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GENETIC DIVERSITY OF SPRINGTAILS (COLLEMBOLA SUBCLASS) BASED ON CYTOCHROME OXIDASE SUBUNIT I (COI) GENES IN MALANG

KERAGAMAN GENETIK EKOR PEGAS (SUBKELAS COLLEMBOLA) BERDASARKAN GEN CYTOCHROME OXIDASE SUBUNIT I (COI) DI MALANG

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ABSTRACT

The springtails belong to the Collembola subclass of insect class and are a mesofauna insect that acts as a detritivore and bio-indicator in the ecosystem. Genetic diversity is influenced by the total genetic variation present in genes coding for species or organisms. COI gene is a gene that is useful as a genetic marker for mitochondrial DNA. The genetic diversity of springtails based on mtDNA COI gene sequences is not widely known in Malang. Based on this, the study aims to reveal the genetic diversity of springtails in Malang. This research was conducted by three locations in Malang (Cangar, Bedengan and Tambakasri). Measurements of microclimate (humidity and air temperature) and altitude measurements were carried out at springtails sampling, then carried out DNA isolation process from springtails samples obtained, COI amplification process and sequencing were carried out. After obtaining the sequence data, genetic diversity and polymorphism analysis were carried out. Results of this study were the target genes amplified from all samples of springtails with DNA fragments along 677-683 bp. The sample of springtails had a genetic identity match with Homidia socia Denis and Homidia cingula Börner based on the BLAST analysis, results of genetic diversity analysis showed that samples tested had high genetic diversity, the composition of nucleotide bases A/T was 60,8%, genetic variation in the form of transition substitution (87 sites) and transversion (55 sites), and the results of polymorphism analysis showed the value of 0,18201 nucleotide diversity, number of segregation was 162 sites and sequences conservation was 0,744.

Keywords: *COI, springtails, genetic diversity*

ABSTRAK

Ekor pegas termasuk dalam Subkelas Collembola dari kelas insekta. Ekor pegas merupakan serangga mesofauna yang memiliki peran sebagai detritivor dan bioindikator kesehatan tanah di ekosistem. Keragaman genetik dipengaruhi oleh jumlah total variasi genetik yang terdapat dalam gen pengkode dari suatu spesies atau organisme. Gen COI merupakan suatu gen yang berguna sebagai penanda genetik dariu DNA mitokondria. Keanekaragaman genetik ekor pegas berdasarkan sekuens gen COI DNA mitokondria belum banyak diketahui di Indonesia, berdasarkan hal tersebut, penelitian ini bertujuan untuk mengungkap keragaman genetik beberapa ekor pegas yang berada di wilayah Malang, Jawa Timur. Penelitian ini dilakukan di tiga lokasi di wilayah Malang (Cangar, Bedengan dan Tambakasri). Dilakukan pengukuran microclimate (kelembaban dan suhu udara) dan pengukuran ketinggian pada lokasi pengambilan sampel ekor pegas, kemudian dilakukan proses isolasi DNA dari sampel ekor pegas yang didapat, dilakukan amplifikasi gen COI dan proses sekuensing. Setelah mendapat data sekuens dilakukan analisis diversitas genetik dan analisis polymorfisme. Hasil dari penelitian ini adalah gen target hasil amplifikasi dari seluruh sampel ekor pegas dengan fragmen DNA sepanjang 677-683 bp. Sampel ekor pegas yang diteliti memiliki kecocokan identitas genetik dengan Homidia socia Denis dan Homidia cingula Börner berdasarkan analisis BLAST (Basic Local Alignment Search Tool), hasil analisis diversitas genetik menunjukkan bahwa sampel yang diuji memiliki keragaman genetik yang tergolong tinggi, komposisi basa nukleotida A/T sebesar .60,8%, variasi genetik berupa subtitusi transisi (87 sites) dan transversi (55 sites), dan hasil analisis polymorfisme menunjukkan bahwa nilai Nucleotide diversity sebesar 0,18201, Number of polymorphic (segregating) sites sebesar 162 sites dan sequence conservation sebesar 0,744.

Kata kunci: *COI, Ekor pegas, keragaman genetik*

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INTRODUCTION

The springtails or Collembola come from the Greek terms, that is, *colle* (= glue) and *embolon* (= piston) [1]. The Collembola subclass is included in the mesofauna group because almost all of its life is in and on the soil surface (litter layer) in addition, the springtails are soil microarthropods because it has a body size range from 0.25 mm to 8.00 mm [1, 2]. In the world, there are about 6000 species from 500 genera that have been described [3], meanwhile in Indonesia, about 250 species have been identified from 124 genera from 17 families [2]. A large proportion of certain springtail populations that feed on mycorrhizal roots can stimulate symbiont growth and promote plant growth. In agricultural land, it can suppress pathogen attacks [4]. Springtails also play a role in the food cycle as a decomposer of organic matter or detritivores [1, 5].

Conventional identification of springtails to species mostly uses morphological data with microscopic observations, and it takes a long time to assess and collect data on this community [6]. In addition, the use of DNA coding from springtails, using the portion of mtDNA cytochrome oxidase subunit I (COI), revealed that there is often a bias (cryptics species) between morphologically identical specimens from different regions, indicating that conventional microscopic identification can lead to identification errors and effect on biodiversity data [7].

Molecular studies for animal recognition including insects using mitochondrial DNA segments as source of genetic data. Mitochondria are steric or elongated, rod-like structures surrounded by an inner membrane and an outer membrane [8]. The outer mitochondrial membrane is smooth, while the inner membrane is curved into sheets or tubules called cristae that extend into the inner chamber (matrix) [9]. Mitochondrial DNA (mtDNA) is used to study genetic variation and intraspecies kinship relationships. The mtDNA gene widely used as a marker in the analysis of genetic variation is the Cytochrome C Oxidase I and II (COI and COII) genes. Cytochrome Oxidase subunit I (COI) and Cytochrome Oxidase subunit II (COII) gene, which acts as a barcode DNA for animal species [7].

The COI gene is one of the genes in mitochondrial DNA (mtDNA) which plays an important role in energy production, so its base is conservative. COI gene markers have been used to identify the breeding results of almost all animals, both intraspecies and interspecies [7]. COI has many advantages for studying genetic characteristics because it has few deletions and insertions in the sequence. In addition, the amino acid composition of the protein encoded in the COI gene rarely undergoes substitution so that the COI gene is stable and can be used as a phylogenetic marker, but the bases in the triple codon are still changing and are silent, i.e., base changes that do not change the type of amino acid [10].

Springtail's genetic diversity data in Indonesia is still unknown because researchers have studied more of springtail in terms of species diversity. Genetic studies using DNA molecules as genetic markers in diversity studies, identification, and taxonomy have long been used because they show more natural characteristics [11]. The advantages of molecular data are widely used in studying evolution, population structure, gene flow, hybridization, biogeography, and phylogenetic relationships of an animal species and support the use of mtDNA as a very strong genetic marker [12].

The location that will be used as a research location is the Malang area which Malang Region has diverse landscapes that can provide dispersal barriers for springtails. Central Malang Regency is an area with a plateau surrounded by several mountains and valleys with an altitude of about 250-500 meters above sea level (masl). The southern part of Malang Regency is an area of limestone hills called South Malang Karst at an altitude of 0-650 masl. The northern part of Malang Regency is the Arjuno-Welirang mountain slope area at an altitude of 600-2700 masl. In the eastern part is the Tengger-Semeru slope area, stretching from north to south at an altitude of 500-3600 masl, and the western part is the Kawi-Arjuno slope area at an altitude of 500-3300 masl [13].

However, there have been many studies of springtails in terms of species diversity based on morphology. Therefore, it is necessary to conduct a study that examines the genetic diversity of springtails based on the mitochondrial DNA sequence of the cytochrome oxidase subunit I (COI) in Malang. This study is useful and aims to provide information about the genetic diversity of springtails and can be used as a reference for the genetic database of springtails in Indonesia.

METHODS

Specimen sampling. Sampling springtails using techniques pitfall trap [14] and samples preserve using a vial bottle filled with 70% alcohol then stored in a freezer in -20^{0}C until use. The selection of research locations was based on altitude and where there were litter or springtails food sources by purposive sampling method [15], then data microclimate collection is temperature

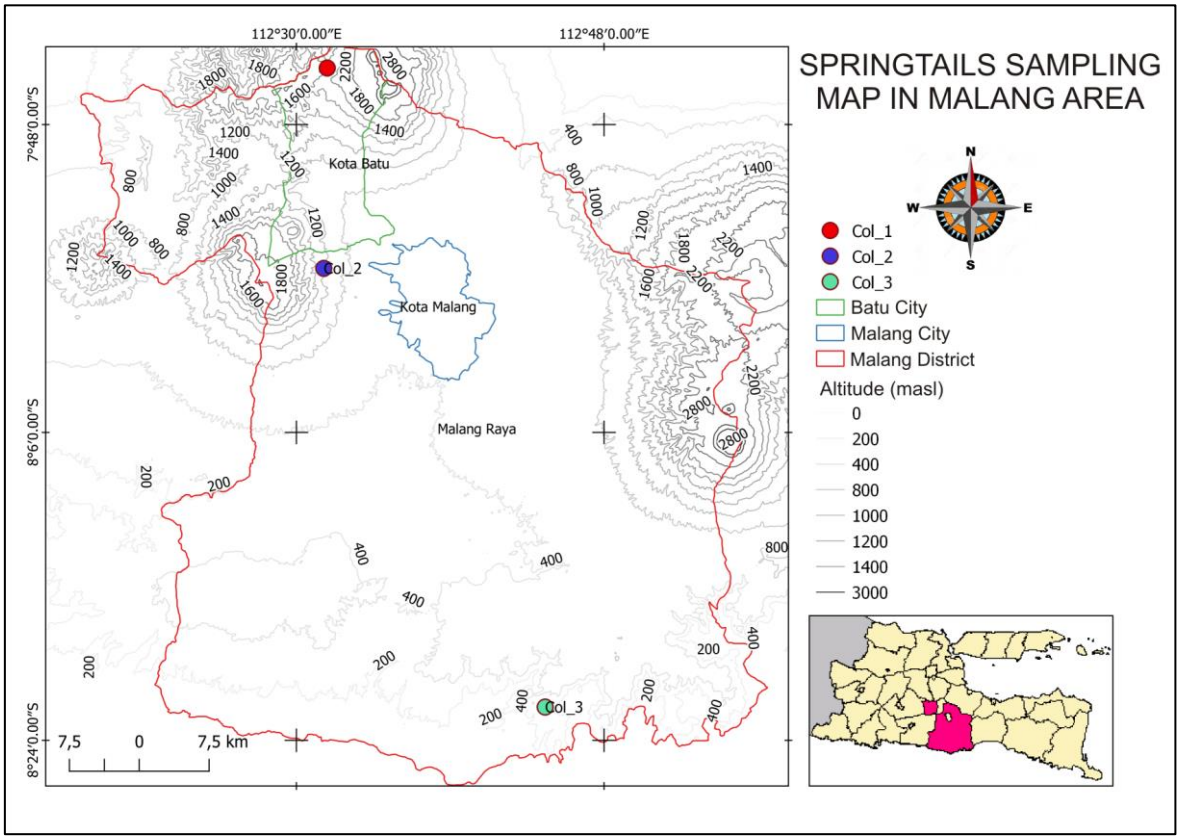

Figure 1. Research sampling location. Col 1= Cangar; Col 3 = Bedengan; Col 3 = Tambakasri, Sumbermanjing Wetan

and humidity as well as the characteristics of each region. Sampling was carried out at three locations in Malang, namely Batu, Malang, and South Malang.

Molecular procedures. Springtail DNA samples were taken from the whole body [16]. DNA was extracted from a single individual from each population following the method described in [17], with slight modifications due to the small specimen size. Single individuals (live, frozen, or preserved with alcohol) were mashed in a homogenized buffer. In the refining process, 200 µl GT1 buffer was added to the sample and then refined again. The mashed sample was added with 20 µl Proteinase K and 200 µl buffer GT2 homogenized using a vortex. Samples were incubated at 56 ºC for 10 minutes, during which the tube was incubated back and forth every 5 minutes. Next, 200 µl of absolute ethanol was added and vortexed. Samples were centrifuged at 13.000 rpm for 1 minute. Discarded the flow-through and added 500 µl W1 buffer, then centrifuged for 1 minute at 13.000 rpm. Then discarded the flow-through and added 700 µl W2 buffer and centrifuged for 1 minute at 13,000 rpm. Discarded the flow-through again and centrifuged again for 2 minutes at 13,000 rpm. Samples that have been centrifuged are taken as much as 1.5 ml of the supernatant and transferred the DNA spin column to a new tube of 1.5 ml then added 50 – 100 µl *elution* incubation buffer at room temperature for 1 minute, then centrifuged for 1 minute at a speed of 13,000 rpm. Discarded *spin column* DNA and stored at -20ºC for a few days, -70ºC for long-term storage.

DNA visualization was carried out by electrophoresis in a horizontal electrophoresis bath using 1% agarose gel. Agarose gel was made by dissolving agarose in 1X TBE buffer and heating until homogeneous, and the solution became clear. The agarose solution was then poured into an electrophoresis bath that was previously installed with a molding comb and waited for it to harden (15-20 minutes). Next, electrophoresis was carried out for 45 minutes at a voltage of 50 volts (long-running time depends on gel concentration and voltage). After electrophoresis, the agarose gel was immersed in Ethidium Bromide (EtBr) solution for 5 seconds then immersed in distilled water for 15 minutes to wash or minimize EtBr contaminants. After that, the DNA was visualized using a UV transilluminator. The results of visualization of genomic DNA were seen based on the presence of the sample genomic DNA band. Good isolation results can be continued in the following method (PCR amplification).

All COI genes were amplified using two primers, forward LCO1490COL 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and

reverse HCO2198COL 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' [18] using profiles the following: denaturation at 94°C (1 min 10 sec), annealing at 45°C (1 min 10 sec), and extension at 72°C (1 min 30 sec). In addition, three microliters of sample from the PCR product and two microliters of loading dye were run on 1% agarose gel to determine the presence and size of the amplified DNA.

PCR product was continued with the sequencing stage. DNA sequencing was performed to determine the nucleotide sequence in the COI region. Sequence data analysis was also carried out to determine several characters of the sequence of nucleotide bases in the tail of the spring analyzed, including calculating the base composition. Sequencing is done using a service *sequencing 1st BASE Laboratories*, Malaysia.

Genetic analysis. Sequenced nitrogen bases were seen using ABI sequences Scanner v.10 to determine the QV peak, BLAST (Basic Locale Alignment Search Tool) [19], and analysis of the percentage of nucleotide bases using *Molecular Evolutionary Genetics Analysis* (MEGA) [20], then for polymorphism data analysis using DnaSP 5.0 software [21, 22].

RESULTS AND DISCUSSION

Research location characteristics. Characterization of the research location is based on the altitude and geographical elements of the Malang area, which is surrounded by mountains (ring of fire) and the coast in the south [23]. The state of the landscape (landform) of the three research locations has its characteristics and the microclimates parameter (Table 1). The first location of data collection is specifically indicated by characteristics of this location have a medium density of undergrowth, with dry litter due to open land, and data collection coincides with the dry season. The second location is dominated by pine trees, medium litter thickness, close to the river ecosystem.

Table 1. Microclimate of research locations

Location and Coordinates	Altitude (masl)	Humidty (%)	Temp (ºC)
Cangar S07º 44'41.7" E112º 31'48.2"	1687	61	25.2
Bedengan S07º 56'24.5" E112º 31'36.0"	992	55	27.2
Tambakasri S08º 22'03.2" E112º 44'33.1"	377	58	29.1

The third location is in the Tambakasri area, Sumbermanjing Wetan, with a coffee plantation owned by a resident, the density of undergrowth is moderate, and almost no litter. Temperature and humidity parameters are very important for insect life in a habitat. Temperature affects the metabolic processes and activity of insects, but the influence is related to the geographical area [24]. In addition, the altitude parameter also affects changes in humidity. Besides, air humidity can also affect insect activity (distribution and metabolism) [25, 26, 27]. Therefore, geographical location and environmental conditions such as temperature, humidity, and altitude can affect the diversity of species [28] and repeat patterns in biogeographic theory and geographic isolation. Genetic drift is an elevation gradient in species richness [29].

The selection of research sites was based on parameters other than height, the litter factor as a food source for springtails, so it can be ascertained that at the selected location, there are springtails. In addition, litter is used as a parameter for site selection because it is a food source for springtails. Litter is part of the plant that has fallen to the ground and undergoes a decomposition process, judging by the condition of the leaves that are not fresh. Fungi play a role in the litter decomposition process, and microfungi are a source of food for the springtails [30].

Genetic diversity of springtails. The results of sampling obtained one specimen for each research location. Visualization of the amplified target gene of all samples showed fragments along 600-750 bp.

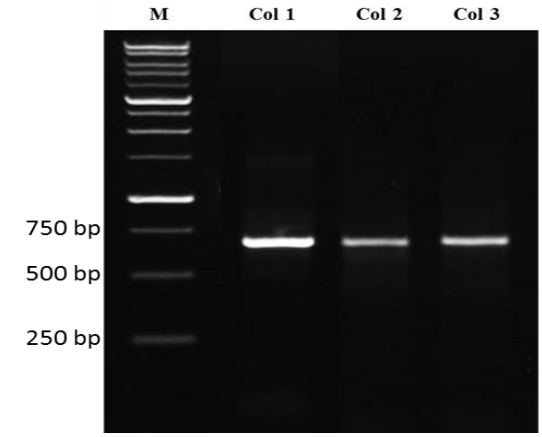

Figure 2. Electropherogram of gene amplicons Cytochrome Oxidase subunit I (COI) from springtail samples. Lane Col 1= Cangar; Col 3 = Bedengan; Col 3 = Tambakasri, Sumbermanjing Wetan. M= 1kb marker (Thermo Scientific).

Amplicon sequencing of the three springtail samples resulted in fragments with a length range of 677-683 bp. Then, BLAST analysis was carried out to compare the sequence of samples analyzed

with the GenBank database. BLAST sequence analysis of springtail species based on the COI mtDNA gene with the NCBI database resulted in identification matches with the percentage of 96.76% - 99.67%. The results of the BLAST analysis (Appendix 1) showed that the Col_1 species had similarities with the *Homidia socia* Denis species (KJ873638.1) with an identity match percentage of 96.76%, the Col_2 species had similarities with the *Homidia cingula* Börner species (KJ923193.1) by 99.38%, and Col_3 had a resemblance to the species *Homidia cingula* Börner (KJ923193.1) similarity. with *Entomobryidae* sp. (KX053098.1) with 99.67% similarity percentage (Table 2). It is known that all specimens obtained are identical to the Entomobryidae family. Entomobryidae family is the largest family of the Collembola subclass with a total percentage of 21% (1130 species, 41 genera) of all identified springtails species in the world, able to adapt to various habitats, but most species live among leaf litter, ground surface, and in the cave [1, 31, 32].

COI fragments from three springtail specimens had T(U) and A base composition values, which were more dominant than C and G basic composition values. The average composition values for each base were T(U) 35.6%, A was 25.2%, C by 20.9%, and G by 18.1% (Table 3). The value of the GC composition of the three samples studied was 39% (630 sites). These results are consistent with the Antarctic springtails study with an average A/T nucleotide base composition of 62.2% [33]. The results of other studies also confirmed that the relative proportion of nucleotide bases in springtail ranged from 58.3-64.6% in each species [34]. In addition, the noncoding structure of a genome, which is the site of replication and transcription, has a rich region, that is A+T-rich region [35]. Nucleotide bases A and T are more dominant because they are more tolerant of incompatibility than bases G or C, thereby lowering the level of specificity (the ability to indicate which individual is a match from several more suitable individuals) [36].

Alignment of the COI gene sequences of the three analyzed specimens resulted in nucleotide sites with a number of 634 characters. Of the 634 characters, 468 were identified as conserved region sites (invariable/monomorphic), and 162 were variable (polymorphic) sites. About 142 sites are singleton variable sites (two variants), nucleotide diversity is 0.18201, and sequence conservation is 0.744. In addition, the three samples of springtail sequences show variations in nucleotide bases at 142 sites, and there are four gaps from a total site of 634 (Table 4). Variations occur due to nucleotide base substitution; these changes occur in the form of transitional substitution and transversion. Based on the results of analysis, polymorphism there were 87 sites (Table 5) undergoing transitional substitution, namely 16 sites (AG), 28 sites (CT), seven sites (GA), and 36 sites (TC). Transversion substitutions occurred at a total of 55 sites (Table 6) with consecutive frequency, namely seven sites (AC), 24 sites (AT), two sites (CA), three sites (GC), two sites (CG), 11 sites (TA), and six sites (TG). This indicates that the COI gene sequences in the three samples of the springtail studied found quite a lot of variation, thus indicating a fairly high genetic diversity.

Table 2. Result of BLAST analysis based on NCBI database

No	Code	Location	NCBI Database		
			Identify species	Identity	Accession
1.	Col_1	Cangar	*Homidia socia* Denis	96,76%	KJ873638.1
2.	Col_2	Bedengan	*Homidia cingula* Börner	99,38%	KJ923193.1
3.	Col_3	Tambakasri	*Entomobryidae* sp.	99,67%	KX053098.1

Table 3. The nucleotide base composition of the COI gene sequences in the springtails sample

No.	Specimen	Fragment Length (bp)	%			
			T(U)	C	A	G
1.	Col 1 ID	683	37,6	18,4	26,9	17,0
2.	Col 2 ID	683	35,1	20,8	25,6	18,4
3.	Col 3 ID	677	34,3	23,5	23,3	18,9
Average			35,6	20,9	25,2	18,1

Table 4. Parameters of COI gene polymorphism of springtails specimen

No	Type	Total (sites)
1.	Number of polymorphic (segregating) sites	162
2.	Nucleotide diversity	Pi : 0,18201
3.	Sequence conservation	C : 0,744
4.	Variable (polymorphic) sites	162
5.	Singleton variable sites (two variants)	142
6.	Singleton variable sites	162
7.	Invariable (monomorphic) sites	468
8.	Sites with alignment gaps or missing data	4

Table 5. Frequency (%) of transversion base

No	Transversion Base	Frequency	%
1	A-C	7	12.73
2	A-T	24	43.64
3	C-A	2	3.64
4	G-C	3	5.45
5	C-G	2	3.64
6	T-A	11	20.00
7	T-G	6	10.91
Total		55	100.00

Table 6. Frequency (%) of transition base

No	Transition base	Frequency	%
1	A-G	16	18.39
2	C-T	28	32.18
3	G-A	7	8.05
4	T-C	36	41.38
Total		87	100.00

DNA barcodes from selected genes have become a method for identifying species at all ontogenetic stages (describing the origin and development of organisms from fertilized eggs to adult forms), besides this method can increase accuracy in determining species richness by using COI molecular markers (mtDNA), and LSU (Large rDNA Subunit) [18, 37]. The results of springtails genetic diversity analysis scattered in Malang showed high value. It is proven based on the value of the character singleton variable sites. Besides that, it is supported by the results of analysis polymorphism which shows the occurrence of nucleotide base substitution in the form of transitions and transversions. Transition events occur more than transversions. In general, the transition ratio (Ts) to transversion (Tv) is higher in the animal nuclear genome with a probability ratio of 1:2 expected if all substitutions have the same probability, whereas the relative transition rate is higher in mitochondrial DNA [38].

Some of the above molecular things that cause the genetic diversity of the springtail are quite high due to the difference in the location of each specimen, where theoretically. The biogeography of each animal species is distinguished based on the diversity of its habitat and will have an impact on the gene expression of each animal. According to Huang et al. [39], genetic diversity has a relationship with environmental factors in a habitat, where specifically the gradient of an environment (soil type, temperature, humidity, etc.) will affect gene expression or genetic adaptation.

CONCLUSION

Specimen identification using molecular methods showed that there are three species of springtails in the Malang area, *Homidia socia* Denis, *H. cingula* Börner, and *Entomobryidae* sp., third of them belong to the family Entomobryidae. The genetic diversity of springtails based on the COI gene sequences in the Malang area shows high diversity because the COI gene has high nucleotide base substitution, and there is gene expression of each specimen based on biogeography.

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Appendix 1. Sequences comparison between sample and Springtails spesies (Query is Sample, and Subject is Species) from BLAST in NCBI.

1. Col 1 Cangar (sample) and *Homidia socia* Denis (KJ873638.1) from China

Score	Expect	Identities	Gaps	Strand
1053 bits(570)	0.0	613/634(97%)	3/634(0%)	Plus/Plus

```
Query  1    TGATCTGCCAATAGTTGGGACGGCTTTTAGAGTTCTTATCCGACTTAGAATTAGGTCAAC  60
            ||||||| | ||||||||||||||||||||||||||||| || |||||||| |||||||||
Sbjct  26   TGATCTG-CTATAGTTGGGACGGCTTTTAGAGTTCTTATTCG-CTTAGAACTAGGTCAAC  83

Query  61   CAGGAAGTTTTATTGGTGACGATCAAATTTATAATGTAATAGTAACTGCTCATGCTTTTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  84   CAGGAAGTTTTATTGGTGACGATCAAATTTATAATGTAATAGTAACTGCTCATGCTTTTA  143

Query  121  TTATAAttttttttATAGTTATACCAATTATAATTGGAGGGTTTGGAAATTGACTTGTAC  180
            |||||||||||||||||||||||||||||||||||||||| ||||| ||||||||||||||
Sbjct  144  TTATAATTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGTAATTGACTTGTAC  203

Query  181  CCTTAATAATTGGAGCCCCTGATATGGCTTTCCCCCGAATAAATAATATAAGATTTTGAT  240
            |||||||||||||||||||||||| ||||||||||||||||||||||||| || ||||
Sbjct  204  CCTTAATAATTGGAGCCCCTGATATAGCTTTCCCCCGAATAAATAATATAAGTTTNTGAT  263

Query  241  TGCTTCCCCCTTCTCTAACTCTATTACTAACAGGAGGACTAGTAGAAAGAGGAGCAGGAA  300
            |||||||||| ||| ||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  264  TGCTTCCCCCATCTTTAACTTTATTACTAACAGGAGGACTAGTAGAAAGAGGAGCAGGAA  323

Query  301  CTGGATGAACTGTTTACCCTCCTTTAGCTTCTAGGAATTGCTCACTCAGGCGCATCAGTT  360
            | |||||||||||||||| |||||||||||| |||||||||||| ||||||||||||||
Sbjct  324  CAGGATGAACTGTTTACCCACCTTTAGCTTCT-GGAATTGCTCACGCAGGCGCATCAGTT  382

Query  361  GATTTATCAATTTTTAGTCTTCATTTAGCCGGAGCTTCTTCAATTTTAGGGGCTGTTAAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  383  GATTTATCAATTTTTAGTCTTCATTTAGCCGGAGCTTCTTCAATTTTAGGGGCTGTTAAT  442

Query  421  TTTATTACTACCATTATTAATATACGAACCCCAGGTATATCTTGAGACCAAACTCCTTTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  443  TTTATTACTACCATTATTAATATACGAACCCCAGGTATATCTTGAGACCAAACTCCTTTA  502

Query  481  TTTGTTTGATCTGTTTTCTTAACAGCTATCCTACTGTTACTATCCCTTCCAGTTTTAGCT  540
            |||||||||||||||||||||||||||||| || ||||||||||||||||||||||||||
Sbjct  503  TTTGTTTGATCTGTTTTCTTAACAGCTATCCTTCTTTTACTATCCCTTCCAGTTTTAGCT  562

Query  541  GGAGCCATTACTATACTTTTGACCGACCGAAATTTAAATACATCtttttttGACCCAGCA  600
            |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  563  GGAGCCATTACTATACTTTTGACCGACCGTAATTTAAATACATCTTTTTTTGACCCAGCA  622

Query  601  GGAGGAGGAGATCCTATTTTATACCAACATTTAT  634
            ||||| |||||||||||||||||||||||||||
Sbjct  623  GGAGGTGGAGATCCTATTTTATACCAACATTTAT  656
```

2. Col 2 Bedengan (sample) and *Homidia cingula* Börner (KJ923193.1) from China

Score	Expect	Identities	Gaps	Strand
1144 bits(619)	0.0	627/631(99%)	0/631(0%)	Plus/Plus

```
Query  1    TGAGCTGCTATAGTAGGCACTGCATTCAGAGTTCTGATCCGATTAGAATTAGGACAGCCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26   TGAGCTGCTATAGTAGGCACTGCATTCAGAGTTCTGATCCGATTAGAATTAGGACAGCCG  85

Query  61   GGAAGCTTTATTGGAGACGACCAAATCTATAATGTAATAGTTACAGCCCATGCTTTTATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  86   GGAAGCTTTATTGGAGACGACCAAATCTATAATGTAATAGTTACAGCCCATGCTTTTATT  145

Query  121  ATAAttttttttATAGTTATGCCTATTATAATTGGAGGCTTCGGAAATTGATTAGTACCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  146  ATAATTTTTTTTATAGTTATGCCTATTATAATTGGAGGCTTCGGAAATTGATTAGTACCT  205

Query  181  TTAATAATTGGAGCCCCTGACATAGCTTTCCCCCGAATAAATAATATAAGTTTCTGACTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  206  TTAATAATTGGAGCCCCTGACATAGCTTTCCCCCGAATAAATAATATAAGTTTCTGACTA  265

Query  241  CTTCCTCCGTCTTTAACTTTATTATTAACGGGGGGCTTAGTTGAAAGAGGTGCAGGAACC  300
            |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  266  CTTCCTCCGTCTTTAACTTTATTATTAACAGGGGGCTTAGTTGAAAGAGGTGCAGGAACC  325

Query  301  GGATGAACAGTCTATCCCCCTTTAGCAGCAGGAATTGCTCATGCTGGAGCTTCCGTTGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  326  GGATGAACAGTCTATCCCCCTTTAGCAGCAGGAATTGCTCATGCTGGAGCTTCCGTTGAC  385

Query  361  CTTTCAATTTTTAGCCTTCATTTAGCAGGTGCTTCATCTATTTTAGGCGCAGTTAACTTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  386  CTTTCAATTTTTAGCCTTCATTTAGCAGGTGCTTCATCTATTTTAGGCGCAGTTAACTTT  445

Query  421  ATCACAACAATTATTAATATACGAGCCCCAGGTATATCGTGGGATCAAACCCCCTTATTT  480
            |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  446  ATCACAACAATTATTAATATACGAGCCCCAGGTATGTCGTGGGATCAAACCCCCTTATTT  505

Query  481  GTATGGTCCGTATTTTTAACTGCCATTCTACTACTTCTTTCACTCCCTGTTTTAGCAGGA  540
            |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  506  GTATGGTCCGTATTTTTAACTGCCATTCTTCTACTTCTTTCACTCCCTGTTTTAGCAGGT  565

Query  541  GCTATTACCATACTTTTAACAGACCGAAACTTGAATACTTCCTTTTTCGACCCTGCTGGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  566  GCTATTACCATACTTTTAACAGACCGAAACTTGAATACTTCCTTTTTCGACCCTGCTGGG  625

Query  601  GGTGGAGACCCAATCTTGTACCAACACTTAT  631
            ||||||||||||||||||||||||||||||
Sbjct  626  GGTGGAGACCCAATCTTGTACCAACACTTAT  656
```

3. Col 3 Tambakasri (sample) and *Entomobryidae* sp. (KX053098.1) from French Polynesia, Tahiti Island

Score	Expect	Identities	Gaps	Strand
1090 bits(590)	0.0	594/596(99%)	0/596(0%)	Plus/Plus

```
Query  36   AATCCGATTTGAGTTAGGCCAACCAGGTAGCTTTATTGGTGATGACCAAATCTATAACGT  95
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AATCCGATTTGAGTTAGGCCAACCAGGTAGCTTTATTGGTGATGACCAAATCTATAACGT  60

Query  96   AATAGTAACTGCCCACGCTTTCATTATAAtttttttATAGTTATGCCTATTATAATCGG  155
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AATAGTAACTGCCCACGCTTTCATTATAATTTTTTTTATAGTTATGCCTATTATAATCGG  120

Query  156  GGGGTTCGGAAATTGACTAGTCCCCTTAATAATTGGAGCCCCAGACATGGCTTTCCCACG  215
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGGGTTCGGAAATTGACTAGTCCCCTTAATAATTGGAGCCCCAGACATGGCTTTCCCACG  180

Query  216  TATAAATAATATAAGATTTTGACTCCTTCCCCCTTCTCTTACTCTTCTTCTAACAGGGGG  275
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TATAAATAATATAAGATTTTGACTCCTTCCCCCTTCTCTTACTCTTCTTCTAACAGGGGG  240

Query  276  TCTCGTAGAAAGAGGTGCTGGAACTGGATGAACCGTTTATCCTCCTCTTGCATCTAACAT  335
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TCTCGTAGAAAGAGGTGCTGGAACTGGATGAACCGTTTATCCTCCTCTTGCATCTAACAT  300

Query  336  CGCTCACTCCGGGGGCAGCGTTGATTTGTCAATTTTTAGCTTACACCTAGCAGGTGCCTC  395
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CGCTCACTCCGGGGGCAGCGTTGATTTGTCAATTTTTAGCTTACACCTAGCAGGTGCCTC  360

Query  396  CTCAATTCTGGGGGCCGTTAATTTTATCACCACAATTATTAATATGCGGACTCCTGGGAT  455
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CTCAATTCTGGGGGCCGTTAATTTTATCACCACAATTATTAATATGCGGACTCCTGGGAT  420

Query  456  GTCTTGAGATCAAACACCTCTCTTTGTTTGATCAGTCTTTTTAACCGCCATCCTATTGCT  515
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GTCTTGAGATCAAACACCTCTCTTTGTTTGATCAGTCTTTTTAACCGCCATCCTATTGCT  480

Query  516  TCTATCCCTTCCAGTCTTAGCAGGAGCCATCACTATGCTCCTGACAGACCGAAATTTAAA  575
            ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TCTATCCCTCCCAGTCTTAGCAGGAGCCATCACTATGCTCCTGACAGACCGAAATTTAAA  540

Query  576  TACCTCttttttGACCCTGCGGGGGGAGGAGACCCTATTTTATACCAACATTTAT  631
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  541  TACCTCTTTTTTTGACCCTGCAGGGGGAGGAGACCCTATTTTATACCAACATTTAT  596
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