*, 2003	AY379748
		Acrida turrita	H8n	Flook *et al.*, 1999	Z97612
Pyrgormorphidae	Atractomorphinae	*Atractomorpha sinensis*	H3d	Yin *et al.,* 2003	AY379746
Chrotogonidae	Mekongiellinae	*Mekongiella kingdoni*	H2j	Yin *et al.*, 2003	AY379745

random stepwise-addition of 10 replicates each and a branch-swapping algorithm of tree-bisection-reconnection (TBR) and ignoring the uninformative sites. For the data set we run parsimony analyses on all charters unweighted or differentially weighting transitions and transversions (TS/TV=4:5). We run MrBayes3_0b4 with the following specifications: The analysis was performed using the general time-reversible model (GTR) including estimation of invariant sites with a gamma distribution (invgamma). Initial runs were conducted starting 400,000 generations starting with a random tree and employing 4 simultaneous MCMC chains was executed. Every 100th tree was saved into a file.

3. RESULTS

3.1. Description of Data

The sequences of the fifteen species studied by us have deposited in GenBank (accession numbers are showed in Table **1**). The 16S rDNA data sets used for analyses contain 408 aligned sites, Of these sites, 135 were variable sites, 41 were parsimony informative. The average base composition are A: 32.5%, T: 35.7%, G: 12.4% and C: 19.5%, with the A+T contents higher than those of G+C.

Patterns of substitutions among the 30 grasshoppers are shown in Figure **1**. At or near the

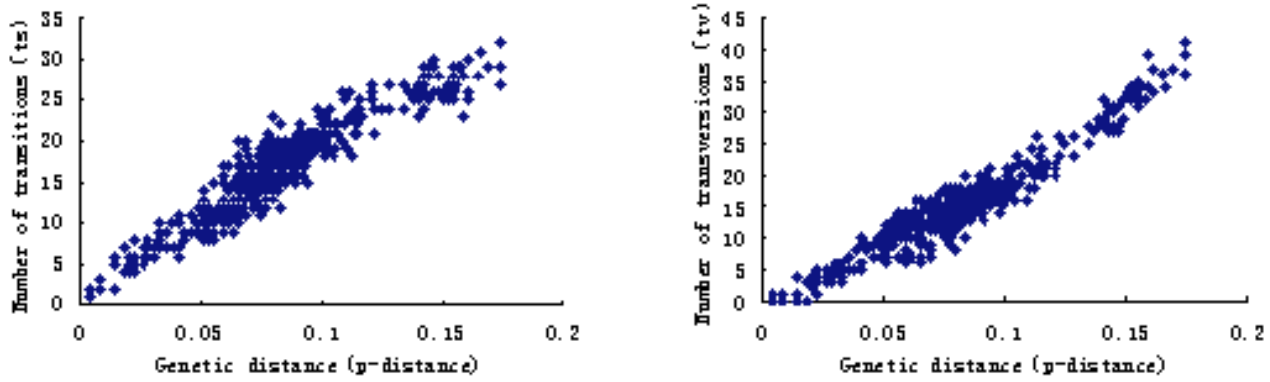

Figure 1: Plots of genetic (p-distance) against number of the transition and the transvertions.

Table 3: Sepuence Divergence (Tamura-nei Distance, below Diagonal) and Numbers of Substitutins (Transition/Transversion, above Diagonal) for Mitochondrial 16SrDNA Sequences of Different Species of Grasshoppers

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	h4651	h6w	h4822	h6d	h7b	h8z	h8n	h5281	h5311	h5091	h5f	h5042	h5192	h5031	h5012	h5011	h5h	h5051	h5074	h5082	h5071	h5072	h5s1	h5s2	h5332	h5331	h5231	h5l	h2j	h3d
1		0.931	0.829	0.951	0.806	0.975	0.722	1.725	1.252	1.546	1.344	1.172	0.941	0.819	1.276	0.983	1.498	1.463	1.312	1.399	1.319	1.249	1.245	1.398	1.175	1.504	1.113	1.041	0.755	0.904
2	0.056		1.037	1.622	0.942	0.974	0.622	1.029	1.245	2.403	1.651	1.811	1.258	1.115	1.615	1.240	2.367	2.131	2.036	2.519	2.131	2.000	2.136	3.092	1.936	2.011	1.547	2.560	1.014	0.981
3	0.072	0.076		1.288	1.246	0.862	0.876	1.187	0.915	1.193	1.467	1.496	1.030	1.169	1.222	1.168	1.562	1.425	1.260	1.498	1.345	1.190	1.176	1.252	1.112	1.115	1.111	0.915	0.992	0.970
4	0.067	0.048	0.078		0.897	0.723	0.569	0.945	1.119	2.722	1.766	1.849	0.955	1.328	1.351	1.227	1.679	1.590	1.557	1.698	1.590	1.630	1.467	1.730	1.403	1.499	1.517	1.963	1.084	1.041
5	0.087	0.062	0.109	0.040		0.741	0.615	0.859	0.866	1.493	1.174	1.175	0.961	1.114	1.119	0.852	1.048	1.119	1.055	1.319	1.050	1.046	1.052	1.326	1.114	0.986	0.935	1.382	0.822	0.806
6	0.085	0.086	0.090	0.079	0.101		0.795	1.033	0.968	1.358	1.290	1.304	1.237	1.304	1.063	1.231	1.600	1.421	1.257	1.495	1.265	1.189	1.397	1.516	1.113	1.339	1.184	0.895	0.712	0.890
7	0.078	0.079	0.096	0.067	0.088	0.043		0.637	0.771	1.118	1.230	1.174	0.831	0.934	0.966	0.718	1.634	1.177	1.053	1.115	1.049	1.046	0.934	1.046	0.925	0.987	1.252	0.771	0.755	1.127
8	0.079	0.059	0.087	0.062	0.086	0.065	0.070		0.751	1.129	1.115	1.116	0.899	1.002	1.116	0.908	1.603	0.967	0.975	1.338	0.967	0.894	1.044	1.197	0.852	0.895	0.744	0.839	0.920	1.046
9	0.096	0.060	0.087	0.073	0.091	0.080	0.076	0.071		1.024	0.936	0.982	0.833	1.048	1.048	1.248	1.356	0.955	0.795	1.218	0.770	0.680	0.790	1.029	0.655	0.856	0.869	0.580	0.989	0.898
10	0.088	0.063	0.082	0.069	0.080	0.089	0.080	0.068	0.048		0.770	0.731	0.788	1.128	1.417	1.119	1.998	1.932	1.555	2.247	1.628	1.728	1.561	2.472	1.636	1.785	1.152	0.915	0.929	0.998
11	0.134	0.108	0.134	0.120	0.126	0.127	0.122	0.116	0.099	0.076		0.505	0.624	1.380	1.256	1.000	1.119	1.277	1.115	1.117	1.196	1.120	0.927	1.190	0.969	0.982	1.124	1.042	0.836	1.219
12	0.124	0.098	0.121	0.107	0.113	0.113	0.112	0.104	0.090	0.064	0.016		0.606	2.065	1.196	1.062	1.128	1.305	1.120	1.125	1.214	1.133	0.909	1.210	0.956	0.971	1.140	1.038	0.815	1.171
13	0.127	0.098	0.139	0.112	0.119	0.116	0.121	0.109	0.099	0.081	0.056	0.042		1.142	1.437	0.834	0.985	0.884	0.897	0.960	0.884	0.806	0.968	1.118	0.683	0.705	0.972	0.804	0.699	0.888
14	0.114	0.086	0.117	0.100	0.110	0.113	0.100	0.109	0.093	0.073	0.056	0.040	0.050		1.205	1.055	1.379	3.044	2.519	2.401	2.563	2.991	1.491	2.146	1.991	1.525	3.584	1.047	0.746	1.129
15	0.143	0.113	0.133	0.128	0.135	0.126	0.124	0.128	0.093	0.097	0.097	0.082	0.091	0.076		1.492	1.543	1.438	1.264	1.196	1.355	1.437	1.584	1.774	1.042	1.547	1.492	1.054	0.615	0.783
16	0.141	0.110	0.123	0.128	0.134	0.122	0.124	0.115	0.102	0.096	0.103	0.094	0.099	0.099	0.099		1.004	1.437	1.266	1.436	1.357	1.203	1.193	1.296	1.203	1.198	1.286	0.777	0.798	0.845
17	0.099	0.089	0.123	0.085	0.094	0.098	0.091	0.097	0.087	0.070	0.091	0.079	0.090	0.081	0.109	0.102		1.626	1.397	1.525	1.427	1.464	1.214	1.689	1.431	1.529	1.585	1.293	0.983	1.172
18	0.112	0.075	0.097	0.083	0.098	0.098	0.106	0.079	0.062	0.053	0.085	0.073	0.070	0.074	0.091	0.090	0.076		1.007	n/c	n/c	n/c	1.431	2.732	1.526	1.279	1.638	1.136	0.877	1.043
19	0.112	0.081	0.096	0.089	0.101	0.098	0.106	0.085	0.062	0.053	0.085	0.073	0.076	0.073	0.090	0.090	0.076	0.005		5.093	1.007	2.028	1.016	1.789	1.013	0.811	1.189	0.933	0.815	0.963
20	0.109	0.084	0.100	0.086	0.107	0.101	0.103	0.094	0.070	0.059	0.079	0.067	0.073	0.062	0.082	0.090	0.073	0.016	0.016		n/c	n/c	1.848	3.443	1.526	2.311	2.275	1.349	0.842	0.998
21	0.106	0.075	0.094	0.083	0.095	0.092	0.100	0.080	0.056	0.048	0.082	0.070	0.070	0.065	0.088	0.088	0.070	0.005	0.005	0.016		n/c	1.220	2.386	1.526	1.015	1.428	0.920	0.877	1.042
22	0.103	0.072	0.088	0.077	0.089	0.089	0.094	0.077	0.053	0.043	0.073	0.062	0.067	0.062	0.091	0.082	0.065	0.008	0.008	0.018	0.005		1.220	2.386	1.691	1.015	1.271	0.906	0.868	1.037
23	0.108	0.083	0.105	0.086	0.100	0.104	0.100	0.088	0.061	0.054	0.094	0.082	0.078	0.079	0.082	0.087	0.070	0.031	0.031	0.037	0.029	0.029		3.070	1.016	2.037	1.158	0.835	0.851	0.898
24	0.102	0.087	0.096	0.080	0.101	0.095	0.094	0.083	0.059	0.054	0.094	0.082	0.084	0.082	0.088	0.079	0.070	0.029	0.029	0.034	0.026	0.026	0.021		1.360	8.274	1.740	1.264	0.869	0.921
25	0.111	0.086	0.096	0.077	0.097	0.098	0.094	0.085	0.061	0.048	0.079	0.067	0.081	0.070	0.082	0.082	0.070	0.026	0.026	0.026	0.026	0.021	0.026	0.018		1.271	1.870	0.921	0.859	0.913
26	0.100	0.072	0.096	0.080	0.092	0.095	0.097	0.076	0.059	0.051	0.091	0.079	0.073	0.073	0.088	0.081	0.073	0.023	0.023	0.034	0.021	0.021	0.023	0.024	0.023		1.020	1.026	0.897	1.041
27	0.120	0.089	0.102	0.094	0.100	0.095	0.103	0.085	0.064	0.057	0.073	0.062	0.078	0.071	0.099	0.091	0.068	0.034	0.034	0.042	0.031	0.023	0.045	0.043	0.037	0.037		0.708	0.945	1.172
28	0.081	0.066	0.087	0.062	0.083	0.077	0.076	0.054	0.059	0.045	0.082	0.070	0.087	0.081	0.105	0.096	0.079	0.056	0.056	0.062	0.050	0.045	0.053	0.053	0.050	0.053	0.059		0.859	1.041
29	0.157	0.176	0.190	0.163	0.160	0.183	0.175	0.156	0.184	0.161	0.188	0.174	0.201	0.183	0.181	0.200	0.177	0.174	0.174	0.171	0.175	0.168	0.177	0.168	0.161	0.164	0.174	0.161		1.275
30	0.171	0.180	0.201	0.160	0.170	0.161	0.160	0.167	0.164	0.155	0.167	0.150	0.181	0.165	0.172	0.194	0.161	0.159	0.158	0.155	0.159	0.153	0.165	0.155	0.149	0.159	0.162	0.159	0.084	

Table 4: Nucleotide Substitution Model Parameter Estimates for Two Indepemdent Bayesian Analyses, Upper Values in each Pair Correspond to Analyses A, Lower Values Correspond to Analyses B

Parameter	Mean	Variance	95% Credibe Interval	
Total tree length(TL)	2.086719	0.126973	1.629000	2.792000
	2.059129	0.178492	1.652000	3.003000
Rate of substitutionr R_{GT}	1.000000	0.000000	1.000000	1.000000
	1.000000	0.000000	1.000000	1.000000
Rate of substitutionr R_{CT}	3.463773	1.890736	1.724435	6.825100
	3.444560	1.279798	1.602340	5.991721
Rate of substitutionr R_{CG}	0.519617	0.108941	0.124913	1.391495
	0.518451	0.075186	0.149706	1.191387
Rate of substitutionr R_{AT}	2.211812	0.380806	1.260839	3.720657
	2.266468	0.479157	1.242698	3.884416
Rate of substitutionr R_{AG}	5.775648	2.346415	3.354162	9.387583
	5.900065	2.721322	3.371619	9.710389
Rate of substitutionr R_{AC}	0.237770	0.029756	0.016618	0.710178
	0.232991	0.023969	0.057124	0.665314
Base frequencies π(A)	0.321654	0.000408	0.283371	0.360295
	0.320725	0.000375	0.282883	0.359047
Base frequencies π(C)	0.109170	0.000235	0.081104	0.138288
	0.108955	0.000199	0.084024	0.136404
Base frequencies π(G)	0.188330	0.000263	0.156357	0.220288
	0.187804	0.000275	0.157418	0.220808
Base frequencies π(T)	0.380846	0.000445	0.339707	0.421572
	0.382516	0.000460	0.342774	0.425312
Shape parameter alpha(G)	0.788424	0.080694	0.375719	1.444650
	0.830107	0.096053	0.360533	1.540739
Pinvar	0.470416	0.005185	0.290860	0.573489
	0.476801	0.005412	0.287587	0.581379

Bharat Book Bureau. Cell-based Assays: Technologies and Global Markets. http://robotics.tmcnet.com/news/2011/12/29/6022837.htm

10% value TS began to level off, indicating saturation of TS. Nucleotide variation and substitution patterns were examined using the software package MEGA 6 (Table **3**). The average value of TS/TV is 1.268, and TS is a little higher than TV. The average value of the sequence divergence is 0.090 after the correction of Tamura-nei model.

3.2. Phylogenetic Relationships

Three phylogenetic trees, weighted MP tree (wMP tree), Neighbor-Joining (NJ) tree and Bayesian tree (Figures **2-4**), were reconstructed. The structure of these phylogenetic trees are similar, and the grasshoppers of the Oedipodidae are clustered into one clade except *Trilophidia annulata*. In both MP and NJ trees, the species studied can be clearly classified into five clades as follows: Clade I contains nine species: *Bryodema luctuosum indum, Bryodema gansuensis*, and *Angaracris rhodopa* (Bryodeminae); *Bryodemilla diamesum* and *Bryodemella xizangensis* (Bryodemellinae); and *Celes akitanus*, *Sphingonotus salinus*, *Sphingonotus ningsianus*, *Sphingonotus haitensis*, and *Sphingonotus fuscoirroratus* (Oedipodinae). Clade II contains six species: *locusta migratory*, *Locusta migratoria manilensis*, *Gastrimargus marmoratus*, *Pternoscirta sauteri*, and *Pternoscirta pulchripes* (Locustinae); *Oedaleus decorus asiaticus*, and *Oedipoda coerulescens* (Oedipodinae). Clade III consists of two species, *Parapheurus alliaceus* and *Epacromius coerulies* (Oedipodinae). Clade IV consists

of only one species *Aiolopus thalassinus*. Clade V contains eight species: *Chondracris rosea rosea*, *Xenocatantops humilis, Arcyptera fusca*, *Chorthippus intermedius*, *Dasyhippus peipingensis*, *Acrida cinerea*, *Acrida turrita*, and *Trilophidia annulata*. The difference between wMP tree and NJ tree is mainly showed within the Clade I: *Celes akitanus* cluster with the grasshoppers of the subfamilies Bryodeminae and Bryodemellinae, and at the root of the wMP tree; Bayesian tree is different from the other two trees, as in the Bayesian tree *Parapheurus alliaceus* and *Epacromius coerulies* do not cluster with each other, and *Trilophidia annulata* is at the root ofthe tree.

4. DISCUSSION

4.1. Characteristics of 16S rDNA Sequence

The average base composition in this study are A: 32.5%, T: 35.7%, C: 12.4% and G: 19.5%, with A+T contents (68.2%) higer than these of C+G (31.9%), a pattern that has been seen repeatedly in the mtDNA of insects [7,10,18]. The number of transition is a little than those of transversion (TS/TV=1.268), which is different from the result of our previous study in the family Catantopidae (TS/TV=0.723) (unpublished). The grasshoppers of the Oedipodidae collapsed into one clade except *T. annulata*. However, in our previous study [8], the species of the Catantopidae clustered with the species of other families alternately. To some extent, the results suggested that the species within the Catantopidae are more divergent than those within the Oedipodidae.

4.2. Monophyly of the Oedipodidae

According to the most taxonomic systems, the grasshoppers of the Oedipodidae are classified as a subfamily Oedipodinae of the family Acrididae [5,6], and it consistent with the Orthoptera Species File Online at internet [19].

All the phylogenetic trees did not support the monophyly of the Oedipodidae, because T. annulata did not cluster with the rest grasshoppers analysed in this study. NJ tree and wMP tree suggested that the Oedipodidae is a polyphyly, while Bayesian tree suggested it is paraphyletic. All these relationships are well supported (pp 1.0 on the Figures **2-4**). Considering the previous study on the Acrididae based on 18S

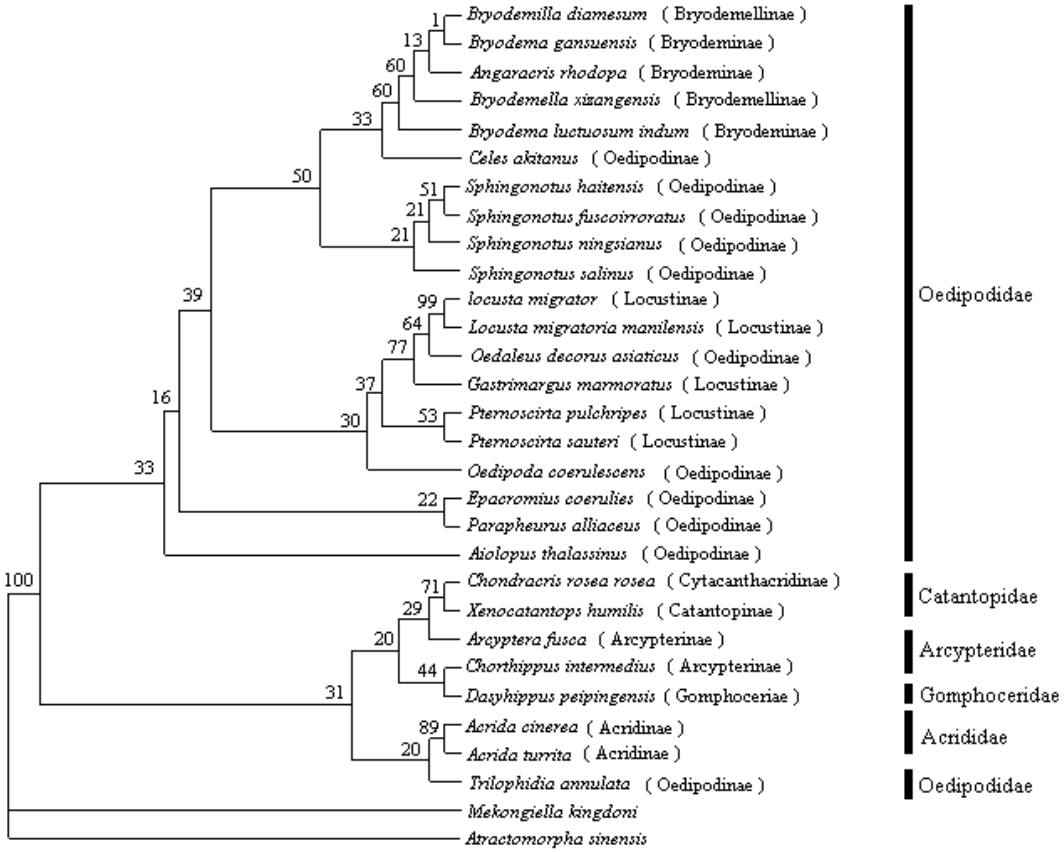

Figure 2: The maximum parsimony tree of weighting 4:5 for ts:tv resulting from analysis of the 16S rDNA sequences of 30 grasshoppers. (Number on nodes correspond to percentage bootstrap values for 1000 replicates).

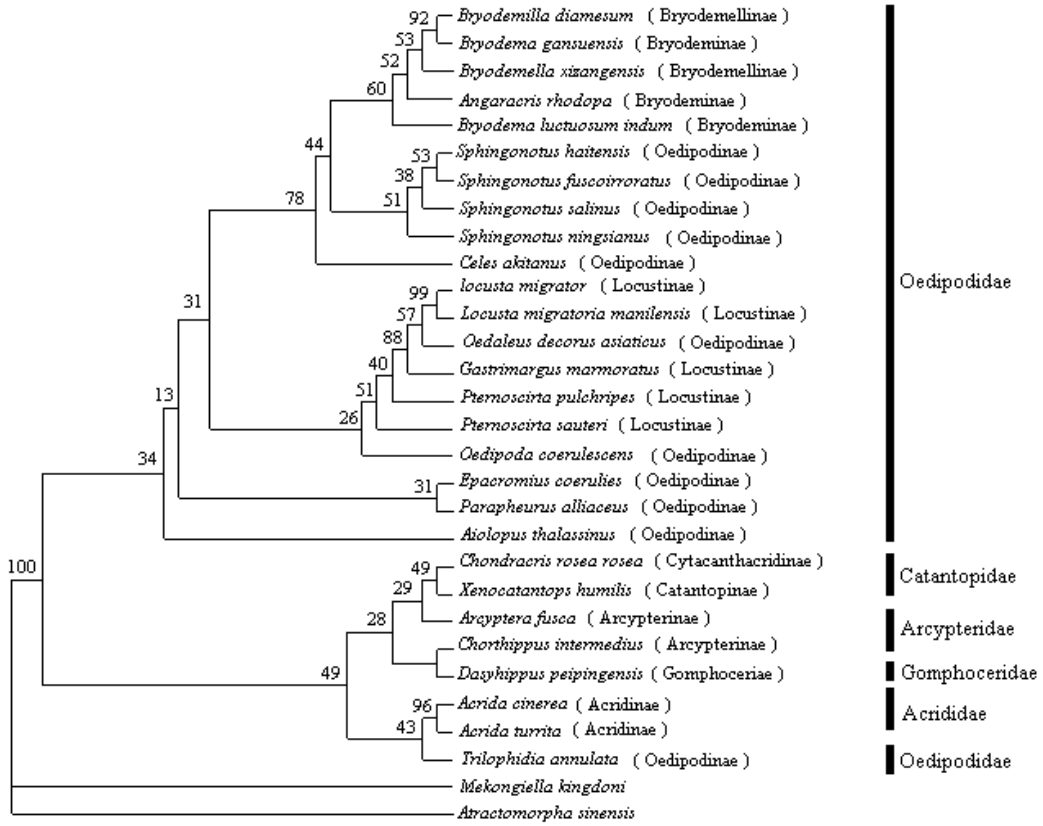

Figure 3: The neighbor-joining tree resulting from analysis of the 16S rDNA sequences of 30 grasshoppers. (Number on nodes correspond to percentage bootstrap values for 1000 replicates).

rDNA [8], we therefore suggested that the grasshoppers of the Oedipodinae should be classified as one subfamily of the Acrididae, but the taxonomic positions of some species need further study.

4.3. The Taxonomic Positions of Bryodeminae and Bryodemellinae

The species of the geus *Bryodemella* has sometimes been treated as one of the genus *Bryodema* based on some morphological characters before Yin [3]. The distinguishable morphological characters in *Bryodemella* are, 1) Elytron with no intercalary vein in medial area; if some times with a weak one, then not serrated. 2) Dorso external carinae of hind femur finely serrated in terminal half, stridulated with thicken longitudinal veins of hindwing. The genus *Bryodemella* was further elevated to a new subfamily, i.e. Bryodemellinae, based on stridulatory apparatus of some gasshoppers in Yin [3]. The three phylogenetic trees in our study showed that the species of the Bryodeminae (*Bryodema gansuensis*, *Bryodema luctuosum indum*, *Angaracris rhodopa*) clustered with those of Bryodemellinae (*Bryodemilla diamesum*, *Bryodemella xizangensis*), which suggested that the two subfamilies were closely related; and the phylogentic analysis did not support the monophyly of the genera *Bryodemella*, *Bryodema* and *Angaracris*, and therefore further proved the invalidity of the subfamily Bryodemellinae.

4.4. Phylogenetic relationships within Oedipodidae

The family Oedipodidae is separated into four subfamilies based on some following morphological characters: dorsal carina of hind femur, and elytron with intercalary vein in medial area and main longitudinal veins of hindwing. The four subfamilies are Locustinae, Bryodemellinae, Bryodeminae and Oedipodinae [3]. But our results did not support the division of the four subfamilies. First, phylogenetic analyses did not support the basic status of the subfamily Bryodemellinae, wMP tree and Baysian trees showed that those species of Bryodemellinae and Bryodeminae firstly cluster with each other, then they cluster with *Celes akitanus* of the subfamily Oedipodinae, so the monophyly of the subfamily Bryodeminae is not supported in our study, and thus further study is necessary. Second, our phylogenetic analyses did not support the monophyly of the subfamilies Locustinae and Oedipodinae. The species of the Locustinae and some species of the

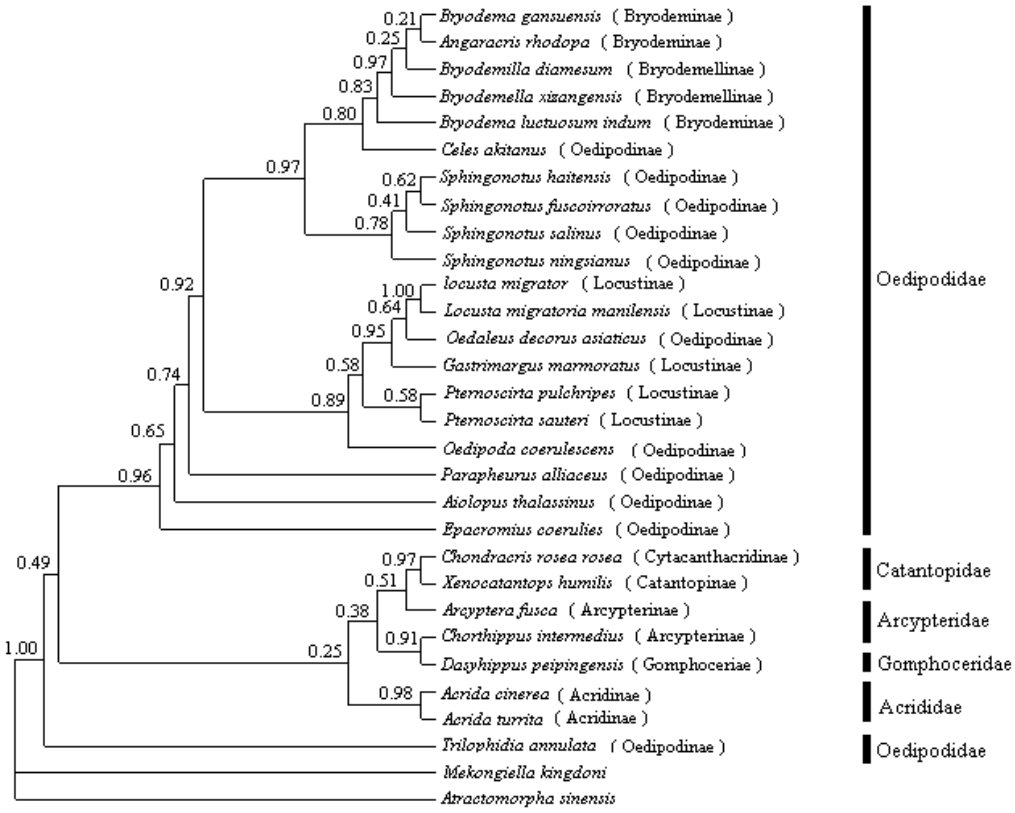

Figure 4: The bayesian tree resulting from analysis 16S rDNA sequences of 30 grasshoppers. (Number on nodes correspond to the values of posterior probability values).

Oedipodinae gathered with each other, the former clade is composed of *Locusta migratory*, *Locusta migratoria manilensis*, *Gastrimargus marmoratus*, *Pternoscirta sauteri*, *Pternoscirta pulchripes*; the latter is composed of *Oedaleus decorus asiaticus*, *Oedipoda coerulescens.* In addition, rest species of the Oedipodinae forms one clade.

C - banding karyotype and the nucleolar organizer region with silver impregnation have been analyzed to eight species of six genera of the Odipodidae [20]. Our results suggested these six generacan be divided into three groups based on their relationships resulting from our analyses. The genus *Locusta* is close related to *Gastrimargus,* together forming a group; there are close relationships among *Epacromius*, *Aiolopus* and *Oedaleus*; the genus *Angaracris* is divergent from the former two groups, independently forming a group.

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