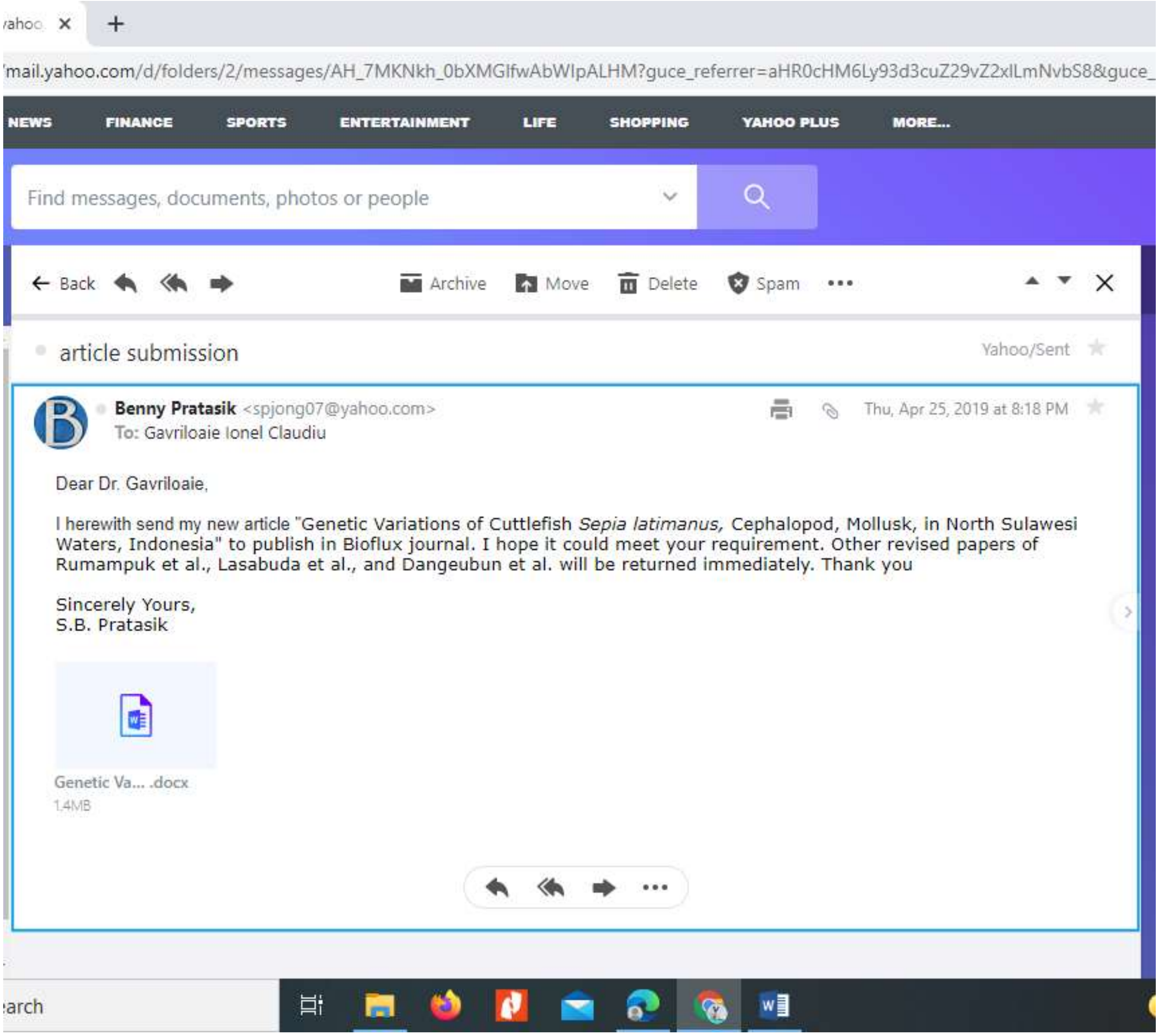

article submission

Benny Pratasik <spjong07@yahoo.com>
To: Gavriloaie Ionel Claudiu

Thu, Apr 25, 2019 at 8:18 PM

Dear Dr. Gavriloaie,

I herewith send my new article "Genetic Variations of Cuttlefish *Sepia latimanus,* Cephalopod, Mollusk, in North Sulawesi Waters, Indonesia" to publish in Bioflux journal. I hope it could meet your requirement. Other revised papers of Rumampuk et al., Lasabuda et al., and Dangeubun et al. will be returned immediately. Thank you

Sincerely Yours,
S.B. Pratasik

Genetic Variations of Cuttlefish *Sepia latimanus,* Cephalopod, Mollusk, in North Sulawesi Waters, Indonesia

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Abstract. This study was aimed at discerning the genetic variations of cuttlefish *Sepia latimanus* in North Sulawesi, using CO1 gene marker. Sampling was conducted in Kalinaung and Likupang, North Minahasa regency, Manado Bay, Manado municipality and Minahasa regency, Poigar, South Minahasa regency, and Basaan, Southeast Minahasa. All samples were put into 95% alcohol and brought to the molecular biology laboratory of Faculty of Fisheries and Marine Sciences for further analysis. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5'taaacttcagggtgaccaaaaaatca-3'. Polymerase chain reaction (PCR) was carried out in 35 cycles at 95^{o}C (30 sec.), 50^{o}C (30 sec.), 72^{o}C (50 sec.). The PCR product was visualized in 1% (b/v) agarosa gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). The chromatogram obtained was edited using Geneious v5.6. The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method and BOLDSystems. The phylogenetic tree was built using Neighbor-Joining Method, and genetic similarity was explained by mutation, haplotype and genetic distance.

Keywords: CO1 gene marker, phylogenetic tree, haplotype, genetic distance.

Introduction. North Sulawesi has many islands surrounded by coral reef ecosystems that provide many different habitats for various marine organisms either as home or shelter of different life stage. Broadclub cuttlefish, *Sepia latimanus*, is one of marine mollusks taking advantages of the coral reef ecosystem as spawning ground, egg placement site, and shelter for the youngsters (Pratasik et al 2017). Species diversity of cuttlefish is very high with more than a hundred species recorded, and their distribution and abundance are highly affected by the availability of suitable habitats and preys, particularly for small individuals (Neves et al 2009; Pratasik et al 2017). Some species live in the water deeper than 400 m, such as *Sepia australis, S. elegans, S. orbignyana and S. hieronis*, and some species occupy shallow waters, such as *S. latimanus*, *S. officinalis,* and *S. pharaonis* (Reid et al 2005). Cuttlefish (and cephalopods in general) are known to have diverse body patterns that can immediately change naturally controlled by its chromatophore system (Hanlon et al 2009) and well known as the most intelligent marine biota with the largest body-brain ratio of all invertebrates (NOVA 2007). These have made many studies have focused on the body pattern alteration-related behavioral aspects (Shohet et al 2006; Barbosa et al 2008; Kelman et al 2008; Allen et al 2009; Chiao et al 2010; Barbosa et al 2011).

Indonesian waters so far holds several species of cuttlefish, such as *Sepia bandensis, S. brevimana, S. latimanus, S. papuensis, S. pharaonis, S. sulcata, S. kiensis, S. senta, Sepiella inermis and S. weberi* (Reid et al 2005), but previous study in Manado Bay and Lembeh Strait found only four species (Pratasik et 2016). Low interest in cuttlefish study could result from sample collection problems as a result of no cuttlefish fisheries in Indonesia, and all cuttlefish catches are bycatch of other fisheries. No cuttlefish fisheries in Indonesia, especially North Sulawesi, make cuttlefish catch generally come from traditional fishermen using handline or speargun in the coral reef areas. *S. latimanus* is main cuttlefish catch in the coral reef ecosystem and has highly market value. *S. latimanus* is the second biggest cuttlefish after *S. apama* with maximum dorsal mantle length of 50 cm and total weight of 10 kg (Reid et al 2005). The species often visit coral reefs for spawning and laying eggs (*pers. obs.*). They often come in pairs and select certain coral crevices to insert their eggs (Pratasik et al 2017). Cuttlefish are, in general, economic fisheries resources, but the resources have unclear fisheries status, insufficient data (Reid et al 2005), and belong to threatened species (IUCN 2014). The present study focuses on genetic variations using DNA sequence of mitochondrial genome as DNA barcode. CO1 is the most common mitochondrial DNA gene marker used for species identification (Folmer et al 1994) due to belonging to the most conservative gene among the mitochondrial DNA protein coding genes. It has become a standard tool of molecular taxonomy and identification (Ratnasingham & Hebert 2007). Molecular techniques have been well applied for stock discrimination studies in fisheries (Murphy et al 2002) and can provide the basis for better management of whole populations and therefore, sustainable fisheries.

Method

Sample collection. Cuttlefish samples were collected in Lembeh Strait (Bitung), Basaan (Southeast Minahasa), Manado Bay (Manado), Likupang waters (North Minahasa), Arakan and Poigar (South Minahasa), North Sulawesi. The first two sites represent the southern part of

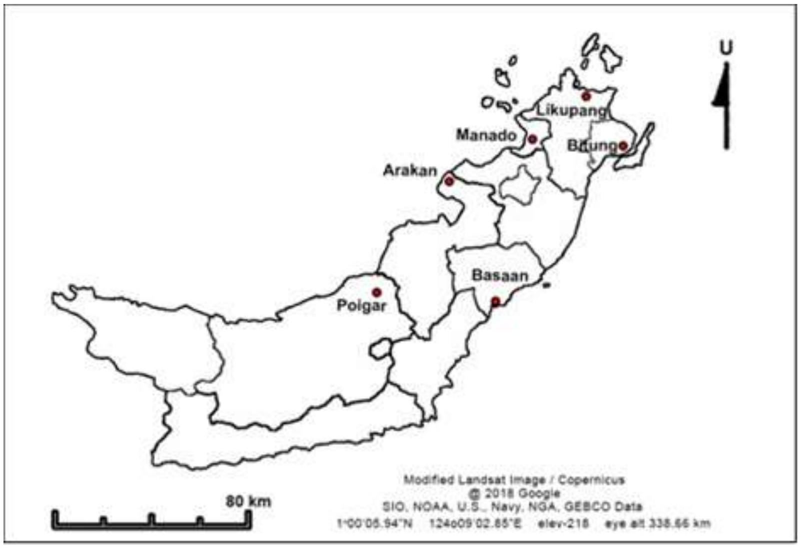

Figure 1. Map of North Sulawesi with sampling points.

North Sulawesi waters, while the second four sites represent the northern part of the island, so that these sites have represented the entire North Sulawesi marine waters. Sampling activities were carried out for 5 months from May to September 2018. Sampling activities employed SCUBA dives over different habitat types and collected from local fishermen. In this study, seven cuttlefish specimens were collected from fishermen's catches, brought to the Biotechnology Laboratory of Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, and all samples were preserved in 95% ethanol and stored at room temperature before DNA extraction.

Extraction, PCR, and sequencing. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5'taaacttcagggtgaccaaaaaatca-3' (Folmer et al 1994). Polymerase chain reaction (PCR) was carried out in 35 cycles at 95°C (30 sec.), 50°C (30 sec.), 72°C (50 sec.). The PCR product was visualized in 1% (b/v) agarose gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer).

Data analysis. The chromatogram obtained was edited using Geneious v5.6 (Drummond et al 2012). The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method (Altschul et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007). The phylogenetic tree was built using Neighbor-Joining Method of MEGA X (Kumar et al 2018). Similarity index was also calculated. The analysis of nucleotide base sequence and haplotype variations between *S. latimanus* from different localities used DnaSP Ver.6.12.03 computer program (Rozas, et al 2017).

Results and Discussion. During the study, 7 individuals of *Sepia latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not taken, so that all samples were obtained from fishermen's catches. Sampling in Poigar, South Minahasa, using beach seine and SCUBA dives, did not obtain any sample as well. This condition reveals that sufficient sampling technique is required for cuttlefish studies. In the past, cuttlefish fishing, such as *S. officinalis* (English Channel) and *S. esculanta* (Japan), used trawl, set net, gill net, trammel net and trap with spawning substrate (Pawson 1995; Arkley et al 1996) by taking advantage of their migration to the spawning areas. In North Sulawesi, *S. latimanus* is generally fished using speargun or jig fishing in the coral reef ecosystem (Pratasik et al 2015). Difficulties in sample collection causes this study collect only few samples.

All cuttlefish collected in North Sulawesi waters, but Manado, were in the same group, while 4 NCBI samples (Indonesia, Japan, Philippines of Aurora_region3) employed as comparison (Figure 1) were in another separate group. This finding reconfirms that geographic distance induces the genetic diversity of *S. latimanus*. However, *S. latimanus* of North Sulawesi has revealed that there are genetic variations and similarities among the same or different locations. *S. latimanus* sample from Bitung waters has genetic similarity to that from Arakan waters. Also, one sample of *S. latimanus* from Likupang waters has genetic similarity to that of Basaan waters. On the other hand, both *S. latimanus* samples from Basaan waters are genetically different as happening for both samples from Likupang waters (Appendix 1).

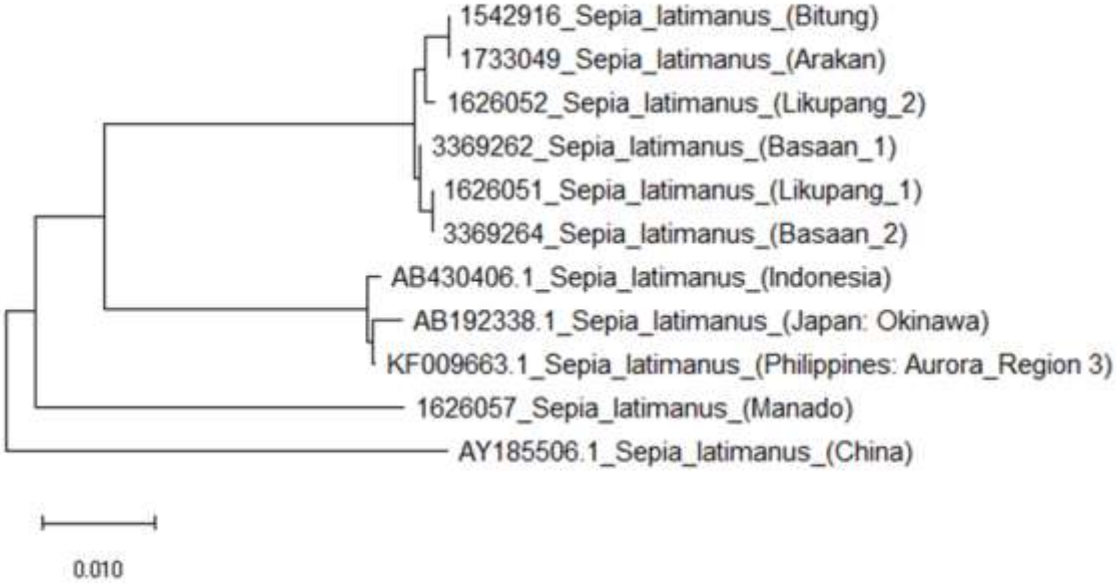

Figure 1. Phylogenetic tree of North Sulawesi *S. latimanus*.

From total numbers of 620 bp, there were 166 polymorphic sites with 186 mutations. Based on variable sites (two variants), there were 73 singleton variable sites with 67 different site positions and 93 parsimony informative sites with 80 site positions. The former was at positions: 2 23 32 33 38 41 56 68 71 77 80 87 99 104 107 113 122 135 140 149 173 179 182 203 212 215 239 254 258 260 263 275 278 279 280 282 293 299 302 332 338 341 347 350 353 371 377 378 384 404 407 410 422 428 431 473 491 497 516 525 526 545 569 587 593 611 618 and the latter at positions 5 8 17 27 44 47 50 53 62 69 72 83 92 98 119 128 131 134 155 158 161 170 177 189 218 224 233 243 251 261 264 266 267 269 272 290 296 317 320 329 336 344 356 359 363 368 374 380 392 393 413 416 435 437 443 452 456 461 470 476 477 485 509 513 515 519 533 539 548 551 566 572 575 578 584 596 602 605 608 617 (Appendix 2).

The present study also found 11 sequences used only formed 9 haplotypes with haplotype diversity (Hd) of 0.9636, haplotype 1 (Bitung and Arakan), 2 (Likupang-1 and Basaan-2), 3 (Likupang-2), 4 (Manado), 5 (Basaan-1), 6 (Japan: Okinawa), 7 (Indonesia), 8 (China), and 9 (Filipina: Aurora-region 3). Different haplotypes of the same localities show that the collected individuals have heteroplasmic-typed mtDNA. It could be seen from individuals from Basaan-1 and Basaan-2 and Likupang-1 and Likupang-2 that form separate haplotypes despite existing in the same locality.

It indicates that *S. latimanus* in North Sulawesi waters has intraspecific population mixture that enables to find different genetic characters at the same locality or same genetic characters at different localities. This condition is also supported by FAO records (Reid et al 2005) that very wide distribution of *S. latimanus* could be a reasonable factor for the population or genetic mixture. This species lives in Indo-Pacific and distributes south Mozambik selatan, entire India ocean, Malacca strait, Melanesia islands, south China sea, Philippine sea, and east China sea, Taipei province and Japan to south Kyushu, Indonesia to northwest and northeast Australia, Palau, Guam, New Caledonia, Fiji and Cocos island (Reid et al 2005).

Genetic mixture, as indicated with haplotype similarity between Likupang 1 and Basaan 2 and between Bitung and Arakan, could be influenced by the ability of *S. latimanus* to move from one place to another. Previous studies have indicated that cuttlefish can inhabit different coastal habitats, such as coral reefs and seagrasses. Habitats are the resources and conditions present in an area to support survival and reproductive success (Leopold 1933). Cuttlefish well known as the chameleon of the sea (Hanlon et al 2009) with well-evolved nervous systems enable to search for extensive trophic niches and migrate to exploit different production systems and prey population, and therefore, they can do long distant migration. Our field observations found that juvenile *S. latimanus* occurred under the seagrass, and near branching corals and sponge. Other evidence also shows that fishermen often take advantage of low tide period to catch cuttlefish in seagrass ecosystem, at least in Likupang waters.

S. latimanus from Manado Bay is also in separate group even though its geographic position occurs between Likupang waters and Arakan waters (Figure 1). It could result from this individual belongs to species complex, a group of closely related organisms that look very similar to the point that the boundaries between them are often unclear. Based on genetic distance, *S. latimanus* from Manado Bay is close to that from Philippine waters (Appendix 1). Manado bay is the northern part of North Sulawesi with series of coral reef-surrounded small islands northwards to southern Philippine. This area belongs to Coral Triangle area. Closer genetic distance of *S. latimanus* between Manado bay and Philippine sea than the same species from other Indonesian waters could result from closer geographic distance between Manado bay to Philippine than other location in Indonesian waters where the sample was collected. This finding is supported by previous studies that increasing geographic distance between patches was associated with increasing genetic distance, while increasing habitat continuity between patches was associated with decreasing genetic distance (Alberto et al 2010). Isolation by distance and isolation by environment are important mechanisms in driving the genetic population structure (Fern'andez et al 2017). Isolation by distance could be used to predict an increase in genetic differentiation with geographic distance (Ramachandran et al 2005), but genetic structure through time is stable (Pascual et al 2016).

Ocean currents are also important factor affecting the marine animal distribution. Therefore, both physical structures and processes are directly related to the swimming and behavioral capabilities of an individual or species. The dynamics of the ocean are highly variable and are driven by multiple forcing factors such as solar insolation, winds, tides and freshwater input. This variability in forcing leads to the development of distinct water masses of different hydrographic properties in both vertical and horizontal dimensions (McManus et al 2011). All these are related with food chain and habitat distribution that influence the availability of living needs of marine animal's dispersal, including cuttlefish, *S. latimanus*.

Conclusion. *Sepia latimanus* of North Sulawesi waters had sufficient genetic variations. These occur between and within localities. Genetic similarity was also present between *S. latimanus* from different localities, such as Basaan-2 and Likupang-1 samples and Bitung and Arakan samples. Eleven nucleotide sequences analyzed formed only 9 haplotypes due to the haplotype similarity between localities, and this condition has reflected genetic mixture. Moreover, *S. latimanus* sample of Manado bay had closer kinship to that of NCBI data for Philippine waters. This condition is supported by habitat and food distribution, ocean currents, and the ability of *S. latimanus* to exploit different niches through behavioral adaptation to different environmental condition.

Acknowledgements.

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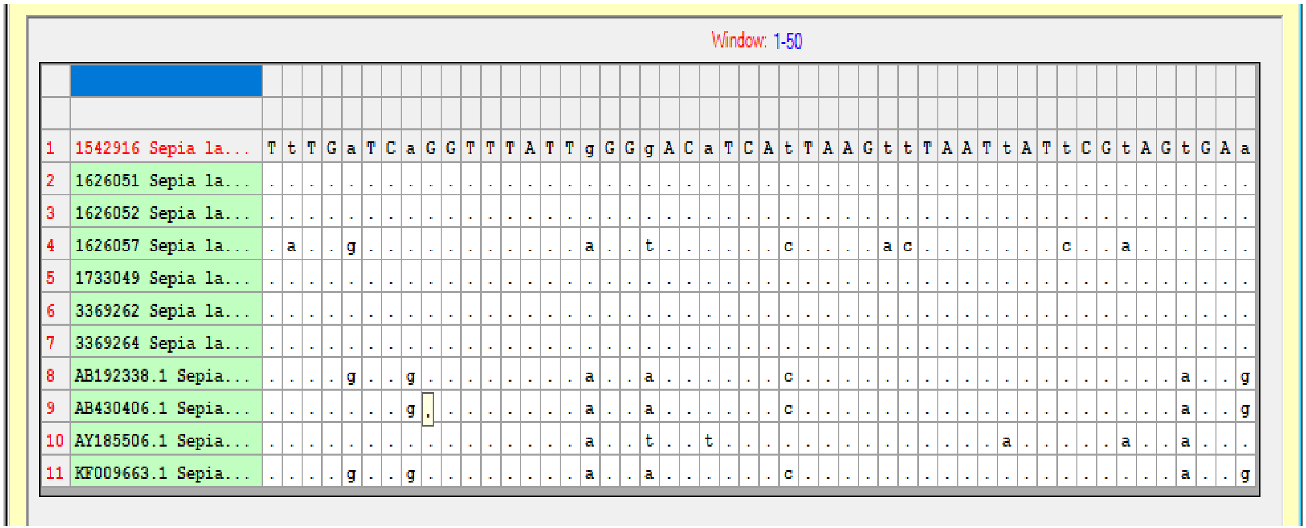
Appendix 1. Genetic distance of cuttlefish *Sepia latimanus* in North Sulawesi waters.

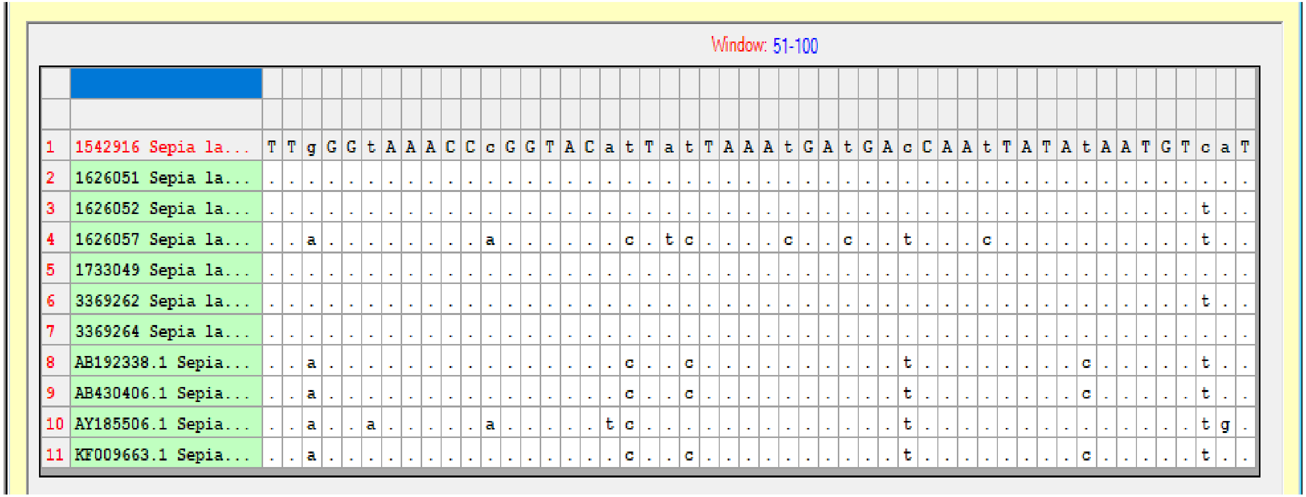
No.		1	2	3	4	5	6	7	8	9	10
1	1542916_*Sepia_latimanus* (Bitung)										
2	1626051_*Sepia_latimanus* (Likupang_1)	0.00435									
3	1626052_*Sepia_latimanus* (Likupang_2)	0.00309	0.00371								
4	1626057_*Sepia_latimanus* (Manado)	0.06984	0.06983	0.07071							
5	1733049_*Sepia_latimanus* (Arakan)	0.00000	0.00435	0.00309	0.06984						
6	3369262_*Sepia_latimanus* (Basaan_1)	0.00435	0.00123	0.00247	0.06820	0.00435					
7	3369264_*Sepia_latimanus* (Basaan_2)	0.00435	0.00000	0.00371	0.06983	0.00435	0.00123				
8	AB192338.1_*Sepia_latimanus* (Japan: Okinawa)	0.05800	0.05627	0.05704	0.06232	0.05800	0.05549	0.05627			
9	AB430406.1_*Sepia_latimanus* (Indonesia)	0.05555	0.05384	0.05461	0.06152	0.05555	0.05307	0.05384	0.00434		
10	AY185506.1_*Sepia_latimanus* (China)	0.07853	0.07747	0.07747	0.07531	0.07853	0.07581	0.07747	0.07725	0.07739	
11	KF009663.1_*Sepia_latimanus* (Philippines:_Aurora_Region_3)	0.05555	0.05384	0.05461	0.05898	0.05555	0.05307	0.05384	0.00309	0.00247	0.07656

Appendix 2. Nucleotide variations of *S. latimanus* in North Sulawesi waters.



Window: 1-50

1	1542916 Sepia la...	T	t	T	G	a	T	C	a	G	G	T	T	T	A	T	T	g	G	G	g	A	C	a	T	C	A	t	T	A	A	G	t	t	T	A	A	T	t	A	T	t	C	G	t	A	G	t	G	A	a
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	g	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	c	.	.	.	.	a	c	.	.	.	.	.	.	.	c	.	.	a	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	g	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	.	.	a	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	g	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g



Window: 51-100

1	1542916 Sepia la...	T	T	g	G	G	t	A	A	A	C	C	c	G	G	T	A	C	a	t	T	a	t	T	A	A	A	t	G	A	t	G	A	c	C	A	A	t	T	A	T	A	t	A	A	T	G	T	c	a	T
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
4	1626057 Sepia la...	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	c	.	t	c	.	.	.	.	c	.	.	c	.	.	t	.	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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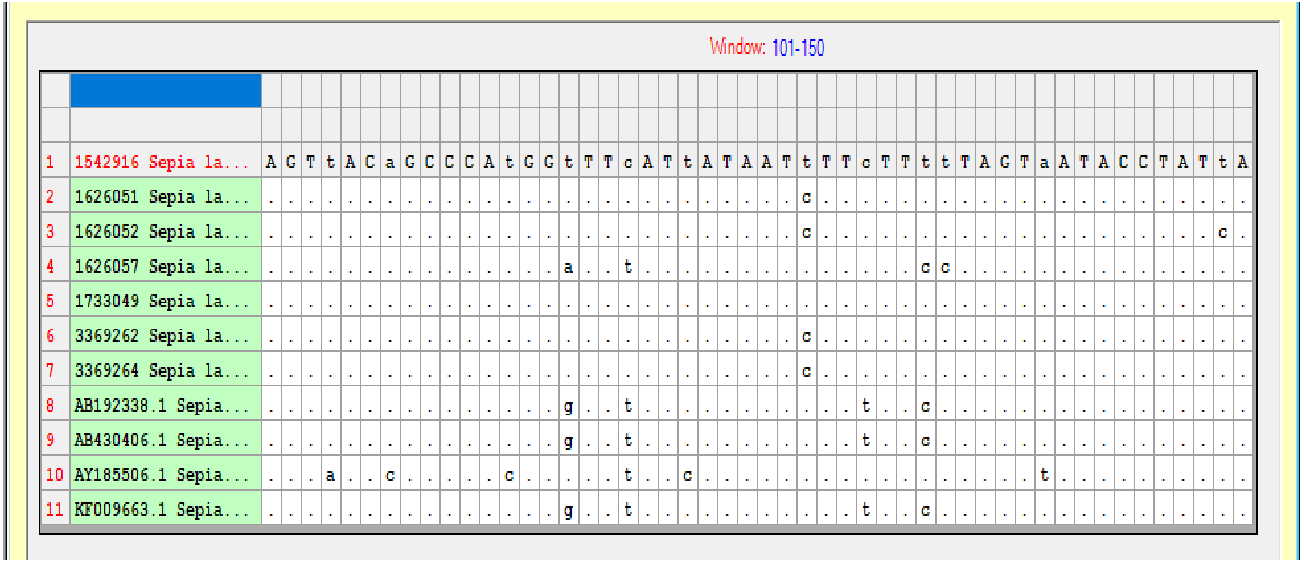
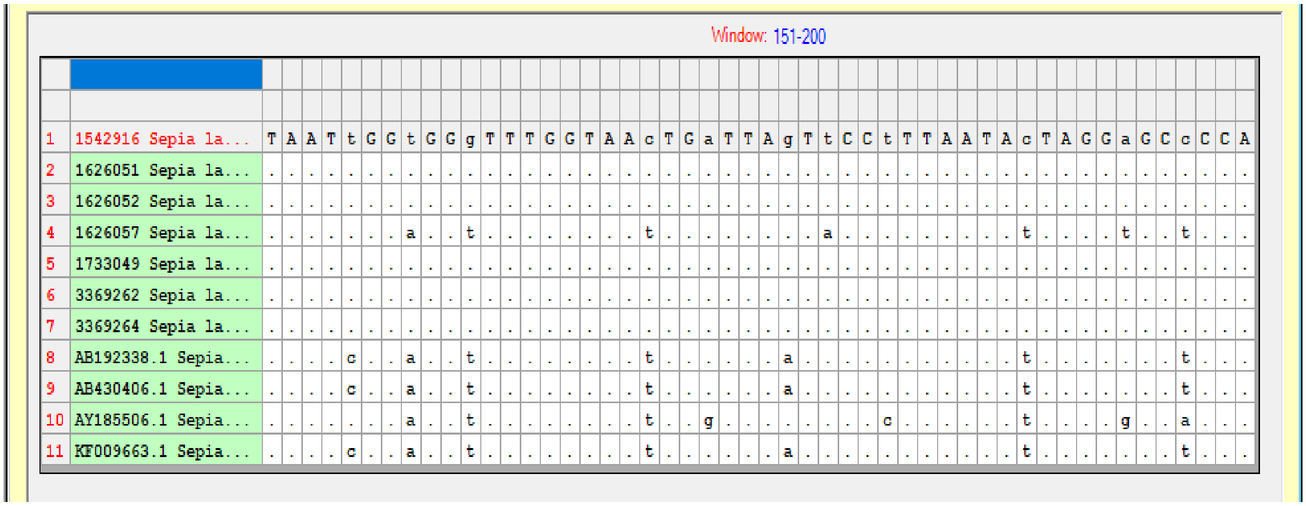
8	AB192338.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.
9	AB430406.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.
10	AY185506.1 Sepia...	.	.	a	.	.	a	.	.	.	.	.	a	.	.	.	.	.	t	c	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	g	.
11	KF009663.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.



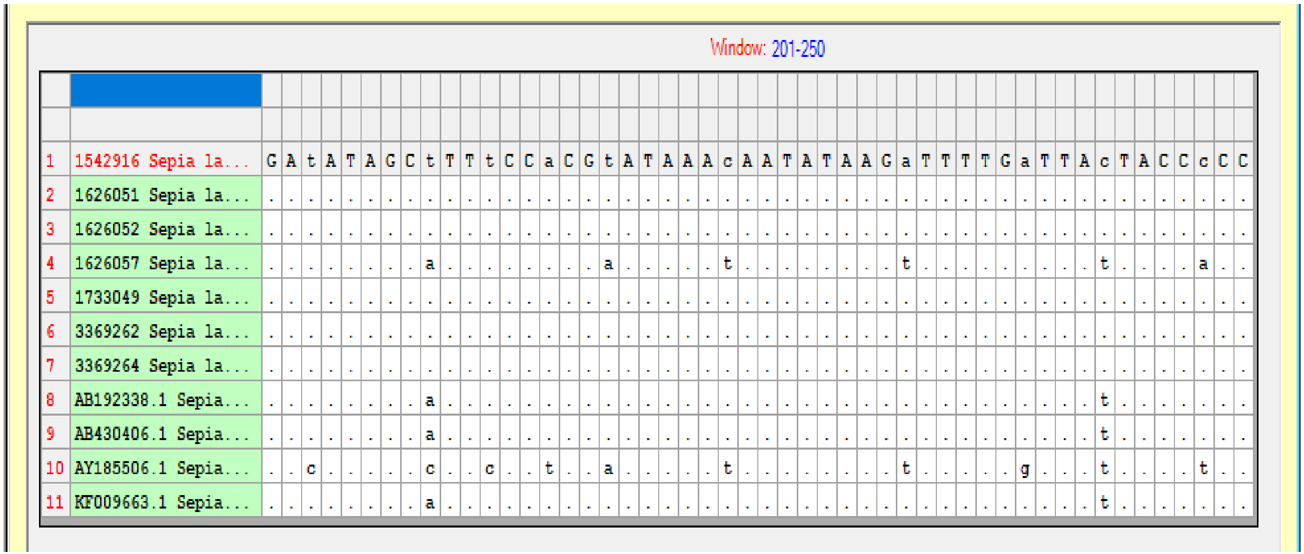
Window: 101-150

1	1542916 Sepia la...	A	G	T	t	A	C	a	G	C	C	C	A	t	G	G	t	T	T	c	A	T	t	A	T	A	A	T	t	T	T	c	T	T	t	t	T	A	G	T	a	A	T	A	C	C	T	A	T	t	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	a	.	.	c	.	.	.	.	.	c	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.



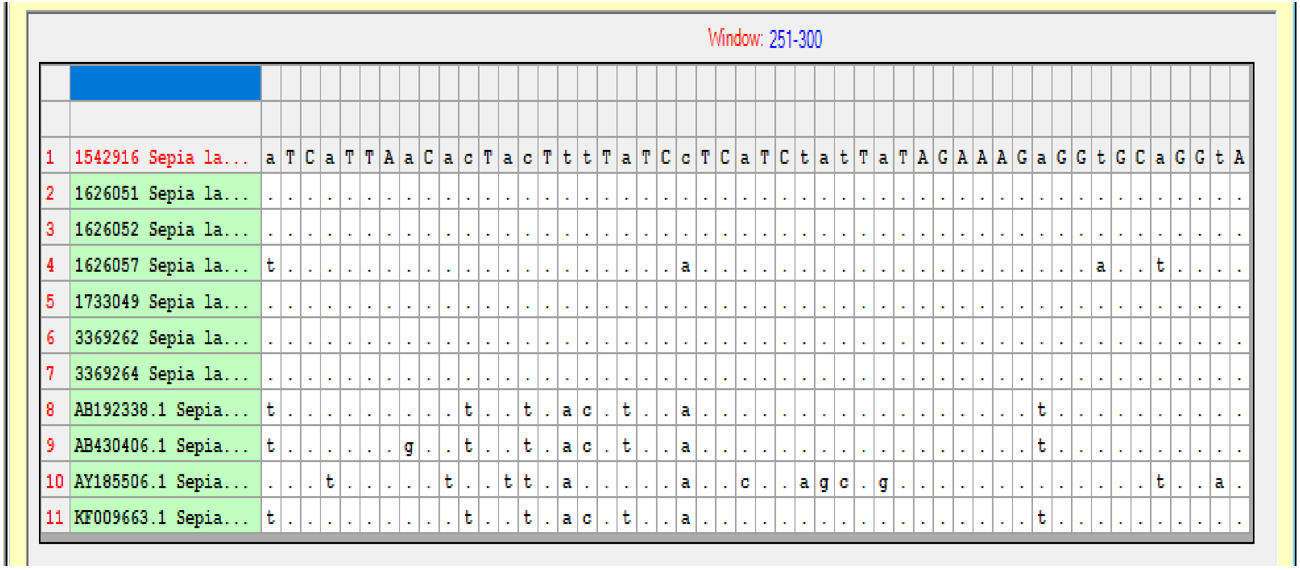
Window: 151-200

1	1542916 Sepia la...	T	A	A	T	t	G	G	t	G	G	g	T	T	T	G	G	T	A	A	c	T	G	a	T	T	A	g	T	t	C	C	t	T	T	A	A	T	A	c	T	A	G	G	a	G	C	c	C	C	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	t	.	.	.	.	t	.	.	t	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	c	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	t	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	c	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	t	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	g	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	t	.	.	.	.	g	.	.	a	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	c	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	t	.	.	.



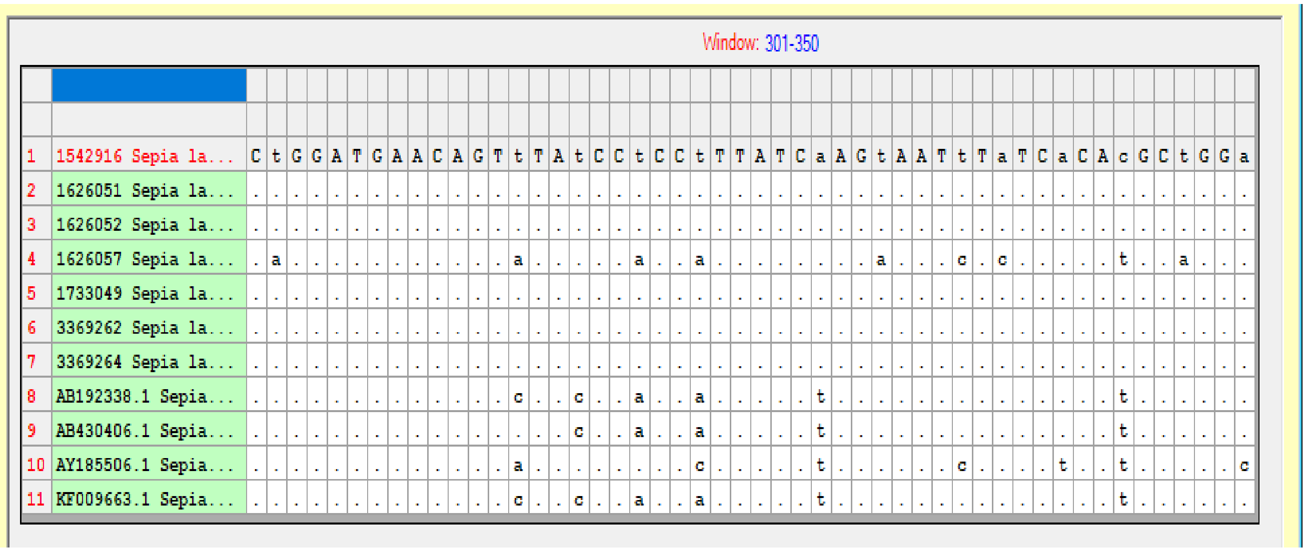
Window: 201-250

1	1542916 Sepia la...	G	A	t	A	T	A	G	C	t	T	T	t	C	C	a	C	G	t	A	T	A	A	A	c	A	A	T	A	T	A	A	G	a	T	T	T	T	G	a	T	T	A	c	T	A	C	C	c	C	C
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	t	.	.	.	.	a	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	c	.	.	.	.	.	c	.	.	c	.	.	t	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	g	.	.	.	t	.	.	.	.	t	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.



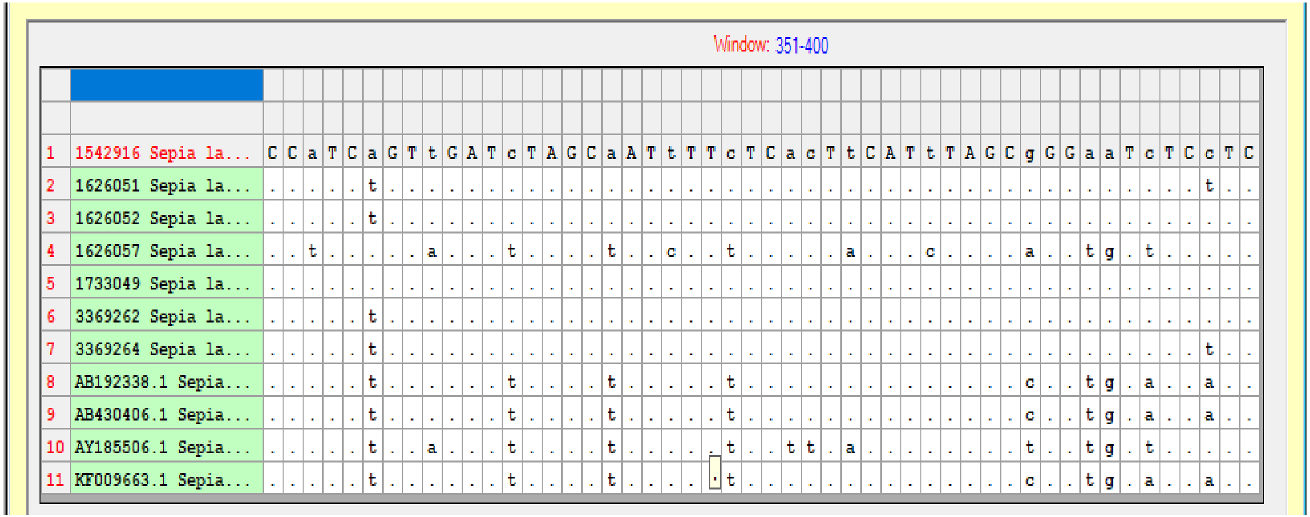
Window: 251-300

1	1542916 Sepia la...	a	T	C	a	T	T	A	a	C	a	c	T	a	c	T	t	t	T	a	T	C	c	T	C	a	T	C	t	a	t	T	a	T	A	G	A	A	A	G	a	G	G	t	G	C	a	G	G	t	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	t	.	.	.	.	.	.	.	.	.	t	.	.	t	.	a	c	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	t	.	.	.	.	.	.	g	.	.	t	.	.	t	.	a	c	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	t	.	.	.	.	.	t	.	.	t	t	.	a	.	.	.	.	.	a	.	.	c	.	.	a	g	c	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	a	.
11	KF009663.1 Sepia...	t	.	.	.	.	.	.	.	.	.	t	.	.	t	.	a	c	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.



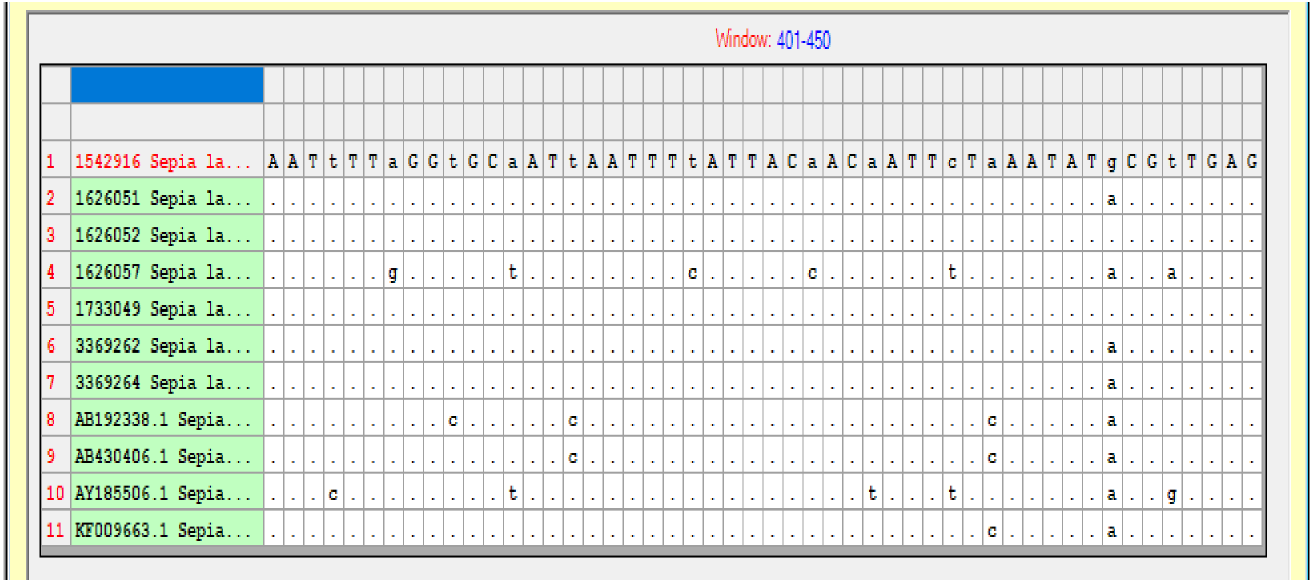
Window: 301-350

1	1542916 Sepia la...	C	t	G	G	A	T	G	A	A	C	A	G	T	t	T	A	t	C	C	t	C	C	t	T	T	A	T	C	a	A	G	t	A	A	T	t	T	a	T	C	a	C	A	c	G	C	t	G	G	a
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	c	.	c	.	.	.	.	.	t	.	.	a	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.	.	.	.	.	c	.	.	.	.	t	.	.	t	.	.	.	.	.	c
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.



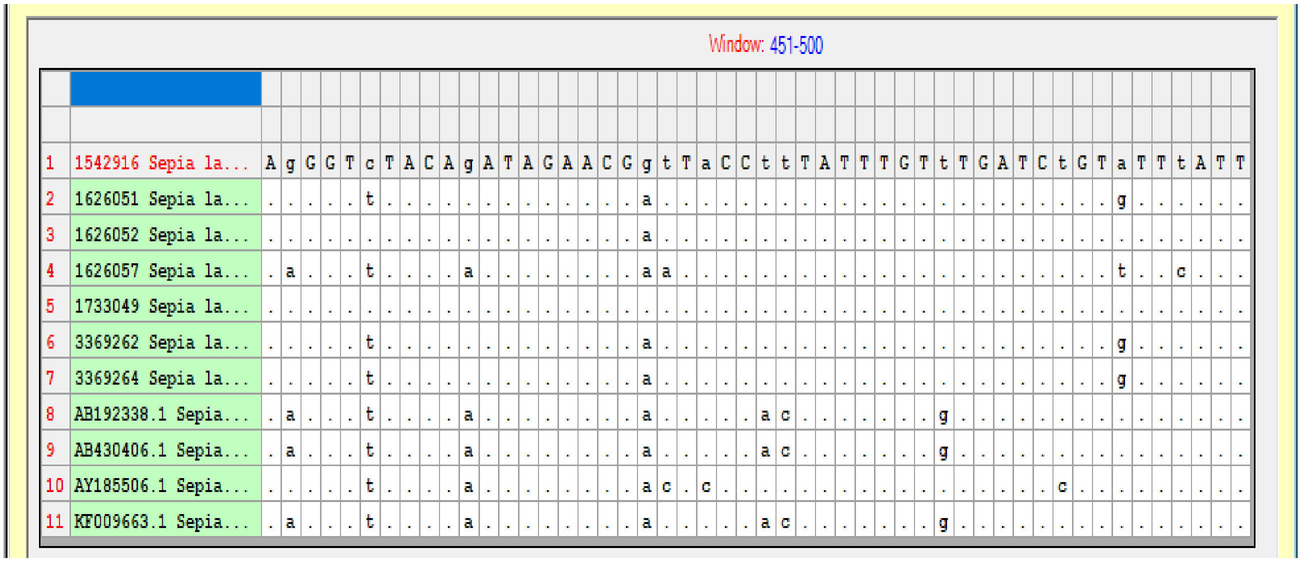
Window: 351-400

1	1542916 Sepia la...	C	C	a	T	C	a	G	T	t	G	A	T	c	T	A	G	C	a	A	T	t	T	T	c	T	C	a	c	T	t	C	A	T	t	T	A	G	C	g	G	G	a	a	T	c	T	C	c	T	C
2	1626051 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
3	1626052 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	t	.	.	.	.	.	a	.	.	.	t	.	.	.	.	t	.	.	c	.	.	t	.	.	.	.	.	a	.	.	.	c	.	.	.	.	a	.	.	t	g	.	t	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	t	.	.	.	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	t	g	.	a	.	.	a	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	t	.	.	.	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	t	g	.	a	.	.	a	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	t	.	.	a	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	t	t	.	a	.	.	.	.	.	.	.	.	t	.	.	t	g	.	t	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	t	.	.	.	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	t	g	.	a	.	.	a	.	.



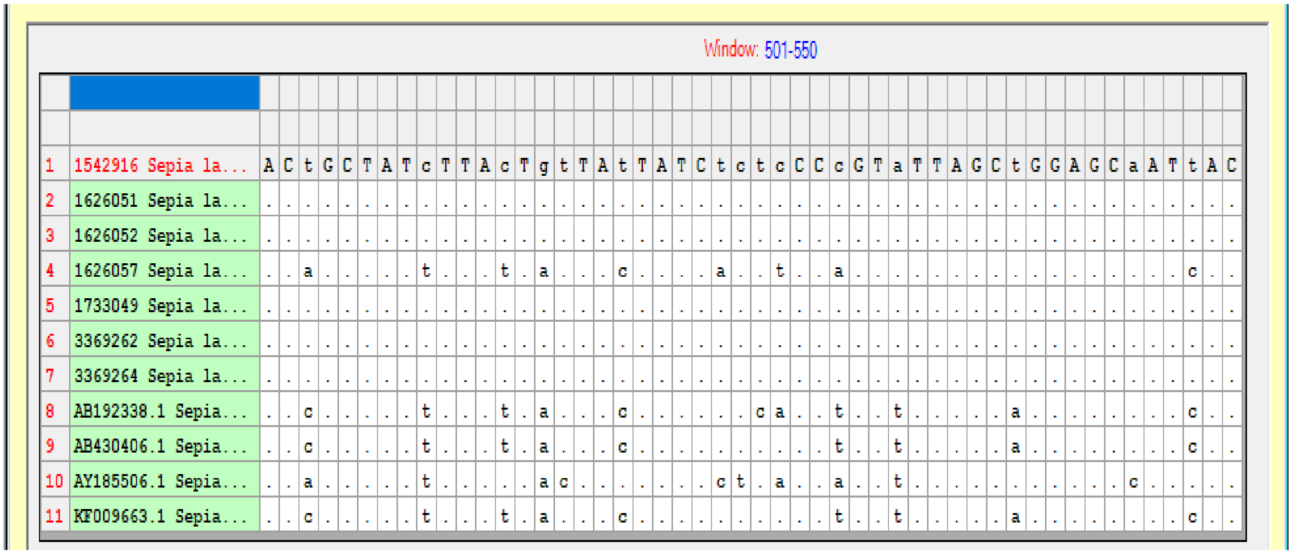
Window: 401-450

1	1542916 Sepia la...	A	A	T	t	T	T	a	G	G	t	G	C	a	A	T	t	A	A	T	T	T	t	A	T	T	A	C	a	A	C	a	A	T	T	c	T	a	A	A	T	A	T	g	C	G	t	T	G	A	G
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	g	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	.	.	.	.	t	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	c	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	t	.	.	.	.	.	.	.	a	.	.	g	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.



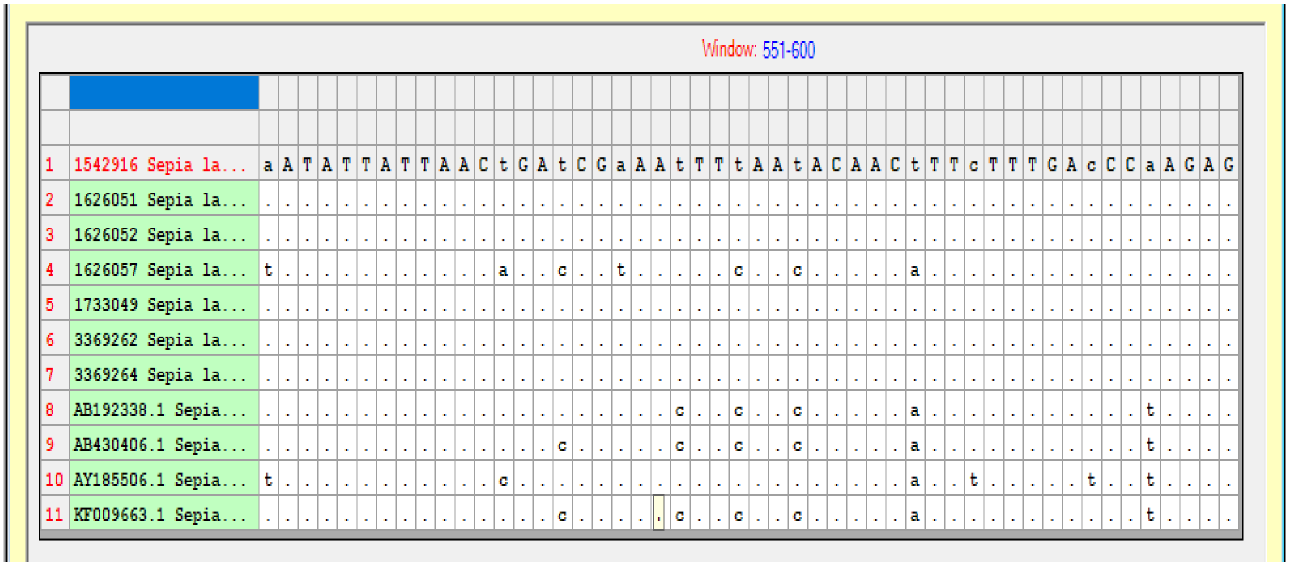
Window: 451-500

1	1542916 Sepia la...	A	g	G	G	T	c	T	A	C	A	g	A	T	A	G	A	A	C	G	g	t	T	a	C	C	t	t	T	A	T	T	T	G	T	t	T	G	A	T	C	t	G	T	a	T	T	t	A	T	T
2	1626051 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	c	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	c	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	c	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.
11	KF009663.1 Sepia...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	c	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.



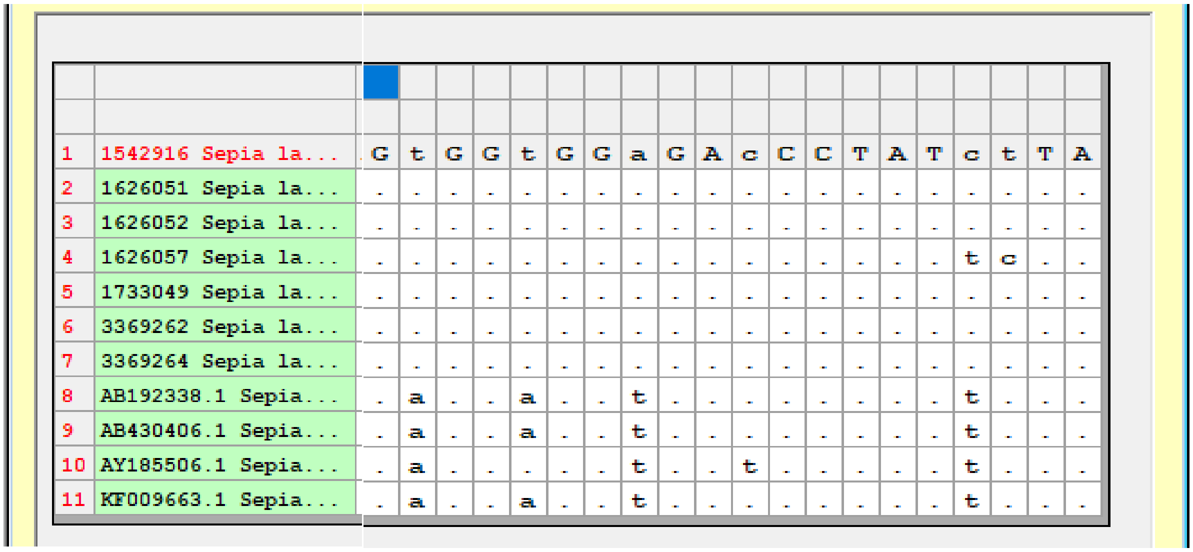
Window: 501-550

1	1542916 Sepia la...	A	C	t	G	C	T	A	T	c	T	T	A	c	T	g	t	T	A	t	T	A	T	C	t	c	t	c	C	C	c	G	T	a	T	T	A	G	C	t	G	G	A	G	C	a	A	T	t	A	C
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	a	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	a	.	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	c	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	.	.	c	a	.	.	t	.	.	t	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.
9	AB430406.1 Sepia...	.	.	c	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	t	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.
10	AY185506.1 Sepia...	.	.	a	.	.	.	.	.	t	.	.	.	.	.	a	c	.	.	.	.	.	.	.	c	t	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	c	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	t	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.



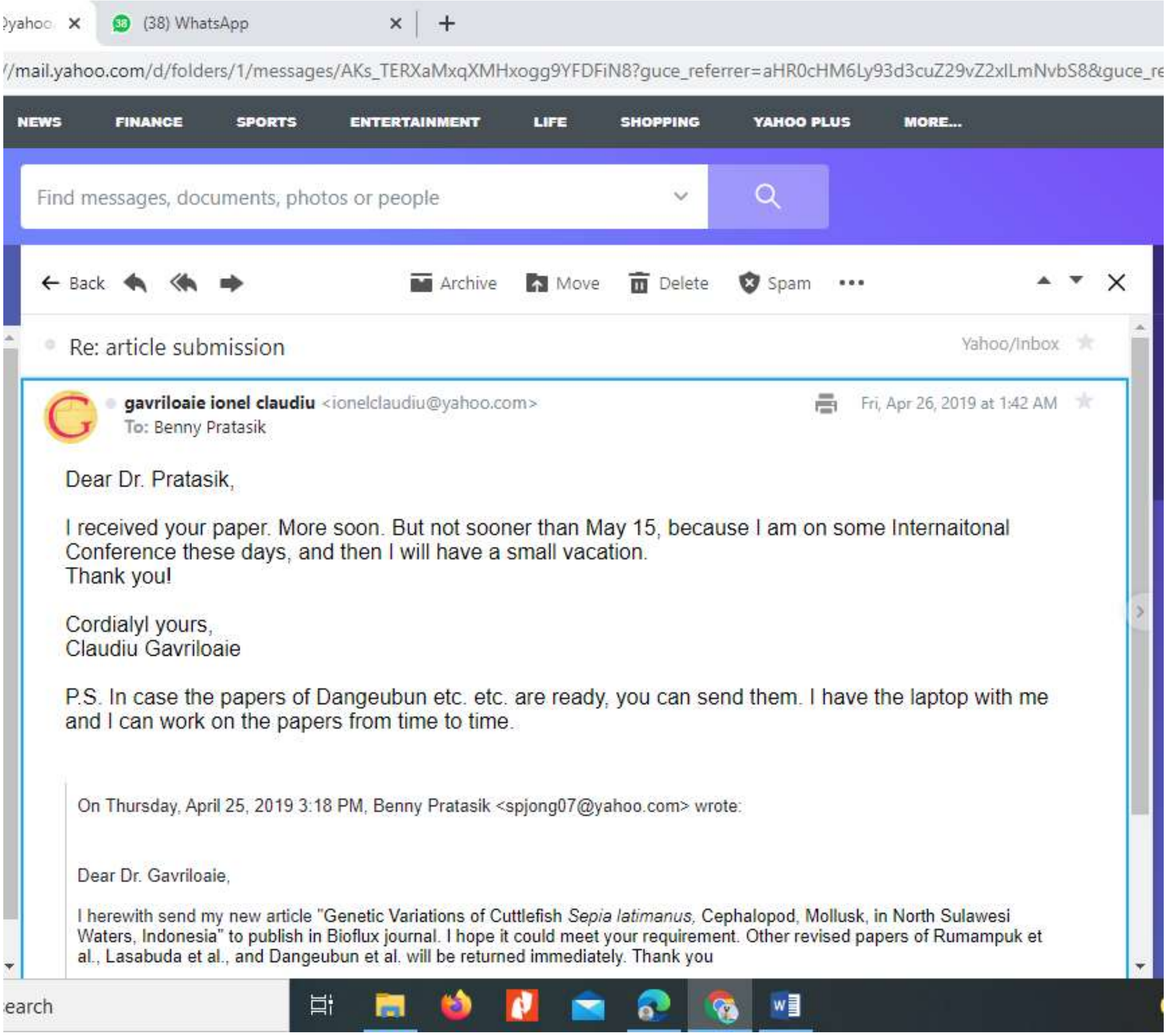
Window: 551-600

1	1542916 Sepia la...	a	A	T	A	T	T	A	T	T	A	A	C	t	G	A	t	C	G	a	A	A	t	T	T	t	A	A	t	A	C	A	A	C	t	T	T	c	T	T	T	G	A	c	C	C	a	A	G	A	G
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	t	.	.	.	.	.	.	.	.	.	.	.	a	.	.	c	.	.	t	.	.	.	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.
10	AY185506.1 Sepia...	t	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	t	.	.	t	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.



1	1542916 Sepia la...	G	t	G	G	t	G	G	a	G	A	c	C	C	T	A	T	c	t	T	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	c	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.
9	AB430406.1 Sepia...	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.
10	AY185506.1 Sepia...	.	a	.	.	.	.	.	t	.	.	t	.	.	.	.	.	t	.	.	.
11	KF009663.1 Sepia...	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.

Notes: 1 = *Sepia latimanus* (Bitung); 2 = *S. latimanus* (Likupang 1); 3 = *S. latimanus* (Likupang 2);4 = *S. latimanus* (Manado); 5 = *S. latimanus* (Arakan); 6 = *S. latimanus* (Basaan 1); 7 = *S. latimanus* (Basaan 2); 8 = *S. latimanus* (Japan: Okinawa); 9 = *S. latimanus* (Indonesia); 10 = *S. latimanus* (China); 11 = *S. latimanus* (Filipina: Aurora-region 3).



Re: article submission

gavriloaie ionel claudiu <ionelclaudiu@yahoo.com>
To: Benny Pratasik
Fri, Apr 26, 2019 at 1:42 AM

Dear Dr. Pratasik,

I received your paper. More soon. But not sooner than May 15, because I am on some Internaitonal Conference these days, and then I will have a small vacation.
Thank you!

Cordialyl yours,
Claudiu Gavriloaie

P.S. In case the papers of Dangeubun etc. etc. are ready, you can send them. I have the laptop with me and I can work on the papers from time to time.

> On Thursday, April 25, 2019 3:18 PM, Benny Pratasik <spjong07@yahoo.com> wrote:
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> Dear Dr. Gavriloaie,
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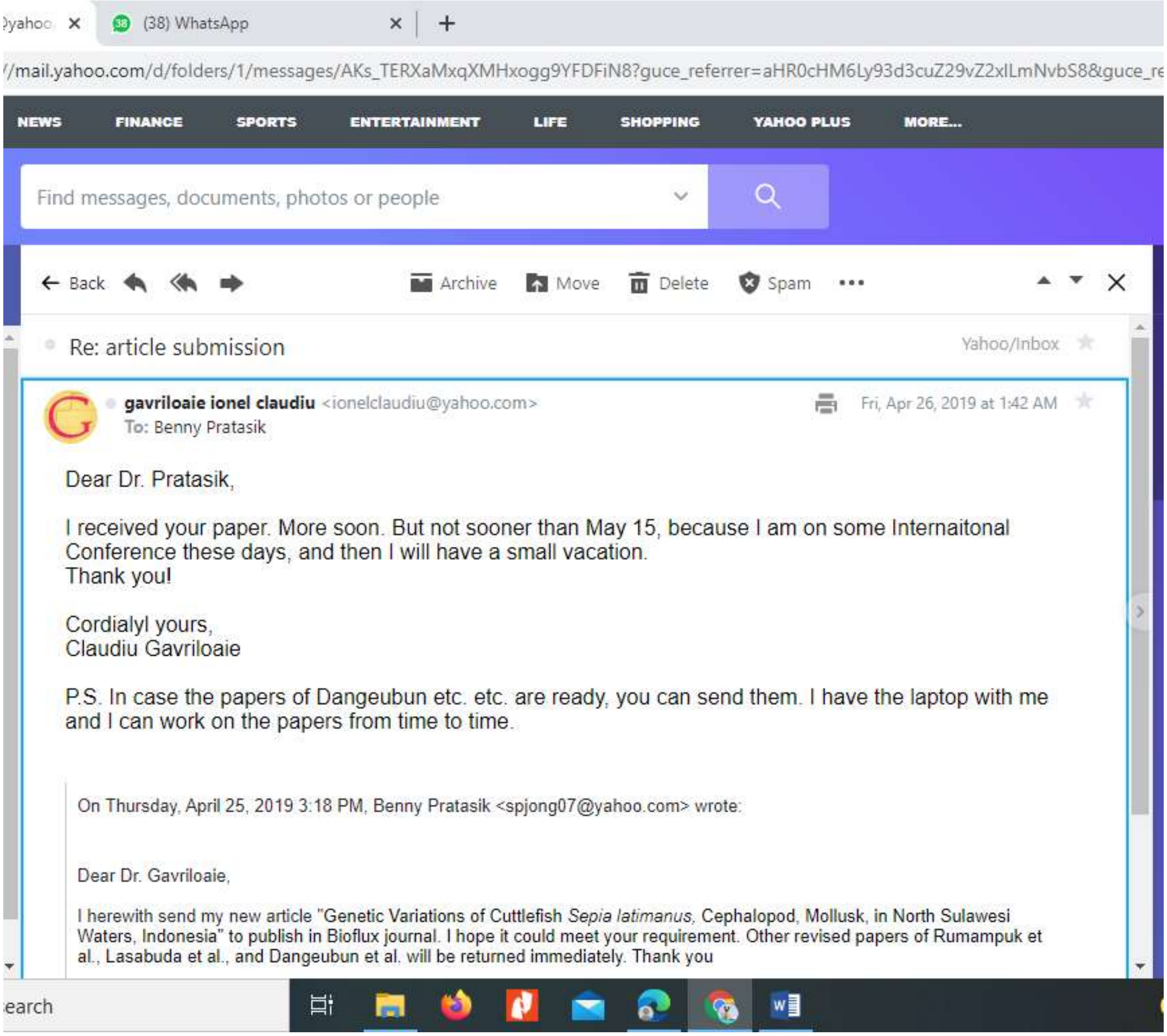
> I herewith send my new article "Genetic Variations of Cuttlefish *Sepia latimanus*, Cephalopod, Mollusk, in North Sulawesi Waters, Indonesia" to publish in Bioflux journal. I hope it could meet your requirement. Other revised papers of Rumampuk et al., Lasabuda et al., and Dangeubun et al. will be returned immediately. Thank you



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Genetic variations of cuttlefish *Sepia latimanus*, cephalopod, mollusk[indra1], in North Sulawesi waters, Indonesia

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Abstract. This study was aimed at discerning the genetic variations of cuttlefish *Sepia latimanus* in North Sulawesi, using CO1 gene marker. Sampling was conducted in Kalinaung and Likupang, North Minahasa regency, Manado Bay, Manado municipality and Minahasa regency, Poigar, South Minahasa regency, and Basaan, Southeast Minahasa. All samples were put into 95% alcohol and brought to the molecular biology laboratory of Faculty of Fisheries and Marine Sciences for further analysis. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5'-ggtcaacaaatcataaagatattgg[indra2]-3' and HCO2198: 5'taaacttcagggtgaccaaaaaatca-3'. Polymerase chain reaction (PCR) was carried out in 35 cycles at 95°C (30 sec), 50°C (30 sec), 72°C (50 sec). The PCR product was visualized in 1% (b/v) agarosa gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). The chromatogram obtained was edited using Geneious v5.6. The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method and BOLDSystems. The phylogenetic tree was built using Neighbor-Joining Method, and genetic similarity was explained by mutation, haplotype and genetic distance.[indra3]

Key Words: CO1 gene marker, phylogenetic tree, haplotype, genetic distance.

Introduction. North Sulawesi has many islands surrounded by coral reef ecosystems that provide many different habitats for various marine organisms either as home or shelter of different life stage. Broadclub cuttlefish, *Sepia latimanus*, is one of marine mollusks taking advantages of the coral reef ecosystem as spawning ground, egg placement site, and shelter for the youngsters (Pratasik et al 2017). Species diversity of cuttlefish is very high with more than a hundred species recorded, and their distribution and abundance are highly affected by the availability of suitable habitats and preys, particularly for small individuals (Neves et al 2009; Pratasik et al 2017). Some species live in the water deeper than 400 m, such as *Sepia australis, S. elegans, S. orbignyana* and [indra4]*S. hieronis*, and some species occupy shallow waters, such as *S. latimanus*, *S. officinalis,* and *S. pharaonis* (Reid et al 2005). Cuttlefish (and cephalopods in general) are known to have diverse body patterns that can immediately naturally change, and controlled by its chromatophore system (Hanlon et al 2009) and well known as the most intelligent marine biota with the largest body-brain ratio of all invertebrates (NOVA 2007[indra5]). These have made many studies have focused on the body pattern alteration-related behavioral aspects (Shohet et al 2006; Barbosa et al 2008; Kelman et al 2008; Allen et al 2009; Chiao et al 2010; Barbosa et al 2011).

Indonesian waters so far hold several species of cuttlefish, such as *Sepia bandensis, S. brevimana, S. kiensis, S. latimanus, S. papuensis, S. pharaonis, S. sulcata, S. senta, Sepiella inermis* and *S. weberi* (Reid et al 2005), but previous study in Manado Bay and Lembeh Strait found only four species (Pratasik et 2016). Low interest in cuttlefish study could result from sample collection problems as a result of no cuttlefish fisheries in Indonesia, and all cuttlefish catches are bycatch of other fisheries. No cuttlefish fisheries in Indonesia, especially North Sulawesi, make cuttlefish catch generally come from traditional fishermen using handline or speargun in the coral reef areas. *S. latimanus* is the main cuttlefish catch in the coral reef ecosystem and has highly market value. *S. latimanus* is the second biggest cuttlefish after *S. apama* with maximum dorsal mantle length of 50 cm and total weight of 10 kg (Reid et al 2005). The species often visit coral reefs for spawning and laying eggs (pers. obs.). They often come in pairs and select certain coral crevices to insert their eggs (Pratasik et al 2017). Cuttlefish are, in general, economic fisheries resources, but the resources have unclear fisheries status, insufficient data (Reid et al 2005), and belong to threatened species (IUCN 2014). The present study focuses on genetic variations using DNA sequence of mitochondrial genome as DNA barcode. CO1 is the most common mitochondrial DNA gene marker used for species identification (Folmer et al 1994) due to belonging to the most conservative gene among the mitochondrial DNA protein coding genes. It has become a standard tool of molecular taxonomy and identification (Ratnasingham & Hebert 2007). Molecular techniques have been well applied for stock discrimination studies in fisheries (Murphy et al 2002) and can provide the basis for better management of whole populations and therefore, sustainable fisheries.[indra6]

Material and Method

Sample collection. Cuttlefish samples were collected in Lembeh Strait (Bitung), Basaan (Southeast Minahasa), Manado Bay (Manado), Likupang waters (North Minahasa), Arakan and Poigar (South Minahasa), North Sulawesi. The first two sites represent the southern part of North Sulawesi waters, while the second four sites represent the northern part of the island, so that these sites have represented the entire North Sulawesi marine waters. Sampling activities were carried out for 5 months from May to September 2018. Sampling activities employed SCUBA dives over different habitat types and collected from local fishermen. In this study, seven cuttlefish specimens were collected from fishermen's catches, brought to the Biotechnology Laboratory of Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, and all samples were preserved in 95% ethanol and stored at room temperature before DNA extraction.

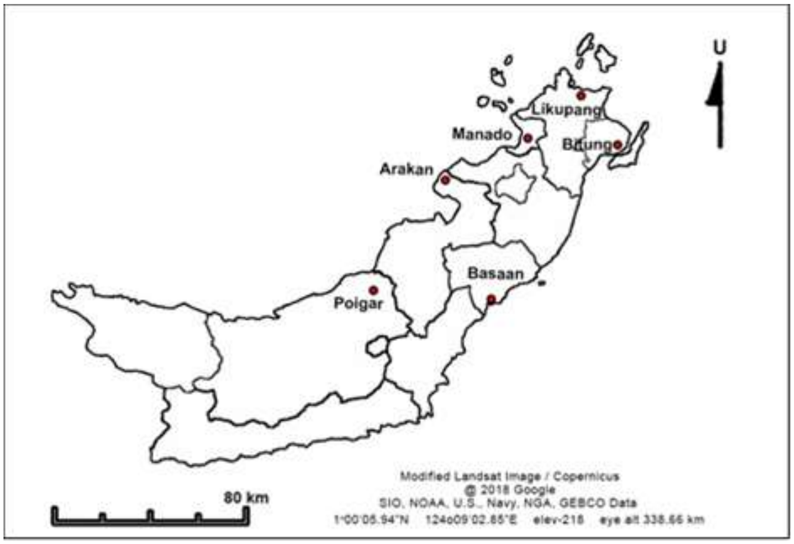

Figure [indra7]1. Map of North Sulawesi with sampling points (marked red).

Extraction, PCR, and sequencing. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5'-ggtcaacaaatcataaagatattgg[indra8]-3' and HCO2198: 5'-taaacttcagggtgaccaaaaaatca-3' (Folmer et al 1994). Polymerase chain reaction (PCR) was carried out in 35 cycles at 95^{o}C (30 sec), 50^{o}C (30 sec), 72^{o}C (50 sec). The PCR product was visualized in 1% (b/v) agarose gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). [indra9]

Data analysis[indra10]. The chromatogram obtained was edited using Geneious v5.6 (Drummond et al 2012). The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method (Altschul et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007). The phylogenetic tree was built using Neighbor-Joining Method of MEGA X (Kumar et al 2018). Similarity index was also calculated. The analysis of nucleotide base sequence and haplotype variations between *S. latimanus* from different localities used DnaSP Ver.6.12.03 computer program (Rozas et al 2017).

Results and Discussion. During the study, 7 individuals of *S. latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not taken, so that all samples were obtained from fishermen's catches. Sampling in Poigar, South Minahasa, using beach seine and SCUBA dives, did not obtain any sample as well. This condition reveals that sufficient sampling technique is required for cuttlefish studies. In the past, cuttlefish fishing, such as *S. officinalis* (English Channel) and *S. esculanta* (Japan), used trawl, set net, gill net, trammel net and trap with spawning substrate (Pawson 1995; Arkley et al 1996) by taking advantage of their migration to the spawning areas. In North Sulawesi, *S. latimanus* is generally fished using speargun or jig fishing in the coral reef ecosystem (Pratasik et al 2015). Difficulties in sample collection causes this study collect only few samples[indra11].

All cuttlefish collected in North Sulawesi waters, but Manado, were in the same group, while 4 NCBI samples (Indonesia, Japan, Philippines of Aurora-region 3) employed as comparison (Figure 2) were in another separate group. This finding reconfirms that geographic distance induces the genetic diversity of *S. latimanus*. However, *S. latimanus* of North Sulawesi has revealed that there are genetic variations and similarities among the same or different locations. *S. latimanus* sample from Bitung waters has genetic similarity to that from Arakan waters. Also, one sample of *S. latimanus* from Likupang waters has genetic similarity to that of Basaan waters. On the other hand, both *S. latimanus* samples from Basaan waters are genetically different as happening for both samples from Likupang waters (Appendix 1[indra12]).

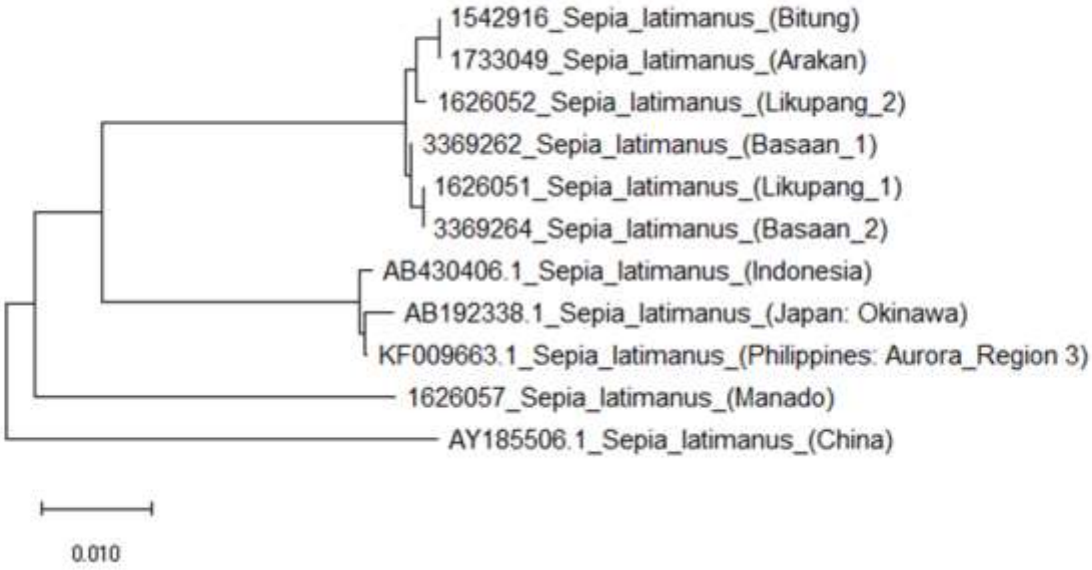

Figure [indra13]2. Phylogenetic tree of North Sulawesi *S. latimanus*.

From total numbers of 620 bp, there were 166 polymorphic sites with 186 mutations. Based on variable sites (two variants), there were 73 singleton variable sites with 67 different site positions and 93 parsimony informative sites with 80 site positions. The former was at positions: 2 23 32 33 38 41 56 68 71 77 80 87 99 104 107 113 122 135 140 149 173 179 182 203 212 215 239 254 258 260 263 275 278 279 280 282 293 299 302 332 338 341 347 350 353 371 377 378 384 404 407 410 422 428 431 473 491 497 516 525 526 545 569 587 593 611 618, and the latter at positions 5 8 17 27 44 47 50 53 62 69 72 83 92 98 119 128 131 134 155 158 161 170 177 189 218 224 233 243 251 261 264 266 267 269 272 290 296 317 320 329 336 344 356 359 363 368 374 380 392 393 413 416 435 437 443 452 456 461 470 476 477 485 509 513 515 519 533 539 548 551 566 572 575 578 584 596 602 605 608 617 (Appendix 2).

The present study also found 11 sequences used only formed 9 haplotypes [indra14]with haplotype diversity (Hd) of 0.9636, haplotype 1 (Bitung and Arakan), 2 (Likupang-1 and Basaan-2), 3 (Likupang-2), 4 (Manado), 5 (Basaan-1), 6 (Japan: Okinawa), 7 (Indonesia), 8 (China), and 9 (Filipina[indra15]: Aurora-region 3). Different haplotypes of the same localities show that the collected individuals have heteroplasmic-typed mtDNA. It could be seen from individuals from Basaan-1 and Basaan-2 and Likupang-1 and Likupang-2 form separate haplotypes despite existing in the same locality.

It indicates that *S. latimanus* in North Sulawesi waters has intraspecific population mixture that enables to find different genetic characters at the same locality or same genetic characters at different localities. This condition is also supported by FAO records (Reid et al 2005) that very wide distribution of *S. latimanus* could be a reasonable factor for the population or genetic mixture. This species lives in Indo-Pacific and distributes south Mozambik selatan[indra16], entire India ocean, Malacca strait, Melanesia islands, south China Sea, Philippines Sea, and east China Sea, Taipei province and Japan to south Kyushu, Indonesia to northwest and northeast Australia, Palau, Guam, New Caledonia, Fiji and Cocos Island (Reid et al 2005).

Genetic mixture, as indicated with haplotype similarity between Likupang 1 and Basaan 2 and between Bitung and Arakan, could be influenced by the ability of *S. latimanus* to move from one place to another. Previous studies have indicated that cuttlefish can inhabit different coastal habitats, such as coral reefs and seagrasses[indra17]. Habitats are the resources and conditions present in an area to support survival and reproductive success (Leopold 1933). Cuttlefish are well known as the chameleon of the sea (Hanlon et al 2009) with well-evolved nervous systems enable to search for extensive trophic niches and migrate to exploit different production systems and prey population, and therefore, they can do long distant migration. Our field observations found that juvenile *S. latimanus* occurred under the seagrass, and near branching corals and sponge. Other evidence also shows that fishermen often take advantage of low tide period to catch cuttlefish in seagrass ecosystem, at least in Likupang waters. [indra18]

S. latimanus from Manado Bay is also in separate group even though its geographic position occurs between Likupang waters and Arakan waters (Figure 1[indra19]). It could result from this individual belongs to species complex, a group of closely related organisms that look very similar to the point that the boundaries between them are often unclear. Based on genetic distance, *S. latimanus* from Manado Bay is close to that from Philippines waters (Appendix 1). Manado bay is the northern part of North Sulawesi with series of coral reef-surrounded small islands northwards to southern Philippines. This area belongs to Coral Triangle area. Closer genetic distance of *S. latimanus* between Manado bay and Philippines Sea than the same species from other Indonesian waters could result from closer geographic distance between Manado bay to Philippines than other location in Indonesian waters where the sample was collected. This finding is supported by previous studies that increasing geographic distance between patches was associated with increasing genetic distance, while increasing habitat continuity between patches was associated with decreasing genetic distance (Alberto et al 2010). Isolation by distance and isolation by environment are important mechanisms in driving the genetic population structure (Fernandez et al 2017). Isolation by distance could be used to predict an increase in genetic differentiation with geographic distance (Ramachandran et al 2005), but genetic structure through time is stable (Pascual et al 2016).

Ocean currents are also an important factor affecting the marine animal distribution. Therefore, both physical structures and processes are directly related to the swimming and behavioral capabilities of an individual or species. The dynamics of the ocean are highly variable and are driven by multiple forcing factors such as solar insolation, winds, tides and freshwater input[indra20]. This variability in forcing leads to the development of distinct water masses of different hydrographic properties in both vertical and horizontal dimensions [indra21](McManus et al 2011). All these are related with food chain and habitat distribution that influence the availability of living needs of marine animal's dispersal, including cuttlefish, *S. latimanus*.

Conclusions. *Sepia latimanus* of North Sulawesi waters had sufficient genetic variations. These occur between and within localities. Genetic similarity was also present between *S. latimanus* from different localities, such as Basaan-2 and Likupang-1 samples and Bitung and Arakan samples. Eleven nucleotide sequences analyzed formed only 9 haplotypes due to the haplotype similarity between localities, and this condition has reflected genetic mixture. Moreover, *S. latimanus* sample of Manado bay had closer kindship to that of NCBI data for Philippines waters. This condition is supported by habitat and food distribution, ocean currents, and the ability of *S. latimanus* to exploit different niches through behavioral adaptation to different environmental condition.

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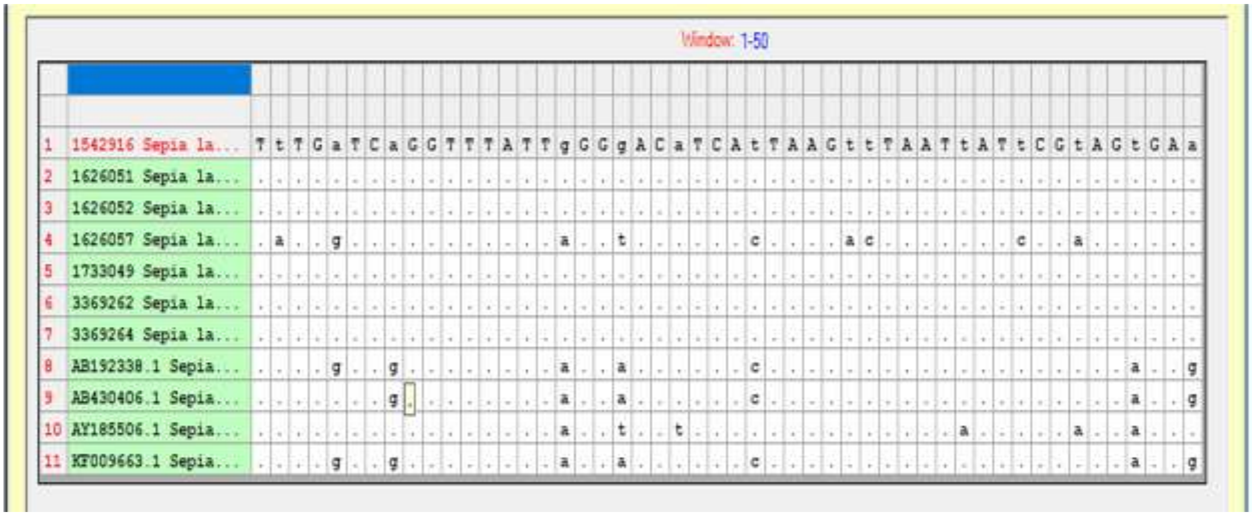
Appendix 1

Genetic distance of cuttlefish *Sepia latimanus* in North Sulawesi waters

No		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	1542916 *Sepia latimanus* (Bitung)										
2	1626051 *Sepia latimanus* (Likupang 1)	0.00435									
3	1626052 *Sepia latimanus* (Likupang 2)	0.00309	0.00371								
4	1626057 *Sepia latimanus* (Manado)	0.06984	0.06983	0.07071							
5	1733049 *Sepia latimanus* (Arakan)	0.00000	0.00435	0.00309	0.06984						
6	3369262 *Sepia latimanus* (Basaan 1)	0.00435	0.00123	0.00247	0.06820	0.00435					
7	3369264 *Sepia latimanus* (Basaan 2)	0.00435	0.00000	0.00371	0.06983	0.00435	0.00123				
8	AB192338.1 *Sepia latimanus* (Japan: Okinawa)	0.05800	0.05627	0.05704	0.06232	0.05800	0.05549	0.05627			
9	AB430406.1 *Sepia latimanus* (Indonesia)	0.05555	0.05384	0.05461	0.06152	0.05555	0.05307	0.05384	0.00434		
10	AY185506.1 *Sepia latimanus* (China)	0.07853	0.07747	0.07747	0.07531	0.07853	0.07581	0.07747	0.07725	0.07739	
11	KF009663.1 *Sepia latimanus* (Philippines: Aurora Region 3)	0.05555	0.05384	0.05461	0.05898	0.05555	0.05307	0.05384	0.00309	0.00247	0.07656

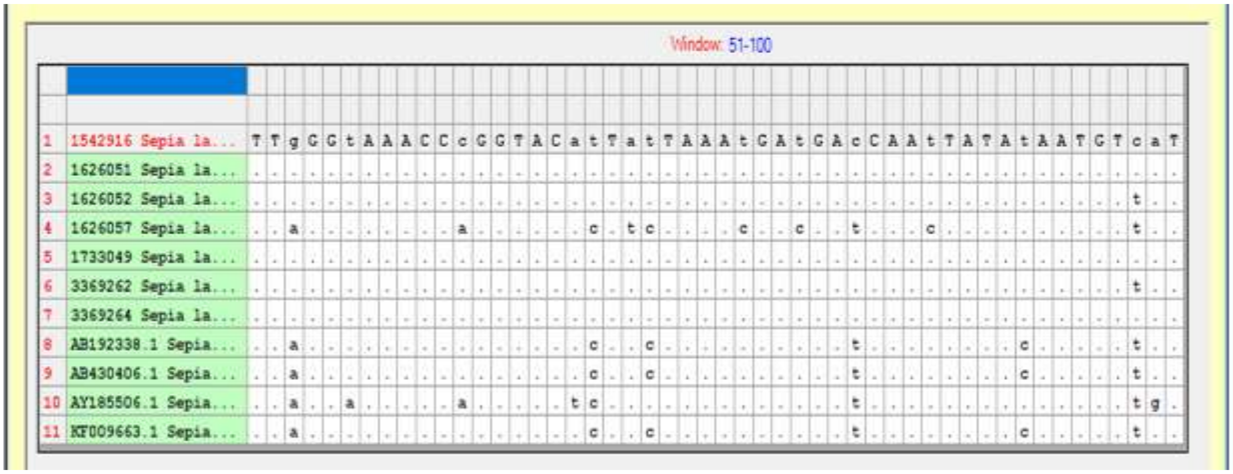
Appendix 2

Nucleotide variations of *S. latimanus* in North Sulawesi waters



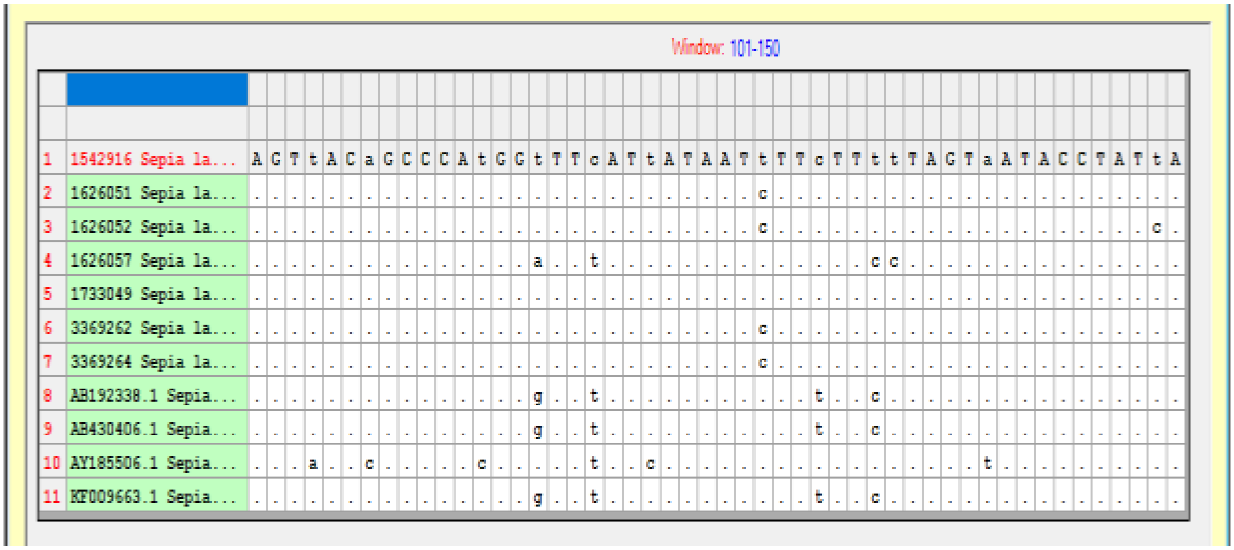
Window: 1-50

1	1542916 Sepia la...	T	t	T	G	a	T	C	a	G	G	T	T	T	A	T	T	g	G	G	g	A	C	a	T	C	A	t	T	A	A	G	t	t	T	A	A	T	t	A	T	t	C	G	t	A	G	t	G	A	a
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	g	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	c	.	.	.	.	a	c	.	.	.	.	.	.	.	c	.	.	a	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	g	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	.	.	a	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	g	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g



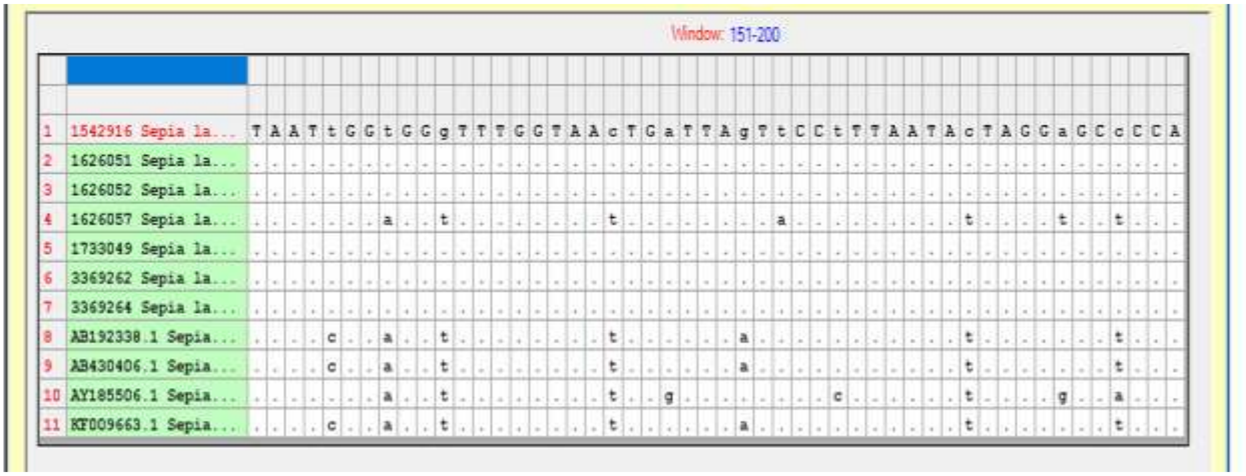
Window: 51-100

1	1542916 Sepia la...	T	T	g	G	G	t	A	A	A	C	C	c	G	G	T	A	C	a	t	T	a	t	T	A	A	A	t	G	A	t	G	A	c	C	A	A	t	T	A	T	A	t	A	A	T	G	T	c	a	T
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
4	1626057 Sepia la...	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	c	.	t	c	.	.	.	.	c	.	.	c	.	.	t	.	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.
9	AB430406.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.
10	AY185506.1 Sepia...	.	.	a	.	.	a	.	.	.	.	.	a	.	.	.	.	.	t	c	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	g	.
11	KF009663.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.



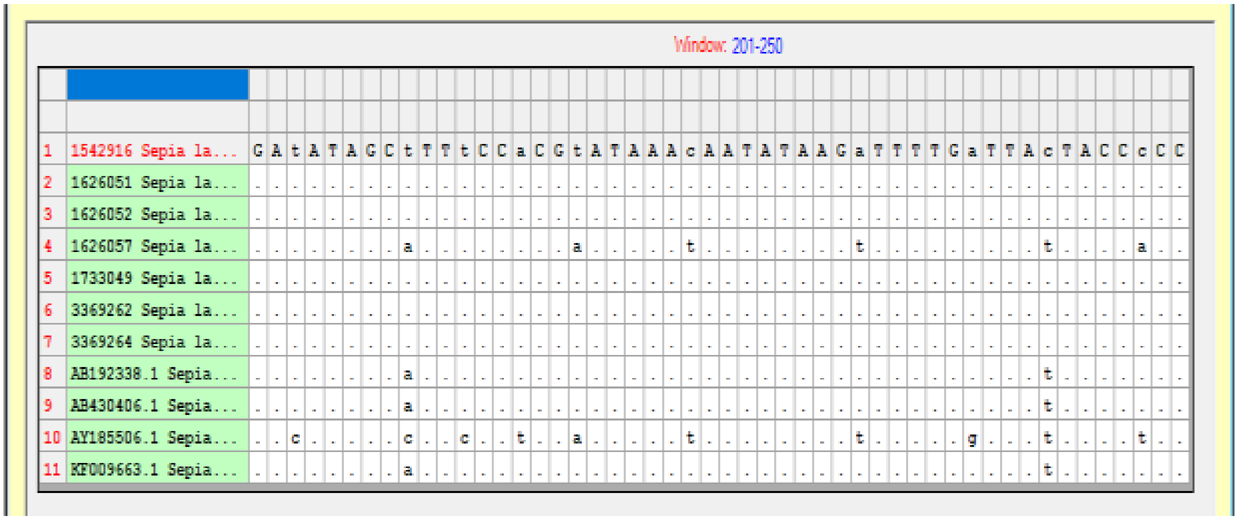
Window: 101-150

1	1542916 Sepia la...	A	G	T	t	A	C	a	G	C	C	C	A	t	G	G	t	T	T	c	A	T	t	A	T	A	A	T	t	T	T	c	T	T	t	t	T	A	G	T	a	A	T	A	C	C	T	A	T	t	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	a	.	.	c	.	.	.	.	.	c	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.



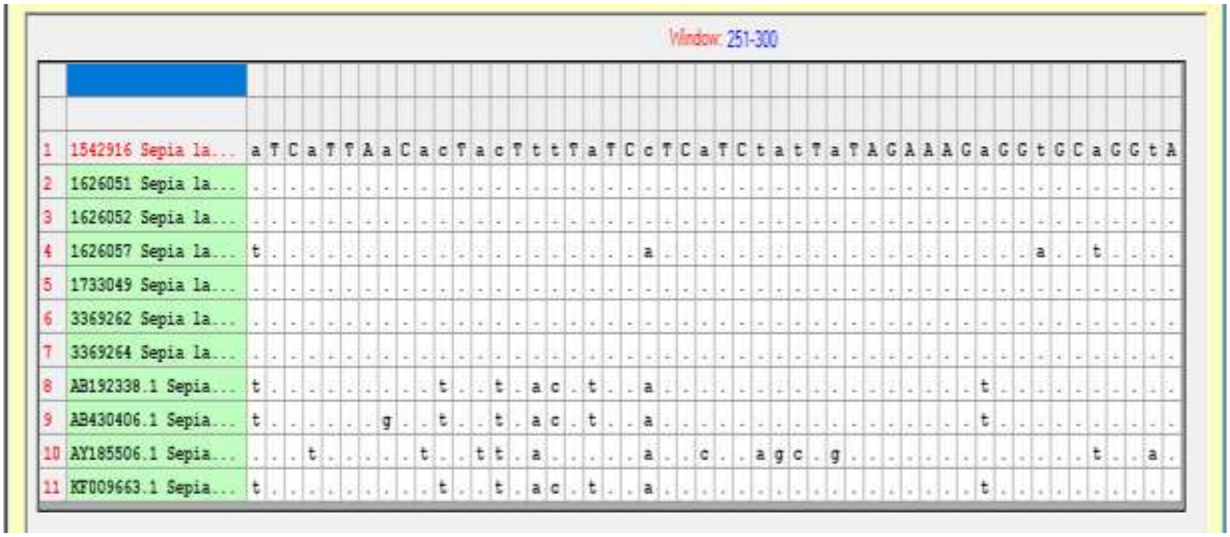
Window: 151-200

1	1542916 Sepia la...	TAATtGGtGGgTTTGGTAAcTGaTTAgTtCCtTTAATAcTAGGaGCcCCA
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	.......a..t........t........a.........t....t..t...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	....c..a..t........t......a...........t.......t...
9	AB430406.1 Sepia...	....c..a..t........t......a...........t.......t...
10	AY185506.1 Sepia...	.......a..t........t..g........c......t....g..a...
11	KF009663.1 Sepia...	....c..a..t........t......a...........t.......t...



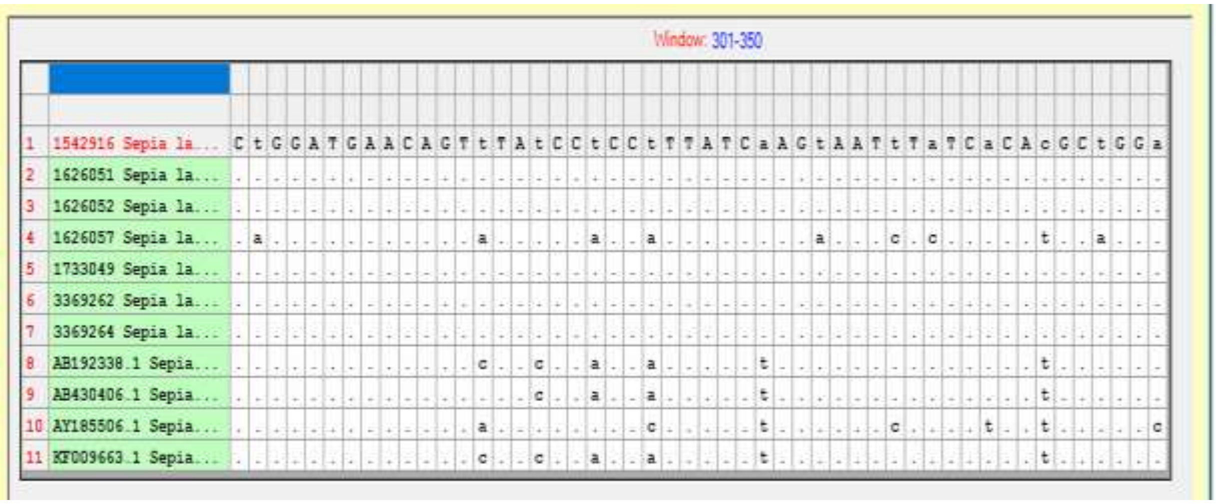
Window: 201-250

1	1542916 Sepia la...	GAtATAGCtTTtCCaCGtATAAAcAATATAAGaTTTTGaTTAcTACCcCC
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	........a........a.....t........t.........t....a..
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	........a.................................t.......
9	AB430406.1 Sepia...	........a.................................t.......
10	AY185506.1 Sepia...	..c.....c..c..t..a.....t........t.....g...t....t..
11	KF009663.1 Sepia...	........a.................................t.......



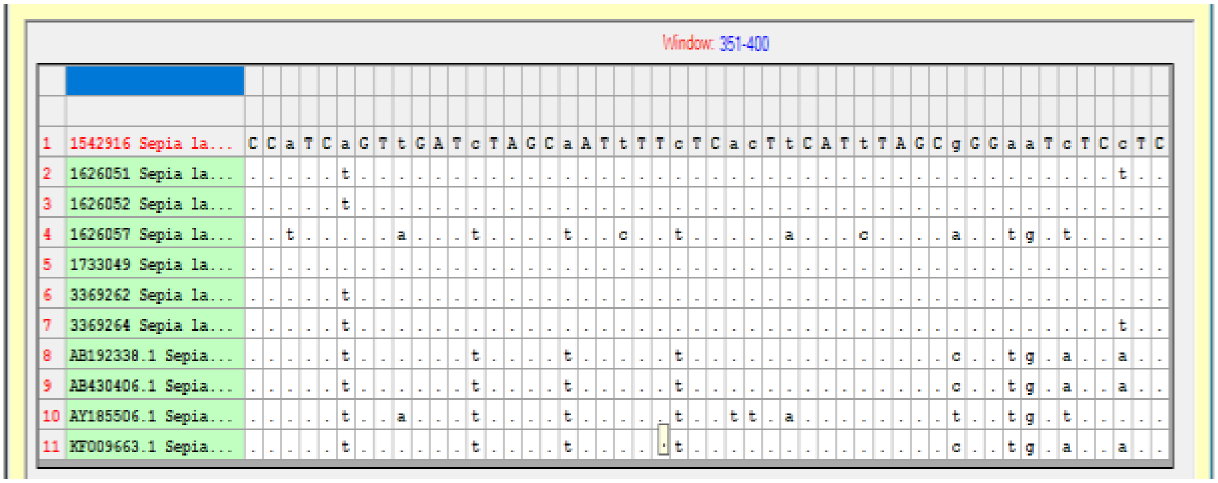
Window: 251-300

1	1542916 Sepia la...	aTCaTTAaCacTacTttTaTCcTCaTCtatTaTAGAAAGaGGtGCaGGtA
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	t...a........................a..t....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	t.........t..t.ac.t..a.................t..........
9	AB430406.1 Sepia...	t......g..t..t.ac.t..a.................t..........
10	AY185506.1 Sepia...	...t.....t..tt.a.....a..c..agc.g.............t..a.
11	KF009663.1 Sepia...	t.........t..t.ac.t..a.................t..........



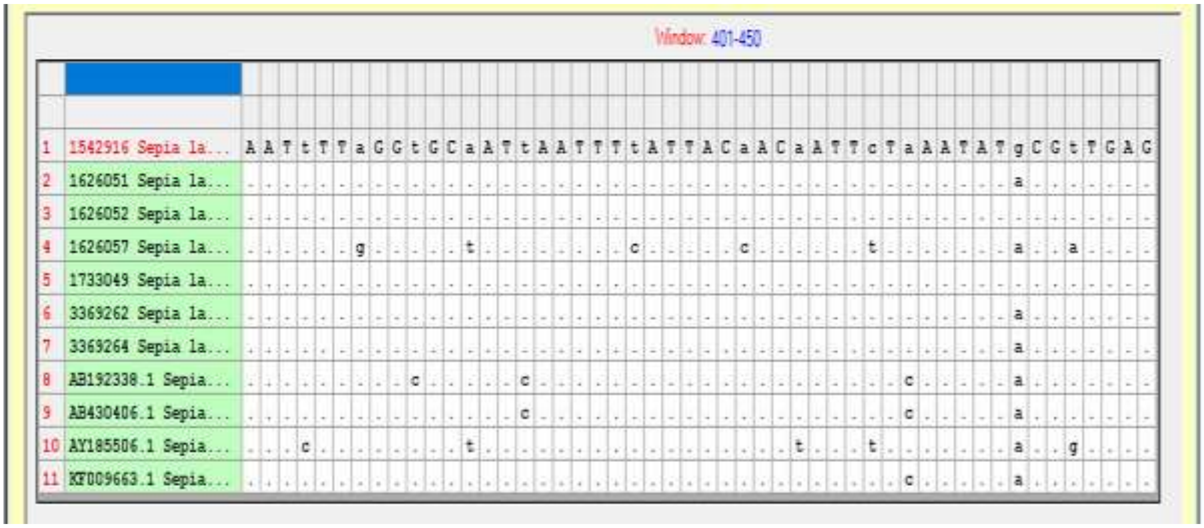
Window: 301-350

1	1542916 Sepia la...	C	t	G	G	A	T	G	A	A	C	A	G	T	t	T	A	t	C	C	t	C	C	t	T	T	A	T	C	a	A	G	t	A	A	T	t	T	a	T	C	a	C	A	c	G	C	t	G	G	a
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	c	.	c	.	.	.	.	.	t	.	.	a	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.	.	.	.	.	c	.	.	.	.	t	.	.	t	.	.	.	.	.	c
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.



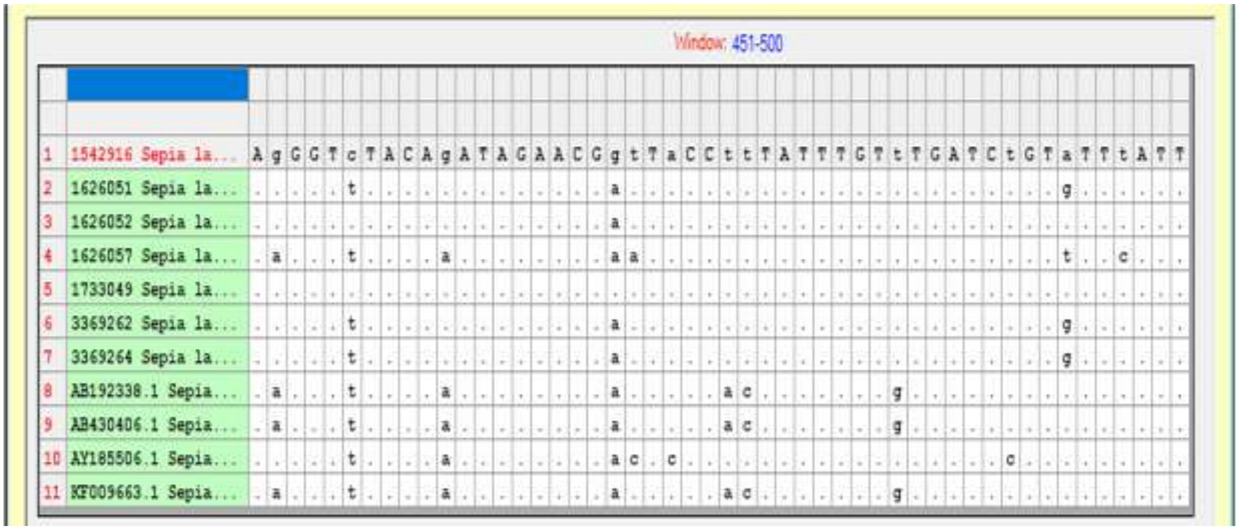
Window: 351-400

1	1542916 Sepia la...	C	C	a	T	C	a	G	T	t	G	A	T	c	T	A	G	C	a	A	T	t	T	T	c	T	C	a	c	T	t	C	A	T	t	T	A	G	C	g	G	G	a	a	T	c	T	C	c	T	C
2	1626051 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
3	1626052 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	t	.	.	.	.	.	a	.	.	.	t	.	.	.	.	t	.	.	c	.	.	t	.	.	.	.	.	a	.	.	.	c	.	.	.	.	a	.	.	t	g	.	t	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	t	.	.	.	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	t	g	.	a	.	.	a	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	t	.	.	.	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	t	g	.	a	.	.	a	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	t	.	.	a	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	t	t	.	a	.	.	.	.	.	.	.	.	t	.	.	t	g	.	t	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	t	.	.	.	.	.	.	t	.	.	.	.	t	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	t	g	.	a	.	.	a	.	.



Window: 401-450

1	1542916 Sepia la...	A	A	T	t	T	T	a	G	G	t	G	C	a	A	T	t	A	A	T	T	T	t	A	T	T	A	C	a	A	C	a	A	T	T	c	T	a	A	A	T	A	T	g	C	G	t	T	G	A	G
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	g	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	.	.	.	.	t	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	c	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	t	.	.	.	.	.	.	.	a	.	.	g	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.



Window: 451-500

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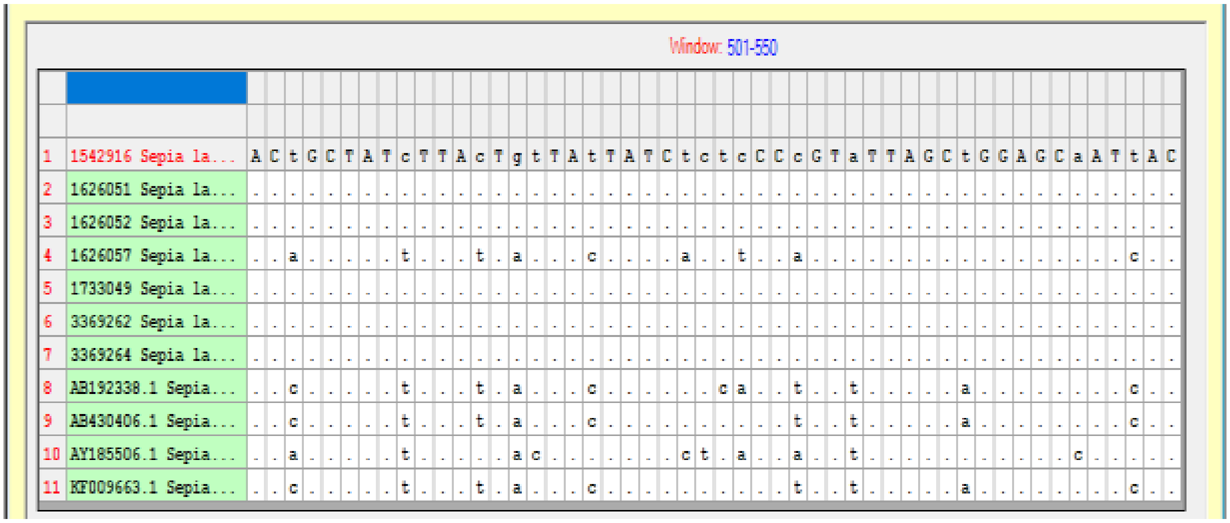
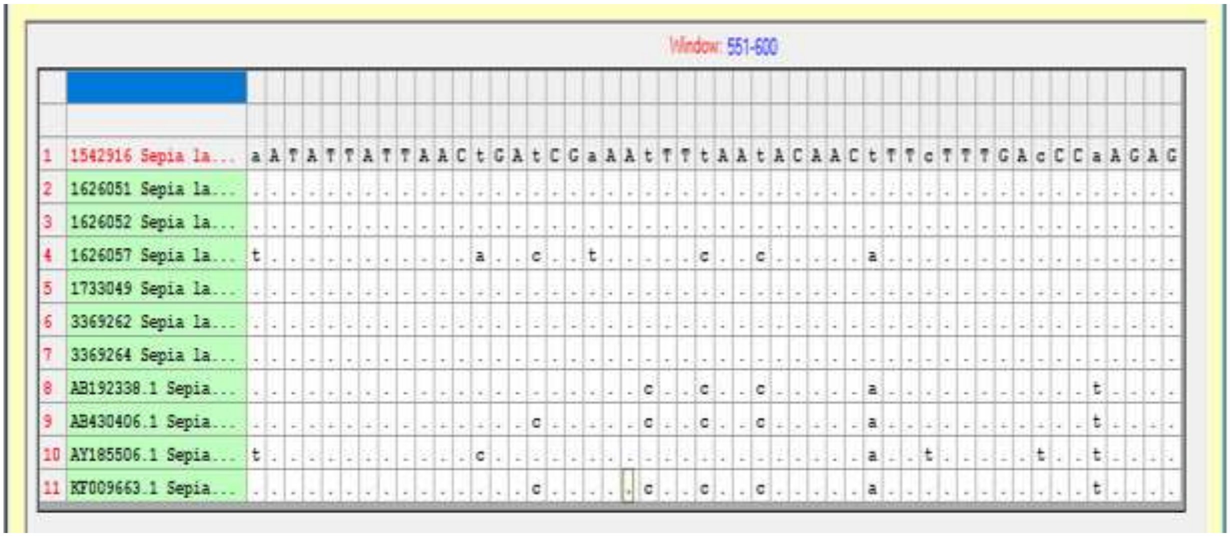
2	1626051 Sepia la...	.....t..............a.......................g......
3	1626052 Sepia la...	...................a..............................
4	1626057 Sepia la...	.a...t....a........aa......................t..c...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	.....t.............a.......................g......
7	3369264 Sepia la...	.....t.............a.......................g......
8	AB192338.1 Sepia...	.a...t....a........a.....ac.......g...............
9	AB430406.1 Sepia...	.a...t....a........a.....ac.......g...............
10	AY185506.1 Sepia...	.....t....a........ac.c.................c.........
11	KF009663.1 Sepia...	.a...t....a........a.....ac.......g...............



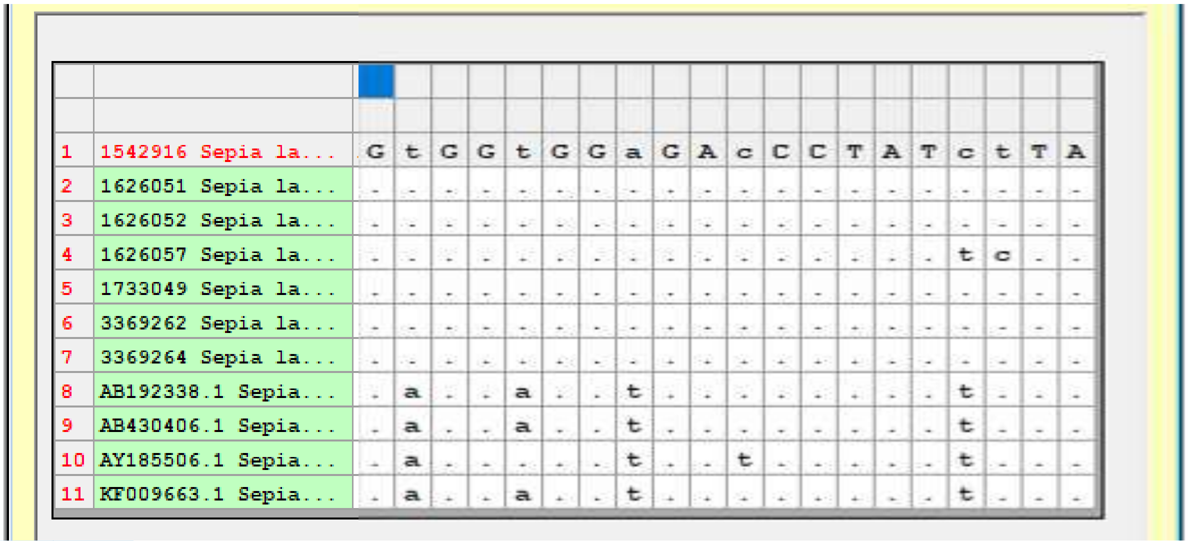
Window: 501-550

1	1542916 Sepia la...	ACtGCTATcTTAcTgtTAtTATCtctcCCcGTaTTAGCtGGAGCaATtAC
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	..a.....t...t.a...c....a..t..a.................c..
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	..c.....t...t.a...c......ca..t..t.....a........c..
9	AB430406.1 Sepia...	..c.....t...t.a...c..........t..t.....a........c..
10	AY185506.1 Sepia...	..a.....t.....ac.......ct.a..a..t...........c.....
11	KF009663.1 Sepia...	..c.....t...t.a...c..........t..t.....a........c..



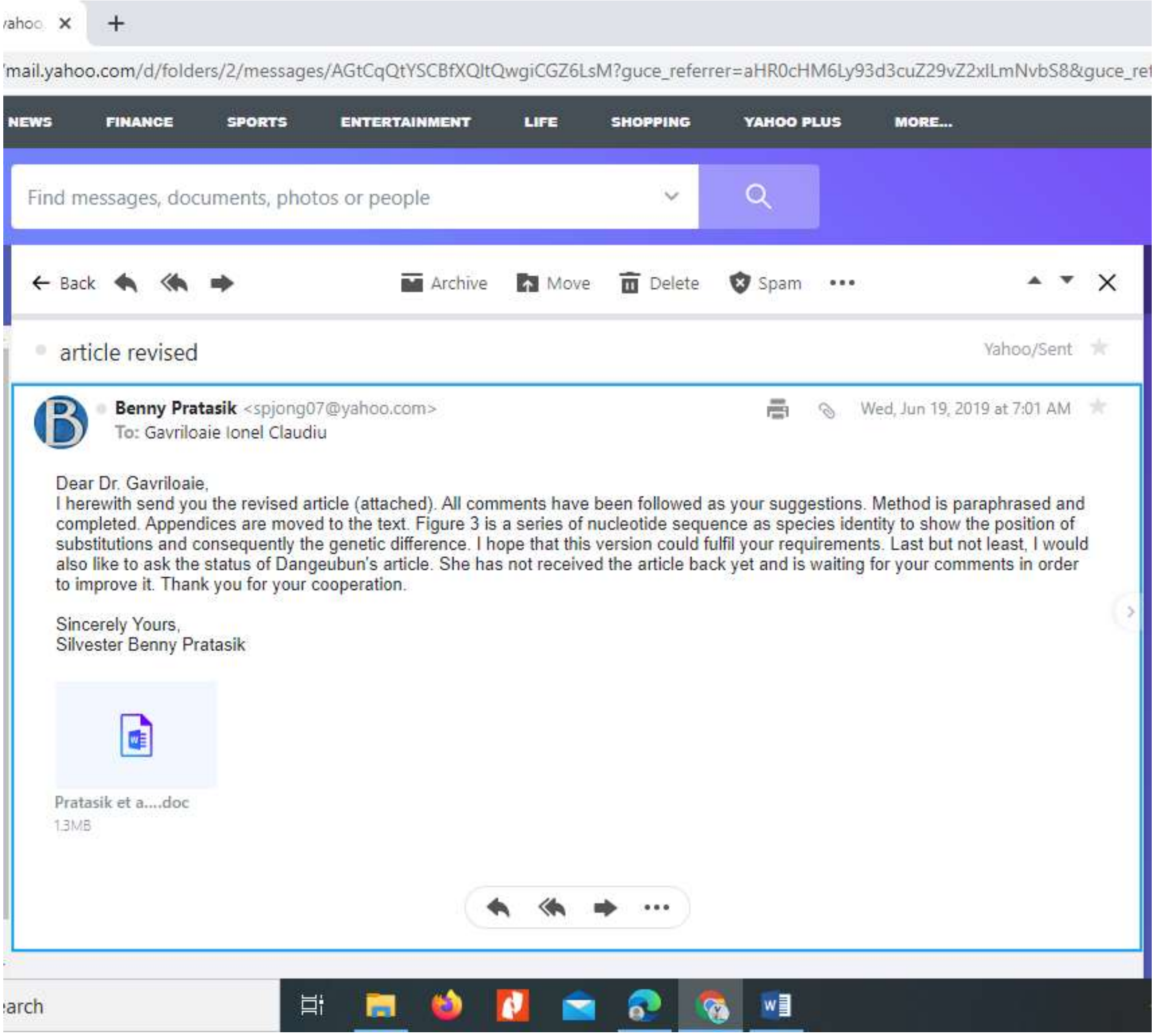
Window: 551-600

1	1542916 Sepia la...	aATATTATTAACtGAtCGaAAtTTtAAtACAACtTTcTTTGAcCCaAGAG
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	t...........a..c..t.....c..c.....a................
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	.....................c..c..c.....a...........t....
9	AB430406.1 Sepia...	...............c.....c..c..c.....a...........t....
10	AY185506.1 Sepia...	t...........c....................a..t.....t..t....
11	KF009663.1 Sepia...	...............c.....c..c..c.....a...........t....



1	1542916 Sepia la...	G	t	G	G	t	G	G	a	G	A	c	C	C	T	A	T	c	t	T	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	c	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.
9	AB430406.1 Sepia...	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.
10	AY185506.1 Sepia...	.	a	.	.	.	.	.	t	.	.	t	.	.	.	.	.	t	.	.	.
11	KF009663.1 Sepia...	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.

Notes[indra29]: 1 = *Sepia latimanus* (Bitung); 2 = *S. latimanus* (Likupang 1); 3 = *S. latimanus* (Likupang 2);4 = *S. latimanus* (Manado); 5 = *S. latimanus* (Arakan); 6 = *S. latimanus* (Basaan 1); 7 = *S. latimanus* (Basaan 2); 8 = *S. latimanus* (Japan: Okinawa); 9 = *S. latimanus* (Indonesia); 10 = *S. latimanus* (China); 11 = *S. latimanus* (Filipina[indra30]: Aurora-region 3).



article revised

Benny Pratasik <spjong07@yahoo.com>
To: Gavriloaie Ionel Claudiu

Wed, Jun 19, 2019 at 7:01 AM

Dear Dr. Gavriloaie,
I herewith send you the revised article (attached). All comments have been followed as your suggestions. Method is paraphrased and completed. Appendices are moved to the text. Figure 3 is a series of nucleotide sequence as species identity to show the position of substitutions and consequently the genetic difference. I hope that this version could fulfil your requirements. Last but not least, I would also like to ask the status of Dangeubun's article. She has not received the article back yet and is waiting for your comments in order to improve it. Thank you for your cooperation.

Sincerely Yours,
Silvester Benny Pratasik

Genetic variations of cuttlefish *Sepia latimanus,* Cephalopoda, Sepiidae, in North Sulawesi waters, Indonesia

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Abstract. This study was aimed at discerning the genetic variations of cuttlefish *Sepia latimanus* in North Sulawesi, using CO1 gene marker. Sampling was conducted in Kalinaung and Likupang, North Minahasa regency, Manado Bay, Manado municipality and Minahasa regency, Poigar, South Minahasa regency, and Basaan, Southeast Minahasa. All samples were put into 95% alcohol and brought to the molecular biology laboratory of Faculty of Fisheries and Marine Sciences for further analysis. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198: 5′TAAACTTCAGGGTGACCAAAAAATCA-3′. Polymerase chain reaction (PCR) was carried out in 35 cycles at 95°C (30 sec), 50°C (30 sec), 72°C (50 sec). The PCR product was visualized in 1% (b/v) agarose gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). The chromatogram obtained was edited using Geneious v5.6. The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method and BOLDSystems. The phylogenetic tree was built using Neighbor-Joining Method, and genetic similarity was explained by mutation, haplotype and genetic distance. This study found genetic variations in *S. latimanus* of North Sulawesi waters between and within localities. Haplotype similarity between localities made eleven nucleotide sequences only form 9 haplotypes, while haplotype difference within locality could result from genetic mixture. Wide distribution of the species is supported by its adaptability to the environmental conditions.

Key Words: CO1 gene marker, phylogenetic tree, haplotype, genetic distance.

Introduction. North Sulawesi has many islands surrounded by coral reef ecosystems that provide many different habitats for various marine organisms either as home or shelter of different life stage. Broadclub cuttlefish, *Sepia latimanus*, is one of marine mollusks taking advantages of the coral reef ecosystem as spawning ground, egg placement site, and shelter for the youngsters (Pratasik et al 2017). Species diversity of cuttlefish is very high with more than a hundred species recorded, and their distribution and abundance are highly affected by the availability of suitable habitats and preys, particularly for small individuals (Neves et al 2009; Pratasik et al 2017). Some species live in the water deeper than 400 m, such as *Sepia australis, S. elegans, S. orbignyana* and *S. hieronis*, and some species occupy shallow waters, such as *S. latimanus*, *S. officinalis,* and *S. pharaonis* (Reid et al 2005). Cuttlefish (and cephalopods in general) are known to have diverse body patterns that can immediately naturally change, and controlled by its chromatophore system (Hanlon et al 2009) and well

known as the most intelligent marine biota with the largest body-brain ratio of all invertebrates (Tricarico et al 2014). These have made many studies have focused on the body pattern alteration-related behavioral aspects (Shohet et al 2006; Barbosa et al 2008; Kelman et al 2008; Allen et al 2009; Chiao et al 2010; Barbosa et al 2012).

Indonesian waters so far hold several species of cuttlefish, such as *Sepia bandensis, S. brevimana, S. kiensis, S. latimanus, S. papuensis, S. pharaonis, S. sulcata, S. senta, Sepiella inermis* and *S. weberi* (Reid et al 2005), but previous study in Manado Bay and Lembeh Strait found only four species (Pratasik et 2016). Low interest in cuttlefish study could result from sample collection problems as a result of no cuttlefish fisheries in Indonesia, and all cuttlefish catches are bycatch of other fisheries. No cuttlefish fisheries in Indonesia, especially North Sulawesi, make cuttlefish catch generally come from traditional fishermen using handline or speargun in the coral reef areas. *S. latimanus* is the main cuttlefish catch in the coral reef ecosystem and has highly market value. *S. latimanus* is the second biggest cuttlefish after *S. apama* with maximum dorsal mantle length of 50 cm and total weight of 10 kg (Reid et al 2005). The species often visit coral reefs for spawning and laying eggs (pers. obs.). They often come in pairs and select certain coral crevices to insert their eggs (Pratasik et al 2017). Cuttlefish are, in general, economic fisheries resources, but the resources have unclear fisheries status, insufficient data (Reid et al 2005), and belong to threatened species (IUCN 2014). The present study focuses on genetic variations using DNA sequence of mitochondrial genome as DNA barcode. CO1 is the most common mitochondrial DNA gene marker used for species identification (Folmer et al 1994) due to belonging to the most conservative gene among the mitochondrial DNA protein coding genes. It has become a standard tool of molecular taxonomy and identification (Ratnasingham & Hebert 2007). Molecular techniques have been well applied for stock discrimination studies in fisheries (Murphy et al 2002) and can provide the basis for better management of whole populations and therefore, sustainable fisheries. This study aims to examine the genetic characteristics of broadclub cuttlefish *S. latimanus* in North Sulawesi waters.

Material and Method

This study was carried out for approximately one-year with 5 months of sample collection, from May to September 2018, using SCUBA dives over different habitat types and obtained from local fishermen Sample collection was done in Lembeh Strait (Bitung), Basaan (Southeast Minahasa), Manado Bay (Manado), Likupang waters (North Minahasa), Arakan and Poigar (South Minahasa), North Sulawesi. (Figure 1).

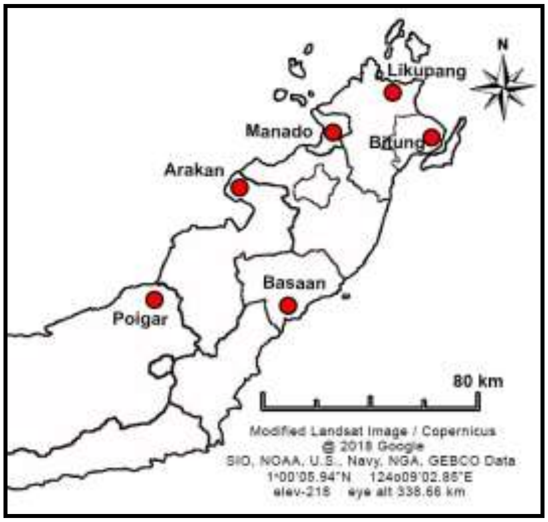


Figure 1. Map of North Sulawesi with sampling points.

The first two sites represent the southern part of North Sulawesi waters, while the second four sites represent the northern part of the island, so that these sites have represented the entire North Sulawesi marine waters. In this study, seven cuttlefish specimens were collected from fishermen's catches and brought to the Biotechnology Laboratory of Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado. For DNA extraction preparation, all samples were preserved in 95% ethanol and kept at room temperature.

Extraction, PCR, and sequencing. Genome DNA was extracted using innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene amplification employed universal primer pairs LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al 1994). Polymerase chain reaction (PCR) was performed in 35 cycles at 95°C (30 sec), 50°C (30 sec), 72°C (50 sec), and the PCR product was visualized in 1% (b/v) agarose gel electrophoresis. To maximize the outcome, a bi-directional sequencing was done using Big Dye© terminator chemistry (Perkin Elmer) through First Base CO (Malaysia).

Data analysis. The chromatogram editing employed Geneious v5.6 (Drummond et al 2012). For species confirmation, BLAST (Basic Local Alignment Search Tools) method was utilized (Altschul et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007), and then the sequences were compared between localities and with the GenBank data. The phylogenetic tree was built using Neighbor-Joining Method of MEGA X and the heterogeneity index was also estimated (Kumar et al 2018). The analysis of nucleotide base sequence and haplotype variations between *S. latimanus* from different localities used DnaSP Ver.6.12.03 computer program (Rozas et al 2017).

Results and Discussion. During the study, 7 individuals of *S. latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not taken, so that all samples were obtained from fishermen's catches. Sampling in Poigar, South Minahasa, using beach seine and SCUBA dives, did not obtain any sample as well. This condition reveals that sufficient sampling technique is required for cuttlefish studies. In the past, cuttlefish fishing, such as *S. officinalis* (English Channel) and *S. esculanta* (Japan), used trawl, set net, gill net, trammel net and trap with spawning substrate (Pawson 1995; Arkley et al 1996) by taking advantage of their migration

Table 1

Heterogeneity index of cuttlefish *Sepia latimanus* in North Sulawesi waters.

No		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	1542916 *Sepia latimanus* (Bitung)										
2	1626051 *Sepia latimanus* (Likupang 1)	0.00435									
3	1626052 *Sepia latimanus* (Likupang 2)	0.00309	0.00371								
4	1626057 *Sepia latimanus* (Manado)	0.06984	0.06983	0.07071							
5	1733049 *Sepia latimanus* (Arakan)	0.00000	0.00435	0.00309	0.06984						
6	3369262 *Sepia latimanus* (Basaan 1)	0.00435	0.00123	0.00247	0.06820	0.00435					
7	3369264 *Sepia latimanus* (Basaan 2)	0.00435	0.00000	0.00371	0.06983	0.00435	0.00123				
8	AB192338.1 *Sepia latimanus* (Japan: Okinawa)	0.05800	0.05627	0.05704	0.06232	0.05800	0.05549	0.05627			
9	AB430406.1 *Sepia latimanus* (Indonesia)	0.05555	0.05384	0.05461	0.06152	0.05555	0.05307	0.05384	0.00434		
10	AY185506.1 *Sepia latimanus* (China)	0.07853	0.07747	0.07747	0.07531	0.07853	0.07581	0.07747	0.07725	0.07739	
11	KF009663.1 *Sepia latimanus* (Philippines: Aurora Region 3)	0.05555	0.05384	0.05461	0.05898	0.05555	0.05307	0.05384	0.00309	0.00247	0.07656

to the spawning areas. In North Sulawesi, *S. latimanus* is generally fished using speargun or jig fishing in the coral reef ecosystem (Pratasik et al 2015). Obtaining sufficient number of samples have become constraint in cuttlefish study, particularly in North Sulawesi, due to no cuttlefish fisheries and unclear distribution of the species, so that this study collected only few samples.

Table 1 demonstrates 4 specimens collected from North Sulawesi waters and 4 others are NCBI data from the genbank (Indonesia, Japan, Philippines of Aurora-region 3) used as comparison, in which the genetic heterogeneity gets bigger with geographic distance, except the specimen of Manado Bay. This condition is supported with group positions, in which the cuttlefish collected in North Sulawesi waters, but Manado, are in the same group, while 4 NCBI samples are in another separate group (Figure 2). This finding reconfirms that geographic distance induces the genetic diversity of *S. latimanus*. However, *S. latimanus* of North Sulawesi has revealed that there are genetic variations and similarities among the same or different locations. *S. latimanus* sample from Bitung waters has genetic similarity to that from Arakan waters. Also, one sample of *S. latimanus* from Likupang waters has genetic similarity to that of Basaan waters. On the other hand, both *S. latimanus* samples from Basaan waters are genetically different as happening for both samples from Likupang waters (Figure 2).

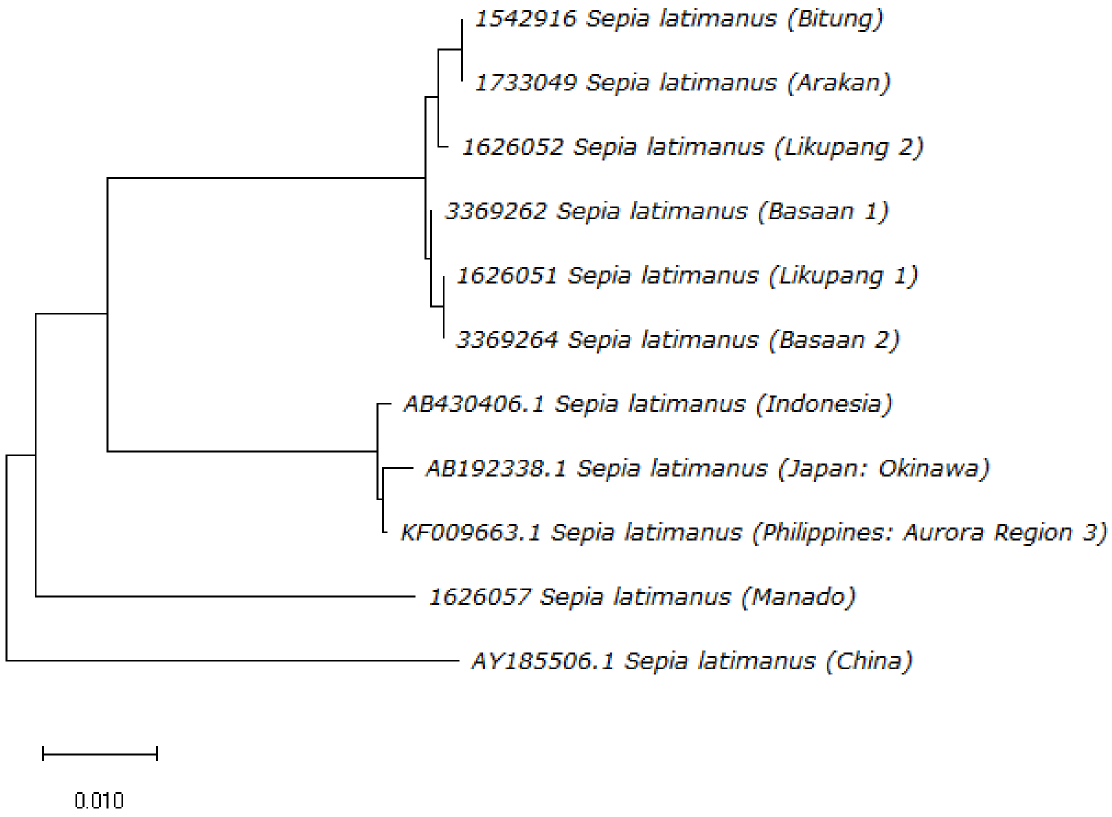
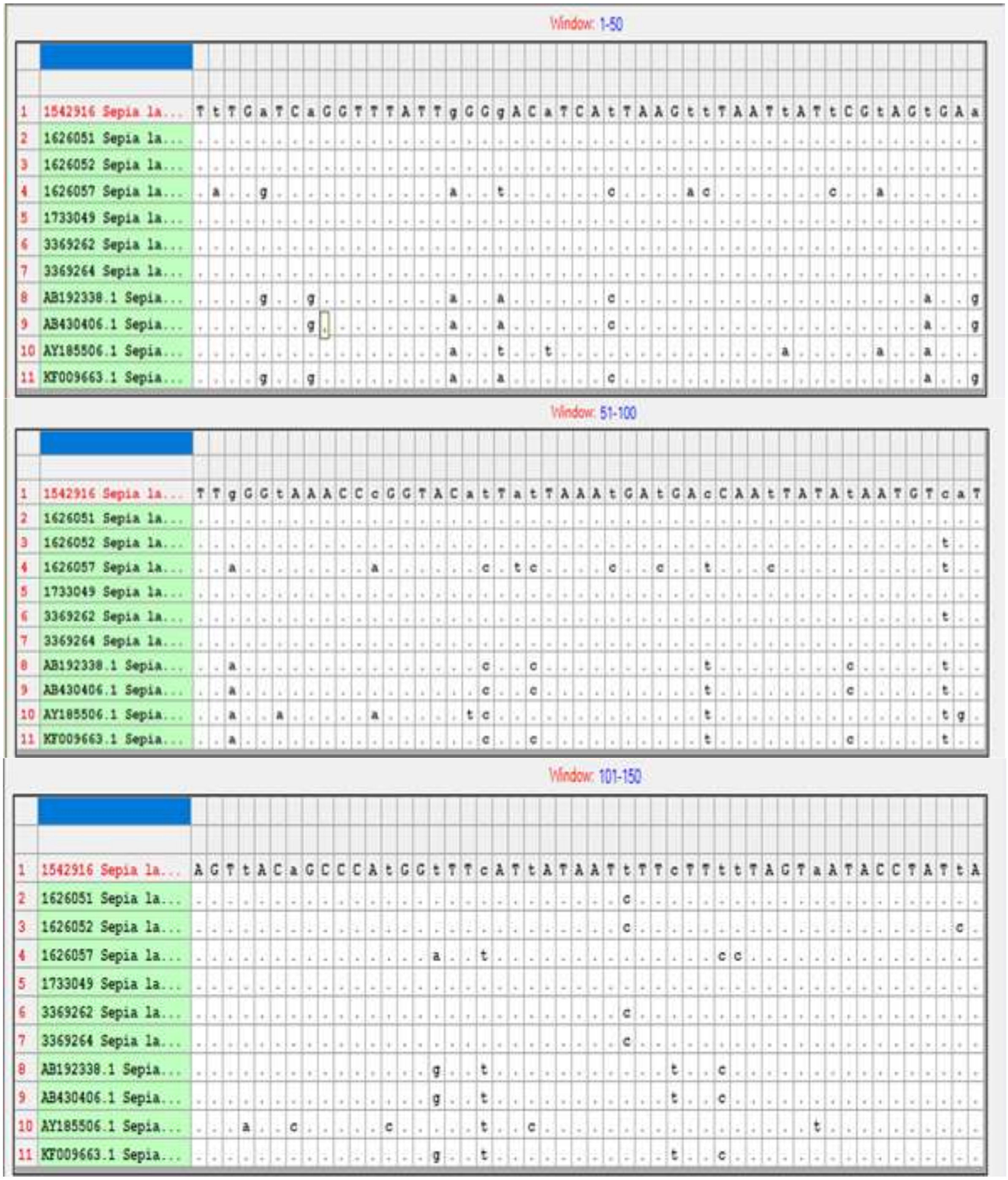


Figure 2. Phylogenetic tree of North Sulawesi *S. latimanus*.

From total numbers of 620 bp, there were 166 polymorphic sites with 186 mutations. Based on variable sites (two variants), there were 73 singleton variable sites with 67 different site positions and 93 parsimony informative sites with 80 site positions. The former was at positions: 2 23 32 33 38 41 56 68 71 77 80 87 99 104 107 113 122 135 140 149

173 179 182 203 212 215 239 254 258 260 263 275 278 279 280 282 293 299 302 332 338 341 347 350 353 371 377 378 384 404 407 410 422 428 431 473 491 497 516 525 526 545 569 587 593 611 618, and the latter at positions 5 8 17 27 44 47 50 53 62 69 72 83 92 98 119 128 131 134 155 158 161 170 177 189 218 224 233 243 251 261 264 266 267 269 272 290 296 317 320 329 336 344 356 359 363 368 374 380 392 393 413 416 435 437 443 452 456 461 470 476 477 485 509 513 515 519 533 539 548 551 566 572 575 578 584 596 602 605 608 617. The variable site positions are obviously shown in the entire nucleotide sequence of *S. latimanus* from both North Sulawesi waters and NCBI data (Figure 3).



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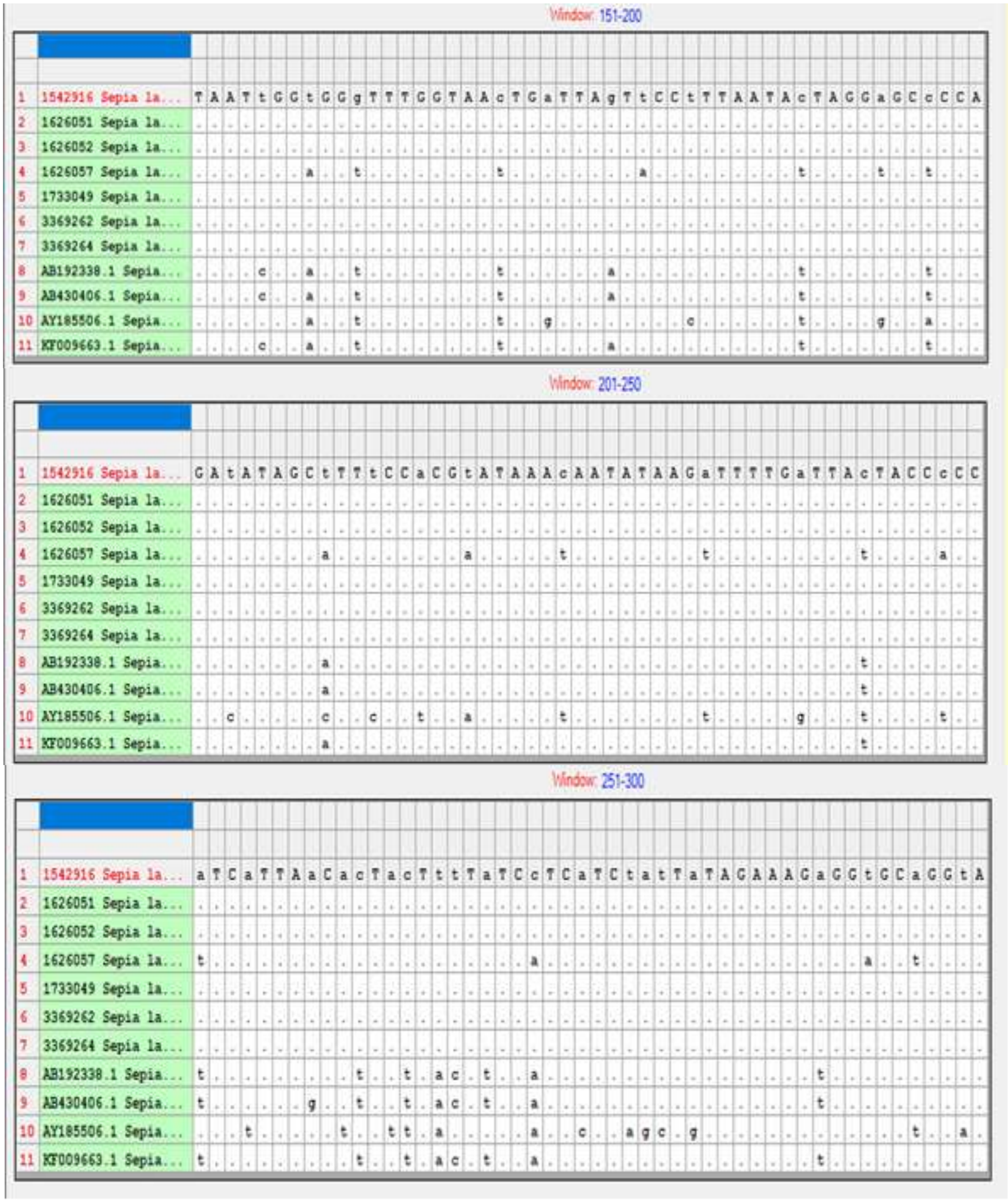
Window: 1-50
1  1542916 Sepia la... TtTGaTCaGGTTTATTgGGgACaTCAtTAAGttTAATtATtCGtAGtGAa
2  1626051 Sepia la... ..................................................
3  1626052 Sepia la... ..................................................
4  1626057 Sepia la... .a..g...........a..t......c....ac.......c..a......
5  1733049 Sepia la... ..................................................
6  3369262 Sepia la... ..................................................
7  3369264 Sepia la... ..................................................
8  AB192338.1 Sepia... ....g..g........a..a......c...................a..g
9  AB430406.1 Sepia... .......g........a..a......c...................a..g
10 AY185506.1 Sepia... ................a..t..t..............a.....a..a...
11 KF009663.1 Sepia... ....g..g........a..a......c...................a..g

Window: 51-100
1  1542916 Sepia la... TTgGGtAAACCcGGTACatTatTAAAtGAtGAcCAAtTATAtAATGTcaT
2  1626051 Sepia la... ..................................................
3  1626052 Sepia la... ...............................................t..
4  1626057 Sepia la... ..a........a......c.tc....c..c..t...c..........t..
5  1733049 Sepia la... ..................................................
6  3369262 Sepia la... ...............................................t..
7  3369264 Sepia la... ..................................................
8  AB192338.1 Sepia... ..a...............c..c..........t........c.....t..
9  AB430406.1 Sepia... ..a...............c..c..........t........c.....t..
10 AY185506.1 Sepia... ..a..a.....a.....tc.............t..............tg.
11 KF009663.1 Sepia... ..a...............c..c..........t........c.....t..

Window: 101-150
1  1542916 Sepia la... AGTtACaGCCCAtGGtTTcATtATAATtTTcTTttTAGTaATACCTATtA
2  1626051 Sepia la... ...........................c......................
3  1626052 Sepia la... ...........................c....................c.
4  1626057 Sepia la... ...............a..t..............cc...............
5  1733049 Sepia la... ..................................................
6  3369262 Sepia la... ...........................c......................
7  3369264 Sepia la... ...........................c......................
8  AB192338.1 Sepia... ...............g..t...........t..c................
9  AB430406.1 Sepia... ...............g..t...........t..c................
10 AY185506.1 Sepia... ...a..c.....c.....t..c.................t..........
11 KF009663.1 Sepia... ...............g..t...........t..c................
```



Window: 151-200

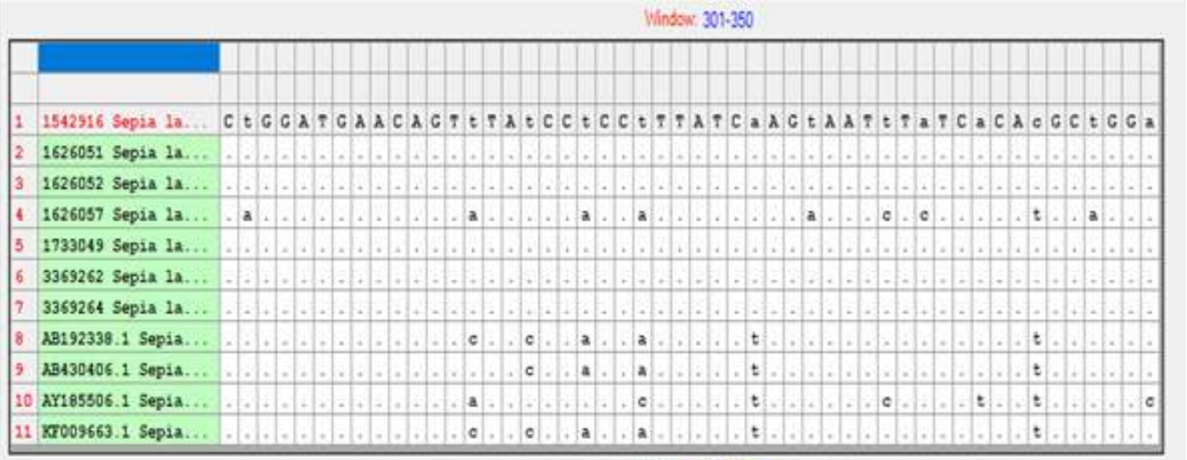
1	1542916 Sepia la...	TAATtGGtGGgTTTGGTAAcTGaTTAgTtCCtTTAATAcTAGGaGCcCCA
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	.......a..t........t........a.........t....t..t...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	....c..a..t........t......a...........t.......t...
9	AB430406.1 Sepia...	....c..a..t........t......a...........t.......t...
10	AY185506.1 Sepia...	.......a..t........t..g........c......t....g..a...
11	KF009663.1 Sepia...	....c..a..t........t......a...........t.......t...

Window: 201-250

1	1542916 Sepia la...	GAtATAGCtTTtCCaCGtATAAAcAATATAAGaTTTTGaTTAcTACCcCC
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	........a........a.....t........t.........t....a..
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	........a.................................t.......
9	AB430406.1 Sepia...	........a.................................t.......
10	AY185506.1 Sepia...	..c.....c..c..t..a.....t........t.....g...t....t..
11	KF009663.1 Sepia...	........a.................................t.......

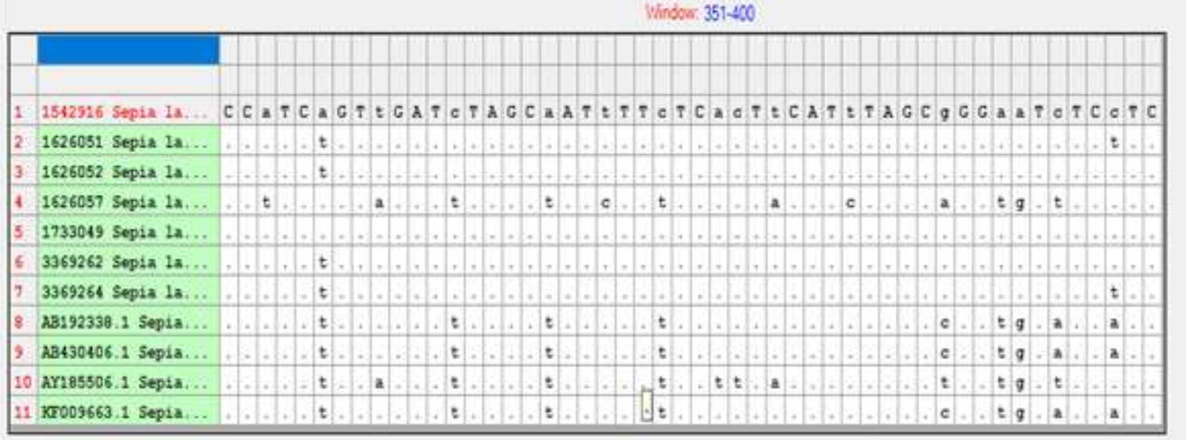
Window: 251-300

1	1542916 Sepia la...	aTCaTTAaCacTacTttTaTCcTCaTCtatTaTAGAAAGaGGtGCaGGtA
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	t....................a....................a..t....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	t.........t..t.act...a.................t..........
9	AB430406.1 Sepia...	t......g..t..t.act...a.................t..........
10	AY185506.1 Sepia...	...t.....t..tt.a.....a..c..agc.g.............t..a.
11	KF009663.1 Sepia...	t.........t..t.act...a.................t..........



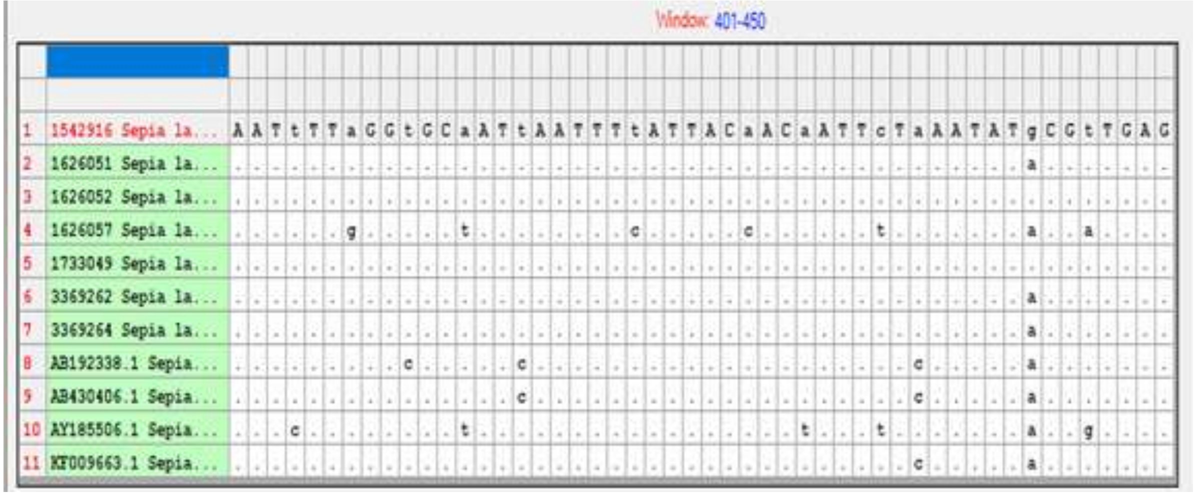
Window: 301-350

1	1542916 Sepia la...	CtGGATGAACAGTtTAtCCtCCtTTATCaAGtAATtTaTCaCAcGCtGGa
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	.a...........a.....a..a........a...c.c.....t..a...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	.............c..c..a..a.....t..............t......
9	AB430406.1 Sepia...	................c..a..a.....t..............t......
10	AY185506.1 Sepia...	.............a........c.....t......c....t..t.....c
11	KF009663.1 Sepia...	.............c..c..a..a.....t..............t......



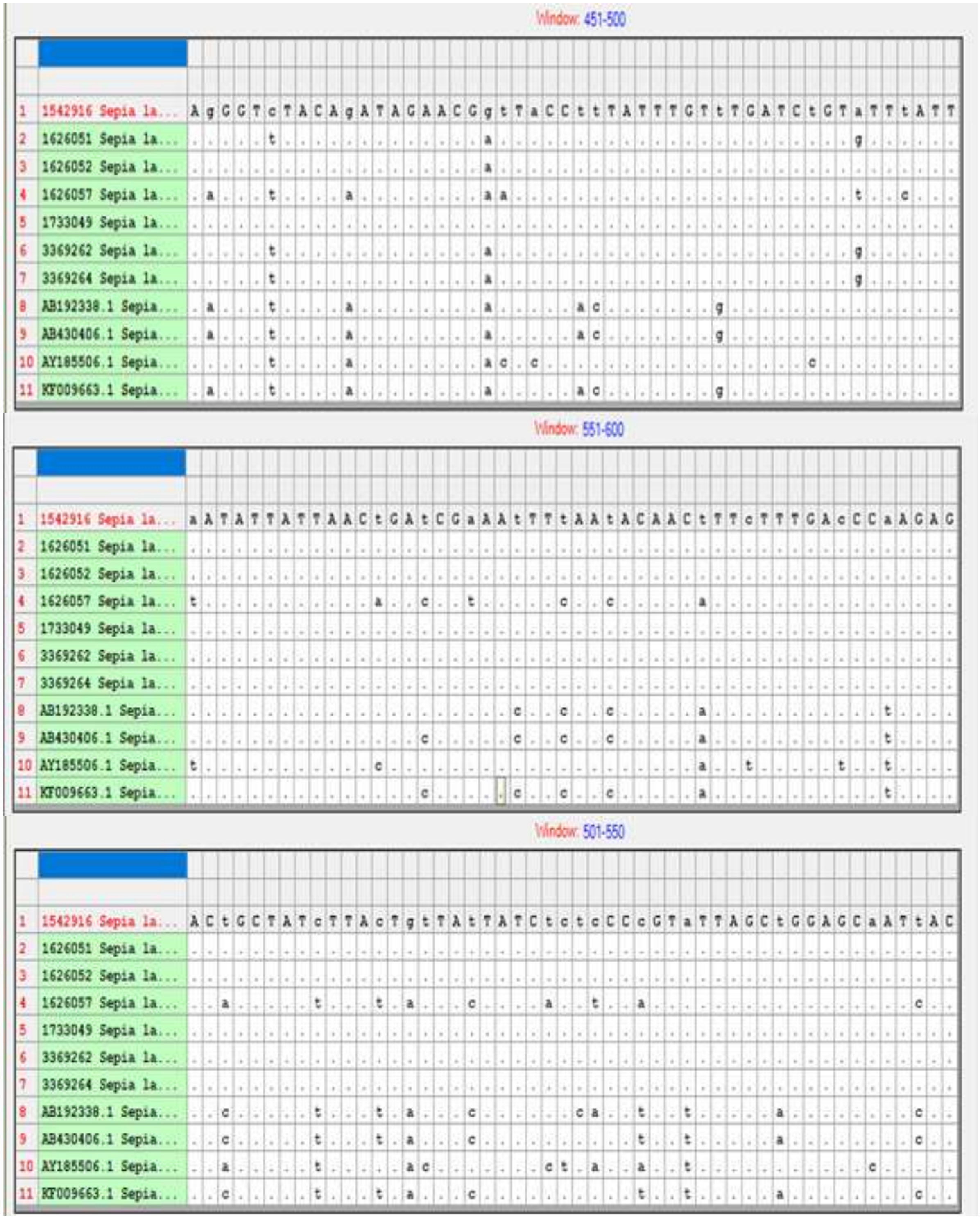
Window: 351-400

1	1542916 Sepia la...	CCaTCaGTtGATcTAGCaATtTTcTCacTtCATtTAGCgGGaaTcTCcTC
2	1626051 Sepia la...	.....t...t..
3	1626052 Sepia la...	.....t..
4	1626057 Sepia la...	..t.....a...t....t..c..t.....a...c....a..tg.t.....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	.....t..
7	3369264 Sepia la...	.....t...t..
8	AB192338.1 Sepia...	.....t......t....t.....t..............c..tg.a..a..
9	AB430406.1 Sepia...	.....t......t....t.....t..............c..tg.a..a..
10	AY185506.1 Sepia...	.....t..a...t....t.....t..tt.a........t..tg.t.....
11	KF009663.1 Sepia...	.....t......t....t.....t..............c..tg.a..a..



Window: 401-450

1	1542916 Sepia la...	AATtTTaGGtGCaATtAATTTtATTACaACaATTcTaAATATgCGtTGAG
2	1626051 Sepia la...	....a.......
3	1626052 Sepia la...	....
4	1626057 Sepia la...	......g.....t........c.....c......t.......a..a....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....a.......
7	3369264 Sepia la...	....a.......
8	AB192338.1 Sepia...	.........c.....c....................c.....a.......
9	AB430406.1 Sepia...	...............c....................c.....a.......
10	AY185506.1 Sepia...	...c........t.................t...t.......a..g....
11	KF009663.1 Sepia...	....................................c.....a.......



Window: 451-500

1	1542916 Sepia la...	A	g	G	G	T	c	T	A	C	A	g	A	T	A	G	A	A	C	G	g	t	T	a	C	C	t	t	T	A	T	T	T	G	T	t	T	G	A	T	C	t	G	T	a	T	T	t	A	T	T
2	1626051 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	c	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	c	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	c	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.
11	KF009663.1 Sepia...	.	a	.	.	.	t	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	c	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Window: 551-600

1	1542916 Sepia la...	a	A	T	A	T	T	A	T	T	A	A	C	t	G	A	t	C	G	a	A	A	t	T	T	t	A	A	t	A	C	A	A	C	t	T	T	c	T	T	T	G	A	c	C	C	a	A	G	A	G
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	t	.	.	.	.	.	.	.	.	.	.	.	a	.	.	c	.	.	t	.	.	.	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.
10	AY185506.1 Sepia...	t	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	t	.	.	t	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	c	.	.	c	.	.	c	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.

Window: 501-550

1	1542916 Sepia la...	A	C	t	G	C	T	A	T	c	T	T	A	c	T	g	t	T	A	t	T	A	T	C	t	c	t	c	C	C	c	G	T	a	T	T	A	G	C	t	G	G	A	G	C	a	A	T	t	A	C
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	a	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	a	.	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	c	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	.	.	c	a	.	.	t	.	.	t	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.
9	AB430406.1 Sepia...	.	.	c	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	t	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.
10	AY185506.1 Sepia...	.	.	a	.	.	.	.	.	t	.	.	.	.	.	a	c	.	.	.	.	.	.	.	c	t	.	a	.	.	a	.	.	t	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	c	.	.	.	.	.	t	.	.	.	t	.	a	.	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	t	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.

Notes: 1 = *Sepia latimanus* (Bitung); 2 = *S. latimanus* (Likupang 1); 3 = *S. latimanus* (Likupang 2);4 = *S. latimanus* (Manado); 5 = *S. latimanus* (Arakan); 6 = *S. latimanus* (Basaan 1); 7 = *S. latimanus* (Basaan 2); 8 = *S. latimanus* (Japan: Okinawa); 9 = *S. latimanus* (Indonesia); 10 = *S. latimanus* (China); 11 = *S. latimanus* (Philippine: Aurora-region 3).

Figure 3. Nucleotide Sequence of *S. latimanus* in North Sulawesi waters.

Nucleotide base substitutions occur in the variable sites, and make the specimens of *S. latimanus* reflect the genetic similarity and difference. Therefore, 11 sequences used in the present study only formed 9 haplotypes with haplotype diversity (Hd) of 0.9636, haplotype 1 (Bitung and Arakan), 2 (Likupang-1 and Basaan-2), 3 (Likupang-2), 4 (Manado), 5 (Basaan-1), 6 (Japan: Okinawa), 7 (Indonesia), 8 (China), and 9 (Philippines: Aurora-region 3). Different haplotypes of the same localities show that the collected individuals have heteroplasmic-typed mtDNA. It could be seen from individuals from Basaan-1 and Basaan-2 and Likupang-1 and Likupang-2 form separate haplotypes despite existing in the same locality.

It indicates that *S. latimanus* in North Sulawesi waters has intraspecific population mixture that enables to find different genetic characters at the same locality or same genetic characters at different localities. This condition is also supported by FAO records (Reid et al 2005) that very wide distribution of *S. latimanus* could be a reasonable factor for the population or genetic mixture. This species lives in Indo-Pacific and distributes south Mozambik, entire Indian ocean, Malacca strait, Melanesia islands, south China Sea, Philippine Sea, and east China Sea, Taipei province and Japan to south Kyushu, Indonesia to northwest and northeast Australia, Palau, Guam, New Caledonia, Fiji and Cocos Island (Reid et al 2005).

Genetic mixture, as indicated with haplotype similarity between Likupang 1 and Basaan 2 and between Bitung and Arakan, could be influenced by the ability of *S. latimanus* to move from one place to another. Previous studies have indicated that cuttlefish can inhabit different coastal habitats in coral reef ecosystem (Reid et al 2005). Habitats are the resources and conditions present in an area to support survival and reproductive success (Leopold 1933). Cuttlefish are well known as the chameleon of the sea (Hanlon et al 2009) with well-evolved nervous systems enable to search for extensive trophic niches and migrate to exploit different production systems and prey population, and therefore, they can do long distant migration. Our field observations found that juvenile *S. latimanus* occurred under the seagrass, and near branching corals and sponge. Other evidence also shows that fishermen often take advantage of low tide period to catch cuttlefish in seagrass ecosystem, at least in Likupang waters (*field obs*).

S. latimanus from Manado Bay is also in separate group even though its geographic position occurs between Likupang waters and Arakan waters (Figure 2). It could result from this individual belongs to species complex, a group of closely related organisms that look very similar to the point that the boundaries between them are often unclear. Based on genetic distance, *S. latimanus* from Manado Bay is close to that from Philippines waters (Table 1). Manado bay is the northern part of North Sulawesi with series of coral reef-surrounded small islands northwards to southern Philippines. This area belongs to Coral Triangle area. Closer genetic distance of *S. latimanus* between Manado bay and Philippines Sea than the same species from other Indonesian waters could result from closer geographic distance between Manado bay to Philippines than other location in Indonesian waters where the sample was collected. This finding is supported by previous studies that increasing geographic distance between patches was associated with increasing genetic distance, while increasing habitat continuity between patches was associated with decreasing genetic distance (Alberto et al 2010). Isolation by distance and isolation by environment are important mechanisms in driving the genetic population structure (Fernandez et al 2017). Isolation by distance could be used to predict an increase in genetic differentiation with geographic distance (Ramachandran et al 2005), but genetic structure through time is stable (Pascual et al 2016).

Ocean currents are also an important factor affecting the distribution of marine animals and plants (Hays, 2017). The dynamics of the ocean are highly variable and are driven by multiple forcing factors such as solar insolation, winds, tides and freshwater input, and this variability influences the development of distinct water masses of different hydrographic properties in both vertical and horizontal dimensions (McManus et al 2011). All these are related with food chain and habitat distribution that impact the availability of living needs of marine animal's dispersal. Therefore, physical structures and processes are directly related to the swimming and behavioral capabilities of an individual or species, such as *S. latimanus*.

Conclusions. *Sepia latimanus* of North Sulawesi waters had sufficient genetic variations. These occur between and within localities. Genetic similarity was also present between *S. latimanus* from different localities, such as Basaan-2 and Likupang-1 samples and Bitung and Arakan samples. Eleven nucleotide sequences analyzed formed only 9 haplotypes due to the haplotype similarity between localities, and this condition has reflected genetic mixture. Moreover, *S. latimanus* sample of Manado bay had closer kindship to that of NCBI data for Philippines waters. This condition is supported by habitat and food distribution, ocean currents, and the ability of *S. latimanus* to exploit different niches through behavioral adaptation to different environmental condition.

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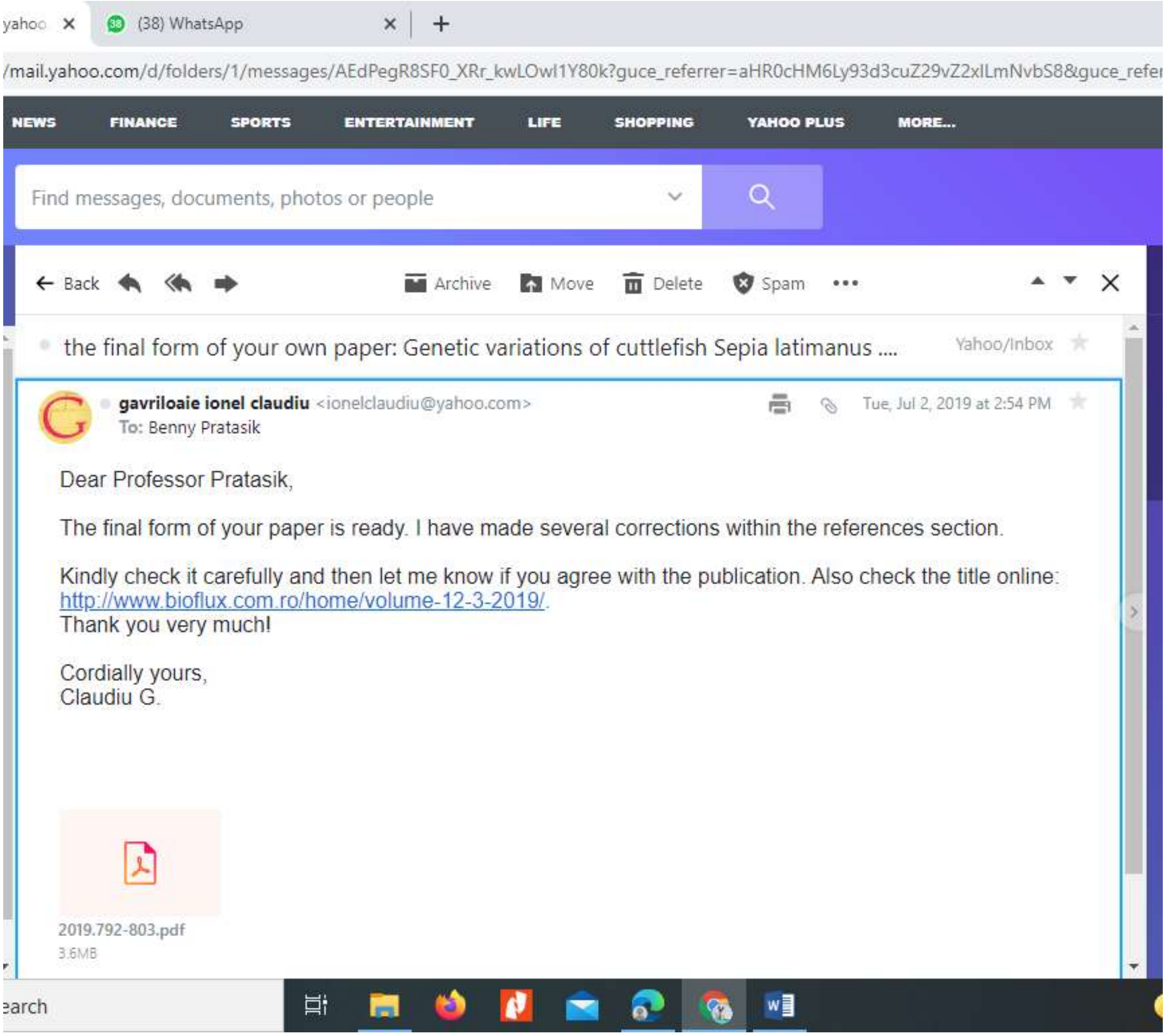

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Genetic variations of cuttlefish *Sepia latimanus* (Cephalopoda, Sepiidae) in North Sulawesi waters, Indonesia

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Abstract. This study was aimed at discerning the genetic variations of cuttlefish *Sepia latimanus* in North Sulawesi, using CO1 gene marker. Sampling was conducted in Kalinaung and Likupang, North Minahasa regency, Manado Bay, Manado municipality and Minahasa regency, Poigar, South Minahasa regency, and Basaan, Southeast Minahasa. All samples were put into 95% alcohol and brought to the molecular biology laboratory of Faculty of Fisheries and Marine Sciences for further analysis. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5’-GGTCAACAAATCATAAAGATATTGG-3’ and HCO2198: 5’TAAACTTCAGGGTGACCAAAAAATCA-3’. Polymerase chain reaction (PCR) was carried out in 35 cycles at 95°C (30 sec), 50°C (30 sec), 72°C (50 sec). The PCR product was visualized in 1% (b/v) agarosa gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). The chromatogram obtained was edited using Geneious v5.6. The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method and BOLDSystems. The phylogenetic tree was built using Neighbor-Joining Method, and genetic similarity was explained by mutation, haplotype and genetic distance. This study found genetic variations in *S. latimanus* of North Sulawesi waters between and within localities. Haplotype similarity between localities made eleven nucleotide sequences only form 9 haplotypes, while haplotype difference within locality could result from genetic mixture. Wide distribution of the species is supported by its adaptability to the environmental conditions.

Key Words: CO1 gene marker, phylogenetic tree, haplotype, genetic distance.

Introduction. North Sulawesi has many islands surrounded by coral reef ecosystems that provide many different habitats for various marine organisms either as home or shelter of different life stage. Broadclub cuttlefish, *Sepia latimanus*, is one of marine mollusks taking advantages of the coral reef ecosystem as spawning ground, egg placement site, and shelter for the youngsters (Pratasik et al 2017). Species diversity of cuttlefish is very high with more than a hundred species recorded, and their distribution and abundance are highly affected by the availability of suitable habitats and preys, particularly for small individuals (Neves et al 2009; Pratasik et al 2017). Some species live in the water deeper than 400 m, such as *Sepia australis, S. elegans, S. orbignyana* and *S. hieronis*, and some species occupy shallow waters, such as *S. latimanus*, *S. officinalis,* and *S. pharaonis* (Reid et al 2005). Cuttlefish (and cephalopods in general) are known to have diverse body patterns that can immediately naturally change, and controlled by its chromatophore system (Hanlon et al 2009) and well

known as the most intelligent marine biota with the largest body-brain ratio of all invertebrates (Tricarico et al 2014). These have made many studies have focused on the body pattern alteration-related behavioral aspects (Shohet et al 2006; Barbosa et al 2008; Kelman et al 2008; Allen et al 2009; Chiao et al 2010; Barbosa et al 2011).

Indonesian waters so far hold several species of cuttlefish, such as *Sepia bandensis, S. brevimana, S. kiensis, S. latimanus, S. papuensis, S. pharaonis, S. sulcata, S. senta, Sepiella inermis* and *S. weberi* (Reid et al 2005), but previous study in Manado Bay and Lembeh Strait found only four species (Pratasik et 2016). Low interest in cuttlefish study could result from sample collection problems as a result of no cuttlefish fisheries in Indonesia, and all cuttlefish catches are bycatch of other fisheries. No cuttlefish fisheries in Indonesia, especially North Sulawesi, make cuttlefish catch generally come from traditional fishermen using handline or speargun in the coral reef areas. *S. latimanus* is the main cuttlefish catch in the coral reef ecosystem and has highly market value. *S. latimanus* is the second biggest cuttlefish after *S. apama* with maximum dorsal mantle length of 50 cm and total weight of 10 kg (Reid et al 2005). The species often visit coral reefs for spawning and laying eggs (pers. obs.). They often come in pairs and select certain coral crevices to insert their eggs (Pratasik et al 2017). Cuttlefish are, in general, economic fisheries resources, but the resources have unclear fisheries status, insufficient data (Reid et al 2005), and belong to threatened species (IUCN 2014). The present study focuses on genetic variations using DNA sequence of mitochondrial genome as DNA barcode. CO1 is the most common mitochondrial DNA gene marker used for species identification (Folmer et al 1994) due to belonging to the most conservative gene among the mitochondrial DNA protein coding genes. It has become a standard tool of molecular taxonomy and identification (Ratnasingham & Hebert 2007). Molecular techniques have been well applied for stock discrimination studies in fisheries (Murphy et al 2002) and can provide the basis for better management of whole populations and therefore, sustainable fisheries. This study aims to examine the genetic characteristics of broadclub cuttlefish *S. latimanus* in North Sulawesi waters.

Material and Method. This study was carried out for approximately one-year with 5 months of sample collection, from May to September 2018, using SCUBA dives over different habitat types and obtained from local fishermen. Sample collection was done in Lembeh Strait (Bitung), Basaan (Southeast Minahasa), Manado Bay (Manado), Likupang waters (North Minahasa), Arakan and Poigar (South Minahasa), North Sulawesi (Figure 1).

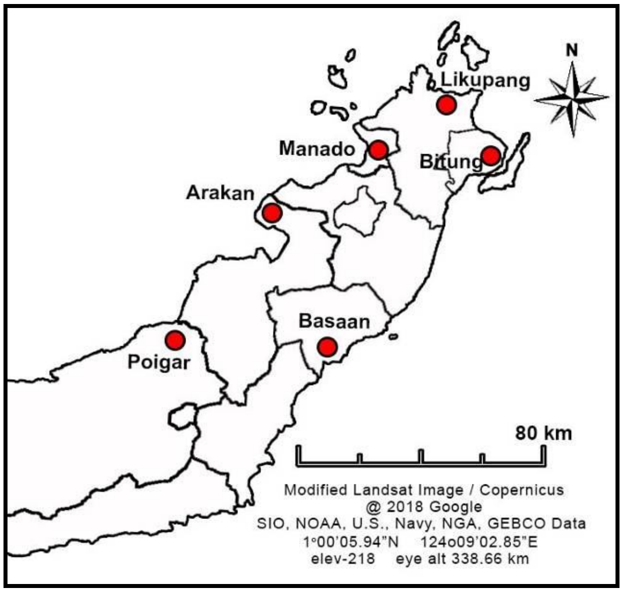


Figure 1. Map of North Sulawesi with sampling points (marked red).

The first two sites represent the southern part of North Sulawesi waters, while the second four sites represent the northern part of the island, so that these sites have represented the entire North Sulawesi marine waters. In this study, seven cuttlefish specimens were collected from fishermen's catches and brought to the Biotechnology Laboratory of Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado. For DNA extraction preparation, all samples were preserved in 95% ethanol and kept at room temperature.

Extraction, PCR, and sequencing. Genome DNA was extracted using innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene amplification employed universal primer pairs LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al 1994). Polymerase chain reaction (PCR) was performed in 35 cycles at 95^{o}C (30 sec), 50^{o}C (30 sec), 72^{o}C (50 sec), and the PCR product was visualized in 1% (b/v) agarose gel electrophoresis. To maximize the outcome, a bi-directional sequencing was done using Big Dye© terminator chemistry (Perkin Elmer) through First Base CO (Malaysia).

Data analysis. The chromatogram editing employed Geneious v5.6 (Drummond et al 2012). For species confirmation, BLAST (Basic Local Alignment Search Tools) method was utilized (Altschul et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007), and then the sequences were compared between localities and with the GenBank data. The phylogenetic tree was built using Neighbor-Joining Method of MEGA X and the heterogeneity index was also estimated (Kumar et al 2018). The analysis of nucleotide base sequence and haplotype variations between *S. latimanus* from different localities used DnaSP Ver.6.12.03 computer program (Rozas et al 2017).

Results and Discussion. During the study, 7 individuals of *S. latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not taken, so that all samples were obtained from fishermen's catches. Sampling in Poigar, South Minahasa, using beach seine and SCUBA dives, did not obtain any sample as well. This condition reveals that sufficient sampling technique is required for cuttlefish studies. In the past, cuttlefish fishing, such as *S. officinalis* (English Channel) and *S. esculanta* (Japan), used trawl, set net, gill net, trammel net and trap with spawning substrate (Pawson 1995; Arkley et al 1996) by taking advantage of their migration to the spawning areas. In North Sulawesi, *S. latimanus* is generally fished using speargun or jig fishing in the coral reef ecosystem (Pratasik et al 2015). Obtaining sufficient number of samples have become constraint in cuttlefish study, particularly in North Sulawesi, due to no cuttlefish fisheries and unclear distribution of the species, so that this study collected only few samples.

Table 1 demonstrates 4 specimens collected from North Sulawesi waters and 4 others are NCBI data from the genbank (Indonesia, Japan, Philippines of Aurora-region 3) used as comparison, in which the genetic heterogeneity gets bigger with geographic distance, except the specimen of Manado Bay. This condition is supported with group positions, in which the cuttlefish collected in North Sulawesi waters, but Manado, are in the same group, while 4 NCBI samples are in another separate group (Figure 2). This finding reconfirms that geographic distance induces the genetic diversity of *S. latimanus*. However, *S. latimanus* of North Sulawesi has revealed that there are genetic variations and similarities among the same or different locations. *S. latimanus* sample from Bitung waters has genetic similarity to that from Arakan waters. Also, one sample of *S. latimanus* from Likupang waters has genetic similarity to that of Basaan waters. On the other hand, both *S. latimanus* samples from Basaan waters are genetically different as happening for both samples from Likupang waters (Figure 2).

Table 1

Genetic distance of cuttlefish *Sepia latimanus* in North Sulawesi waters

No		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	1542916 *Sepia latimanus* (Bitung)										
2	1626051 *Sepia latimanus* (Likupang 1)	0.00435									
3	1626052 *Sepia latimanus* (Likupang 2)	0.00309	0.00371								
4	1626057 *Sepia latimanus* (Manado)	0.06984	0.06983	0.07071							
5	1733049 *Sepia latimanus* (Arakan)	0.00000	0.00435	0.00309	0.06984						
6	3369262 *Sepia latimanus* (Basaan 1)	0.00435	0.00123	0.00247	0.06820	0.00435					
7	3369264 *Sepia latimanus* (Basaan 2)	0.00435	0.00000	0.00371	0.06983	0.00435	0.00123				
8	AB192338.1 *Sepia latimanus* (Japan: Okinawa)	0.05800	0.05627	0.05704	0.06232	0.05800	0.05549	0.05627			
9	AB430406.1 *Sepia latimanus* (Indonesia)	0.05555	0.05384	0.05461	0.06152	0.05555	0.05307	0.05384	0.00434		
10	AY185506.1 *Sepia latimanus* (China)	0.07853	0.07747	0.07747	0.07531	0.07853	0.07581	0.07747	0.07725	0.07739	
11	KF009663.1 *Sepia latimanus* (Philippines: Aurora Region 3)	0.05555	0.05384	0.05461	0.05898	0.05555	0.05307	0.05384	0.00309	0.00247	0.07656

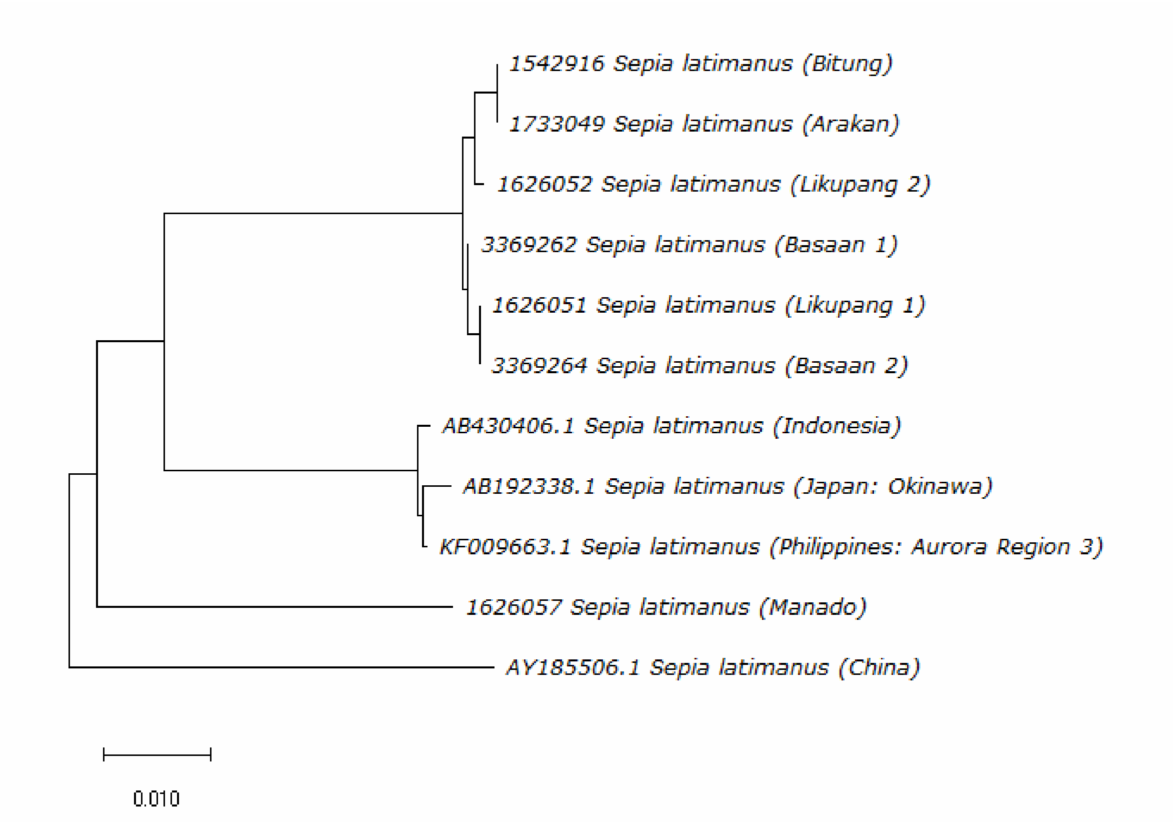
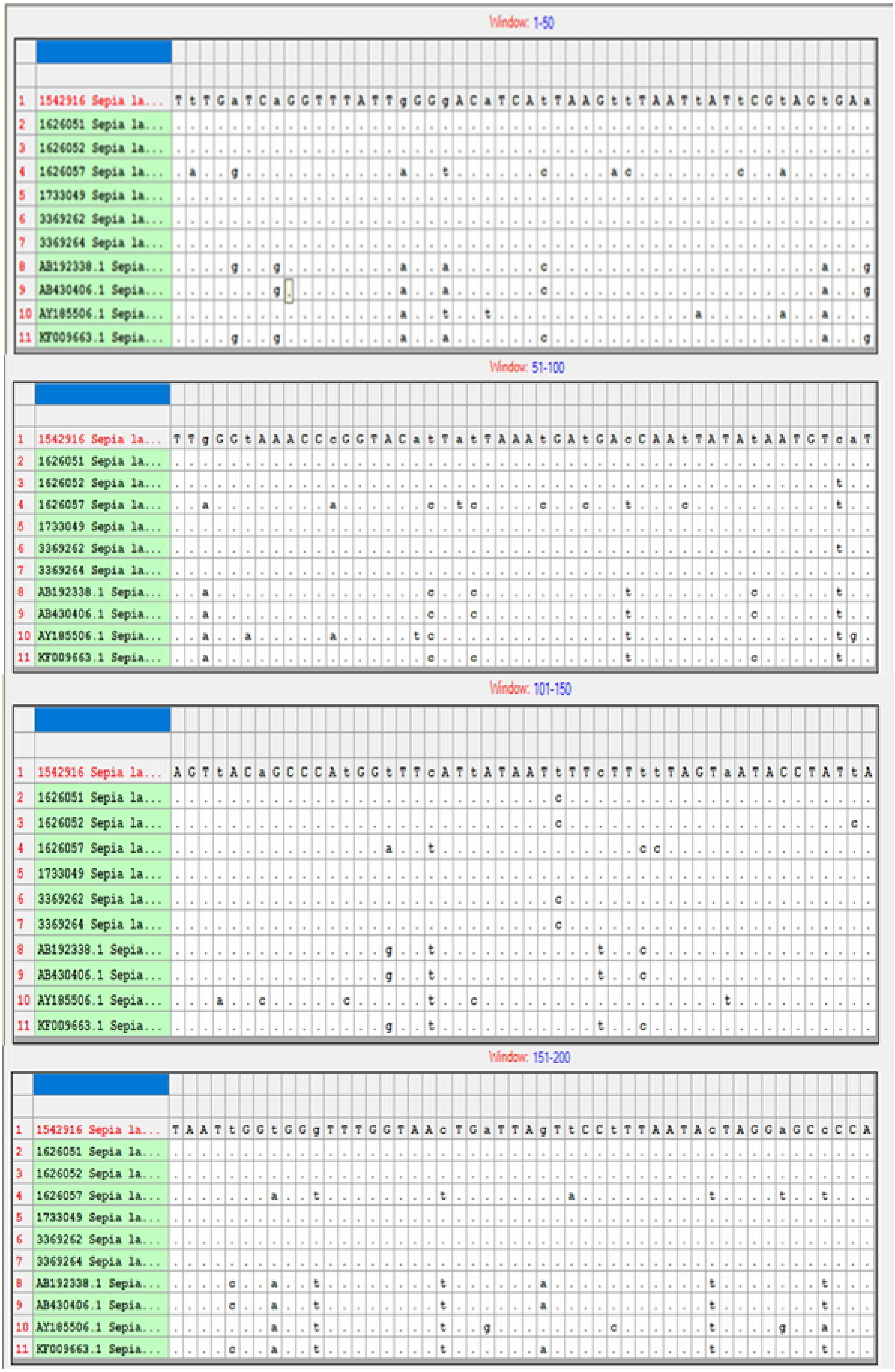


Figure 2. Phylogenetic tree of North Sulawesi *S. latimanus*.

From total numbers of 620 bp, there were 166 polymorphic sites with 186 mutations. Based on variable sites (two variants), there were 73 singleton variable sites with 67 different site positions and 93 parsimony informative sites with 80 site positions. The former was at positions: 2 23 32 33 38 41 56 68 71 77 80 87 99 104 107 113 122 135 140 149 173 179 182 203 212 215 239 254 258 260 263 275 278 279 280 282 293 299 302 332 338 341 347 350 353 371 377 378 384 404 407 410 422 428 431 473 491 497 516 525 526 545 569 587 593 611 618, and the latter at positions 5 8 17 27 44 47 50 53 62 69 72 83 92 98 119 128 131 134 155 158 161 170 177 189 218 224 233 243 251 261 264 266 267 269 272 290 296 317 320 329 336 344 356 359 363 368 374 380 392 393 413 416 435 437 443 452 456 461 470 476 477 485 509 513 515 519 533 539 548 551 566 572 575 578 584 596 602 605 608 617 (Figure 3).

Nucleotide base substitutions occur in the variable sites, and make the specimens of *S. latimanus* reflect the genetic similarity and difference. Therefore, 11 sequences used in the present study only formed 9 haplotypes with haplotype diversity (Hd) of 0.9636, haplotype 1 (Bitung and Arakan), 2 (Likupang-1 and Basaan-2), 3 (Likupang-2), 4 (Manado), 5 (Basaan-1), 6 (Japan: Okinawa), 7 (Indonesia), 8 (China), and 9 (Philippines: Aurora-region 3). Different haplotypes of the same localities show that the collected individuals have heteroplasmic-typed mtDNA. It could be seen from individuals from Basaan-1 and Basaan-2 and Likupang-1 and Likupang-2 form separate haplotypes despite existing in the same locality.



Window: 1-50

1	1542916 Sepia la...	TtTGaTCaGGTTTATTgGGgACaTCAtTAAGttTAATtATtCGtAGtGAa
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	.a..g.........a..t......c....ac.......c..a......
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	....g..g........a..a......c.........................a..g
9	AB430406.1 Sepia...	.......g........a..a......c.........................a..g
10	AY185506.1 Sepia...	................a..t..t..............a.....a..a...
11	KF009663.1 Sepia...	....g..g........a..a......c.........................a..g

Window: 51-100

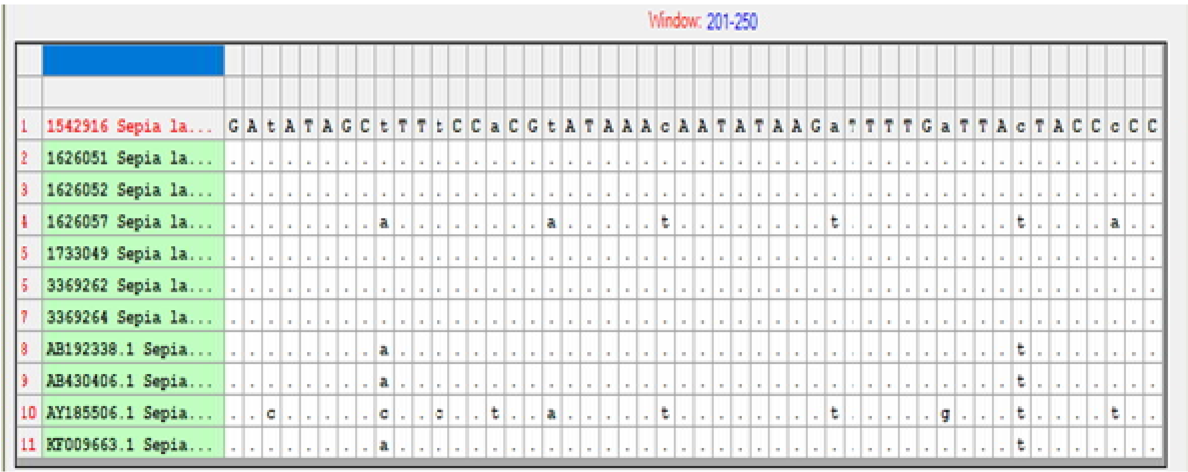
1	1542916 Sepia la...	TTgGGtAAACCcGGTACatTatTAAAtGAtGAcCAAtTATAtAATGTcaT
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....t..
4	1626057 Sepia la...	..a........a......c.tc....c..c..t...c..........t..
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....t..
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	..a...............c..c..........t........c.....t..
9	AB430406.1 Sepia...	..a...............c..c..........t........c.....t..
10	AY185506.1 Sepia...	..a..a.....a.....tc.............t..............tg.
11	KF009663.1 Sepia...	..a...............c..c..........t........c.....t..

Window: 101-150

1	1542916 Sepia la...	AGTtACaGCCCAtGGtTTcATtATAATtTTcTTttTAGTaATACCTATtA
2	1626051 Sepia la...	...........................c......................
3	1626052 Sepia la...	...........................c....................c.
4	1626057 Sepia la...	...............a..t..............cc...............
5	1733049 Sepia la...	....
6	3369262 Sepia la...	...........................c......................
7	3369264 Sepia la...	...........................c......................
8	AB192338.1 Sepia...	...............g..t...........t..c................
9	AB430406.1 Sepia...	...............g..t...........t..c................
10	AY185506.1 Sepia...	...a..c.....c.....t..c.................t..........
11	KF009663.1 Sepia...	...............g..t...........t..c................

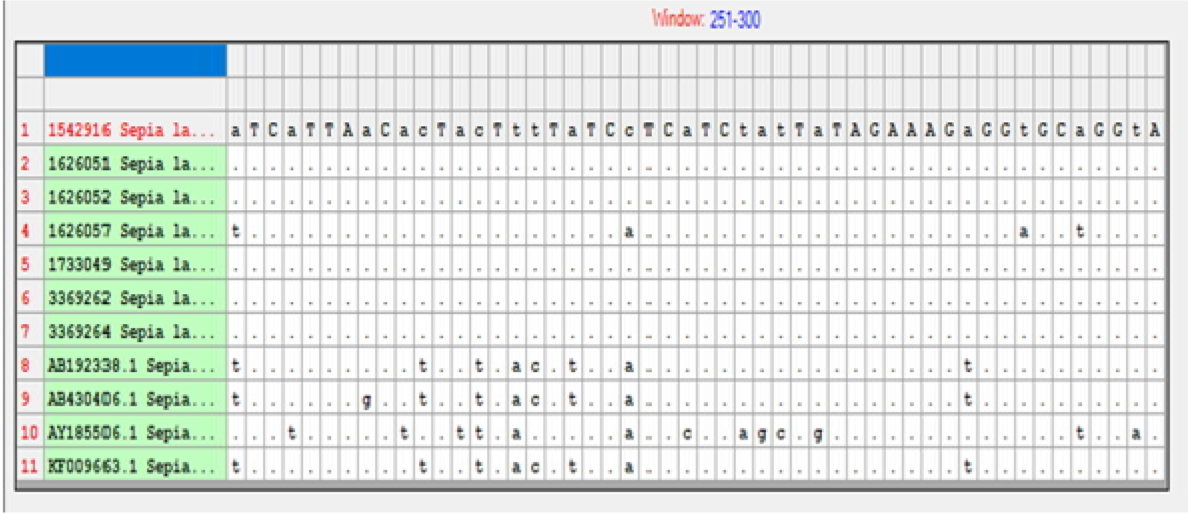
Window: 151-200

1	1542916 Sepia la...	TAATtGGtGGgTTTGGTAAcTGaTTAgTtCCtTTAATAcTAGGaGCcCCA
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	.......a..t........t........a.........t....t..t...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	....c..a..t........t......a...........t.......t...
9	AB430406.1 Sepia...	....c..a..t........t......a...........t.......t...
10	AY185506.1 Sepia...	.......a..t........t..g........c......t....g..a...
11	KF009663.1 Sepia...	....c..a..t........t......a...........t.......t...



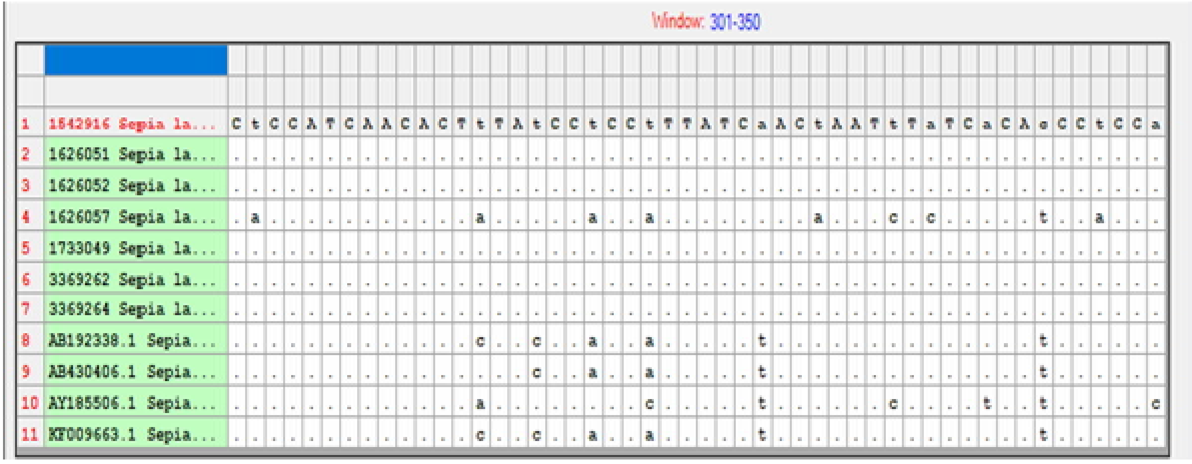
Window: 201-250

1	1542916 Sepia la...	G	A	t	A	T	A	G	C	t	T	T	t	C	C	a	C	G	t	A	T	A	A	A	c	A	A	T	A	T	A	A	G	a	T	T	T	T	G	a	T	T	A	c	T	A	C	C	c	C	C
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	t	.	.	.	.	a	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	c	.	.	.	.	.	c	.	.	c	.	.	t	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	g	.	.	.	t	.	.	.	.	t	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.



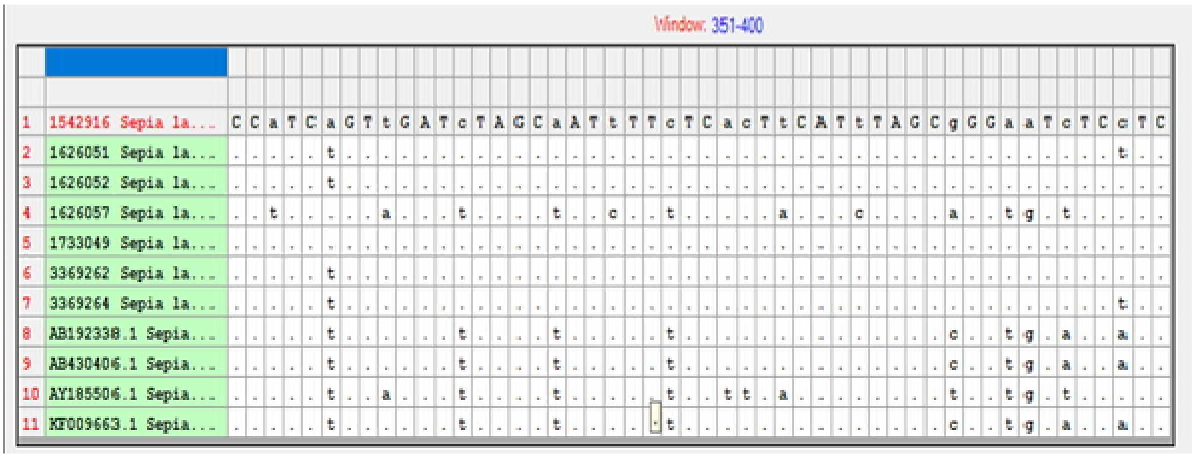
Window: 251-300

1	1542916 Sepia la...	a	T	C	a	T	T	A	a	C	a	c	T	a	c	T	t	t	T	a	T	C	c	T	C	a	T	C	t	a	t	T	a	T	A	G	A	A	A	G	a	G	G	t	G	C	a	G	G	t	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	t	.	.	.	.	.	.	.	.	.	t	.	.	t	.	a	c	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	t	.	.	.	.	.	.	g	.	.	t	.	.	t	.	a	c	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	t	.	.	.	.	.	t	.	.	t	t	.	a	.	.	.	.	.	a	.	.	c	.	.	a	g	c	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	a	.
11	KF009663.1 Sepia...	t	.	.	.	.	.	.	.	.	.	t	.	.	t	.	a	c	.	t	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.



Window: 301-350

1	1542916 Sepia la...	C	t	C	C	A	T	C	A	A	C	A	C	T	t	T	A	t	C	C	t	C	C	t	T	T	A	T	C	a	A	C	t	A	A	T	t	T	a	T	C	a	C	A	c	C	C	t	C	C	a
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	a	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	.	.	a	.	.	.	c	.	c	.	.	.	.	.	t	.	.	a	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.	.	.	.	.	c	.	.	.	.	t	.	.	t	.	.	.	.	.	c
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	a	.	.	a	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.



Window: 351-400

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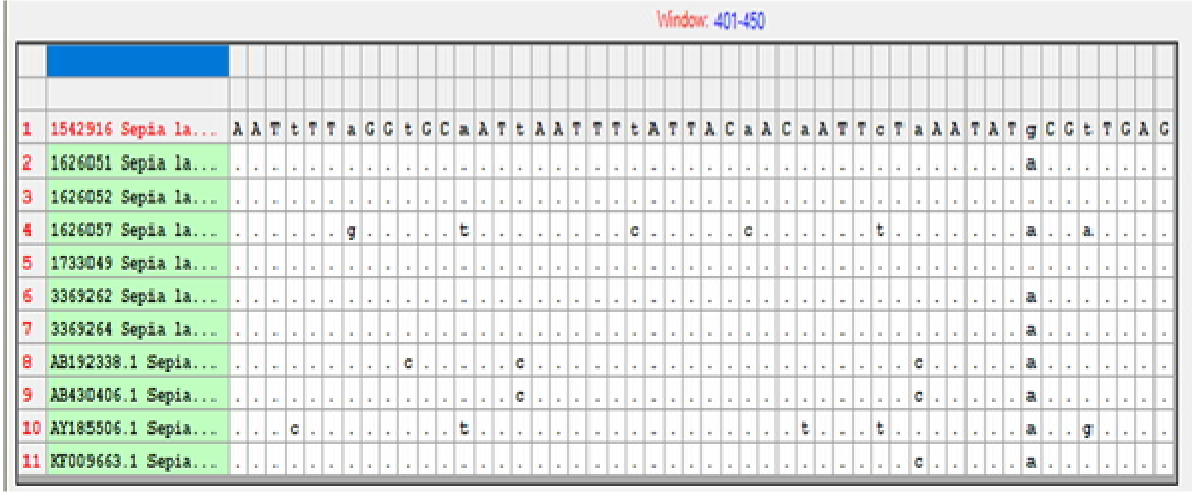
1  1542916 Sepia la...    CCaTCaGTtGATcTAGCaATtTTcTCacTtCATtTAGCgGGaaTcTCcTC
2  1626051 Sepia la...    .....t.........................................t..
3  1626052 Sepia la...    .....t............................................
4  1626057 Sepia la...    ..t.....a...t....t..c..t.....a...c....a..tg.t.....
5  1733049 Sepia la...    ..................................................
6  3369262 Sepia la...    .....t............................................
7  3369264 Sepia la...    .....t.........................................t..
8  AB192338.1 Sepia...    .....t......t....t.....t...............c..tg.a..a..
9  AB430406.1 Sepia...    .....t......t....t.....t...............c..tg.a..a..
10 AY185506.1 Sepia...    .....t..a...t....t.....t..tt.a........t..tg.t.....
11 KF009663.1 Sepia...    .....t......t....t.....t...............c..tg.a..a..
```



Window: 401-450

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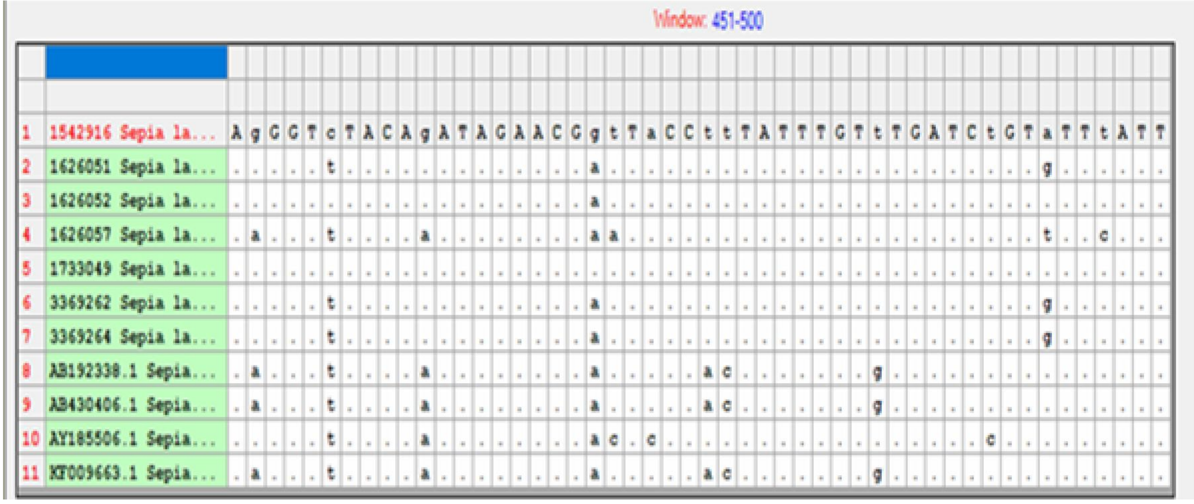
1  1542916 Sepia la...    AATtTTaGGtGCaATtAATTTtATTACaACaATTcTaAAATATgCGtTGAG
2  1626051 Sepia la...    ..........................................a.......
3  1626052 Sepia la...    ..................................................
4  1626057 Sepia la...    ......g.....t........c.....c......t.......a..a....
5  1733049 Sepia la...    ..................................................
6  3369262 Sepia la...    ..........................................a.......
7  3369264 Sepia la...    ..........................................a.......
8  AB192338.1 Sepia...    .........c.....c....................c.....a.......
9  AB430406.1 Sepia...    ...............c....................c.....a.......
10 AY185506.1 Sepia...    ...c........t.................t...t.......a..g....
11 KF009663.1 Sepia...    ....................................c.....a.......
```



Window: 451-500

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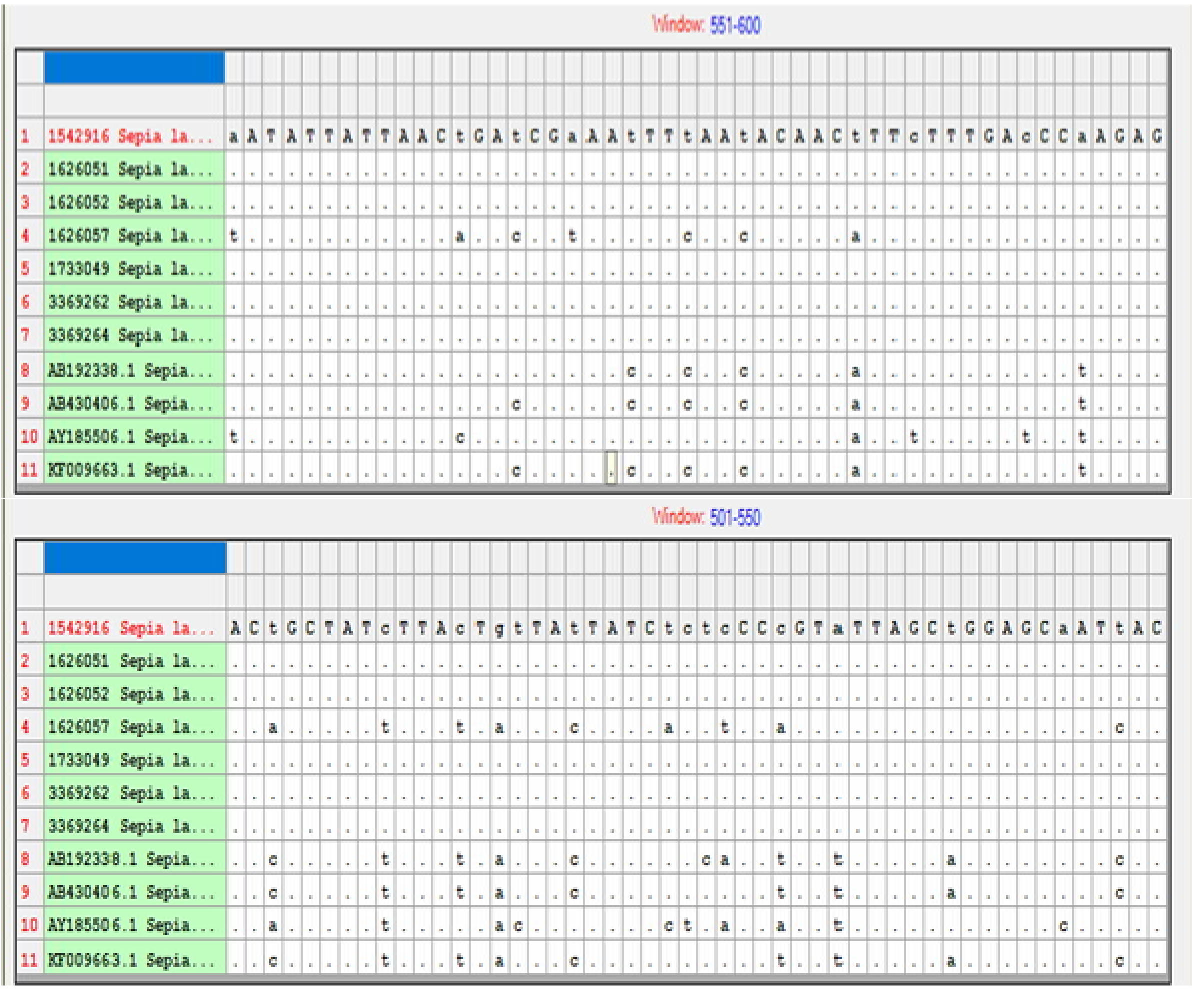
1  1542916 Sepia la...    AgGGTcTACAgATAGAACGgtTaCCttTATTTGTtTGATCtGTaTTtATT
2  1626051 Sepia la...    .....t.............a.......................g......
3  1626052 Sepia la...    ...................a..............................
4  1626057 Sepia la...    .a...t....a........aa......................t..c...
5  1733049 Sepia la...    ..................................................
6  3369262 Sepia la...    .....t.............a.......................g......
7  3369264 Sepia la...    .....t.............a.......................g......
8  AB192338.1 Sepia...    .a...t....a........a.....ac.......g...............
9  AB430406.1 Sepia...    .a...t....a........a.....ac.......g...............
10 AY185506.1 Sepia...    .....t....a........ac.c.................c.........
11 KF009663.1 Sepia...    .a...t....a........a.....ac.......g...............
```



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Window: 551-600

1  1542916 Sepia la...   a A T A T T A T T A A C t G A t C G a A A t T T t A A t A C A A C t T T c T T T G A c C C a A G A G
2  1626051 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
3  1626052 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
4  1626057 Sepia la...   t . . . . . . . . . . . a . . c . . t . . . . . c . . c . . . . . a . . . . . . . . . . . . . . . .
5  1733049 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
6  3369262 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
7  3369264 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
8  AB192338.1 Sepia...   . . . . . . . . . . . . . . . . . . . . . c . . c . . c . . . . . a . . . . . . . . . . . t . . . .
9  AB430406.1 Sepia...   . . . . . . . . . . . . . . . c . . . . . c . . c . . c . . . . . a . . . . . . . . . . . t . . . .
10 AY185506.1 Sepia...   t . . . . . . . . . . . c . . . . . . . . . . . . . . . . . . . . a . . t . . . . . t . . t . . . .
11 KF009663.1 Sepia...   . . . . . . . . . . . . . . . c . . . . . c . . c . . c . . . . . a . . . . . . . . . . . t . . . .

Window: 501-550

1  1542916 Sepia la...   A C t G C T A T c T T A c T g t T A t T A T C t c t c C C c G T a T T A G C t G G A G C a A T t A C
2  1626051 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
3  1626052 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
4  1626057 Sepia la...   . . a . . . . . t . . . t . a . . . c . . . . a . . t . . a . . . . . . . . . . . . . . . . . c . .
5  1733049 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
6  3369262 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
7  3369264 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
8  AB192338.1 Sepia...   . . c . . . . . t . . . t . a . . . c . . . . . . c a . . t . . t . . . . . a . . . . . . . . c . .
9  AB430406.1 Sepia...   . . c . . . . . t . . . t . a . . . c . . . . . . . . . . t . . t . . . . . a . . . . . . . . c . .
10 AY185506.1 Sepia...   . . a . . . . . t . . . . . a c . . . . . . . c t . a . . a . . t . . . . . . . . . . . c . . . . .
11 KF009663.1 Sepia...   . . c . . . . . t . . . t . a . . . c . . . . . . . . . . t . . t . . . . . a . . . . . . . . c . .
```

Figure 3. Nucleotide sequence of *S. latimanus* in North Sulawesi waters (1 = *Sepia latimanus* (Bitung); 2 = *S. latimanus* (Likupang 1); 3 = *S. latimanus* (Likupang 2); 4 = *S. latimanus* (Manado); 5 = *S. latimanus* (Arakan); 6 = *S. latimanus* (Basaan 1); 7 = *S. latimanus* (Basaan 2); 8 = *S. latimanus* (Japan: Okinawa); 9 = *S. latimanus* (Indonesia); 10 = *S. latimanus* (China); 11 = *S. latimanus* (Philippines: Aurora-region 3).

It indicates that *S. latimanus* in North Sulawesi waters has intraspecific population mixture that enables to find different genetic characters at the same locality or same genetic characters at different localities. This condition is also supported by FAO records (Reid et al 2005) that very wide distribution of *S. latimanus* could be a reasonable factor for the population or genetic mixture. This species lives in Indo-Pacific and is distributed in south Mozambik, entire Indian ocean, Malacca strait, Melanesia islands, south China Sea, Philippines Sea, and east China Sea, Taipei province and Japan to south Kyushu, Indonesia to northwest and northeast Australia, Palau, Guam, New Caledonia, Fiji and Cocos Island (Reid et al 2005).

Genetic mixture, as indicated with haplotype similarity between Likupang 1 and Basaan 2 and between Bitung and Arakan, could be influenced by the ability of *S. latimanus* to move from one place to another. Previous studies have indicated that cuttlefish can inhabit different coastal habitats in coral reef ecosystem (Reid et al 2005). Habitats are the resources and conditions present in an area to support survival and reproductive success (Leopold 1933). Cuttlefish are well known as the chameleon of the sea (Hanlon et al 2009) with well-evolved nervous systems enable to search for extensive trophic niches and migrate to exploit different production systems and prey population, and therefore, they can do long distant migration. Our field observations found that juvenile *S. latimanus* occurred under the seagrass, and near branching corals and sponge. Other evidence also shows that fishermen often take advantage of low tide period to catch cuttlefish in seagrass ecosystem, at least in Likupang waters (*field obs*).

S. latimanus from Manado Bay is also in separate group even though its geographic position occurs between Likupang waters and Arakan waters (Figure 2). It could result from this individual belongs to species complex, a group of closely related organisms that look very similar to the point that the boundaries between them are often unclear. Based on genetic distance, *S. latimanus* from Manado Bay is close to that from Philippines waters (Table 1). Manado bay is the northern part of North Sulawesi with series of coral reef-surrounded small islands northwards to southern Philippines. This area belongs to Coral Triangle area. Closer genetic distance of *S. latimanus* between Manado bay and Philippines Sea than the same species from other Indonesian waters could result from closer geographic distance between Manado bay to Philippines than other location in Indonesian waters where the sample was collected. This finding is supported by previous studies that increasing geographic distance between patches was associated with increasing genetic distance, while increasing habitat continuity between patches was associated with decreasing genetic distance (Alberto et al 2010). Isolation by distance and isolation by environment are important mechanisms in driving the genetic population structure (Fernandez et al 2017). Isolation by distance could be used to predict an increase in genetic differentiation with geographic distance (Ramachandran et al 2005), but genetic structure through time is stable (Pascual et al 2016).

Ocean currents are also an important factor affecting the distribution of marine animals and plants (Hays 2017). The dynamics of the ocean are highly variable and are driven by multiple forcing factors such as solar insolation, winds, tides and freshwater input, and this variability influences the development of distinct water masses of different hydrographic properties in both vertical and horizontal dimensions (McManus et al 2012). All these are related with food chain and habitat distribution that impact the availability of living needs of marine animal's dispersal. Therefore, physical structures and processes are directly related to the swimming and behavioral capabilities of an individual or species, such as *S. latimanus*.

Conclusions. *Sepia latimanus* of North Sulawesi waters had sufficient genetic variations. These occur between and within localities. Genetic similarity was also present between *S. latimanus* from different localities, such as Basaan-2 and Likupang-1 samples and Bitung and Arakan samples. Eleven nucleotide sequences analyzed formed only 9 haplotypes due to the haplotype similarity between localities, and this condition has reflected genetic mixture. Moreover, *S. latimanus* sample of Manado bay had closer kindship to that of NCBI data for Philippines waters. This condition is supported by habitat and food distribution, ocean currents, and the ability of *S. latimanus* to exploit different niches through behavioral adaptation to different environmental condition.

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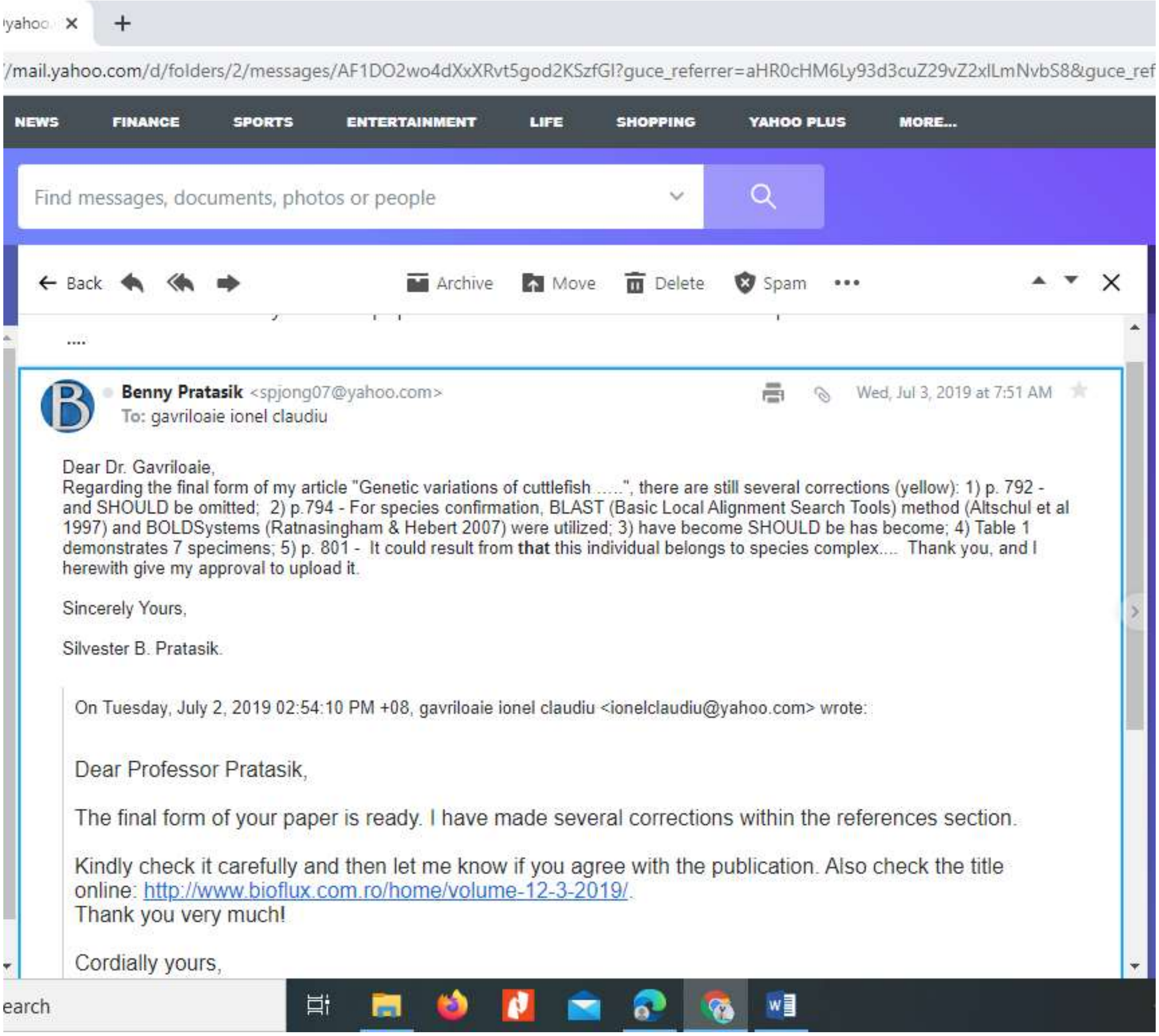


Benny Pratasik <spjong07@yahoo.com>
To: gavriloaie ionel claudiu

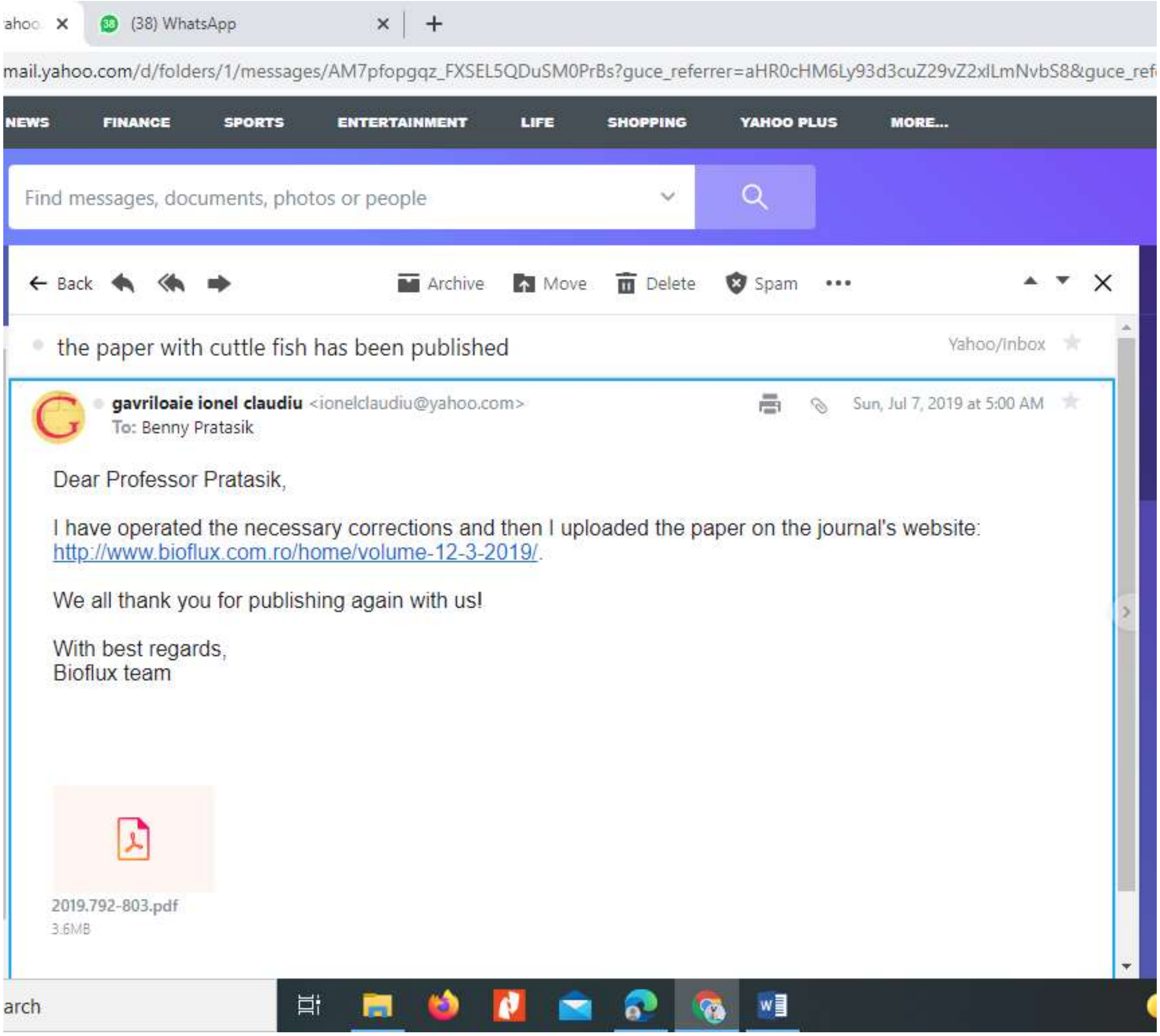
Wed, Jul 3, 2019 at 7:51 AM

Dear Dr. Gavriloaie,
Regarding the final form of my article "Genetic variations of cuttlefish .....", there are still several corrections (yellow): 1) p. 792 - and SHOULD be omitted; 2) p.794 - For species confirmation, BLAST (Basic Local Alignment Search Tools) method (Altschul et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007) were utilized; 3) have become SHOULD be has become; 4) Table 1 demonstrates 7 specimens; 5) p. 801 - It could result from **that** this individual belongs to species complex.... Thank you, and I herewith give my approval to upload it.

Sincerely Yours,

Silvester B. Pratasik.

> On Tuesday, July 2, 2019 02:54:10 PM +08, gavriloaie ionel claudiu <ionelclaudiu@yahoo.com> wrote:
>
> Dear Professor Pratasik,
>
> The final form of your paper is ready. I have made several corrections within the references section.
>
> Kindly check it carefully and then let me know if you agree with the publication. Also check the title online: http://www.bioflux.com.ro/home/volume-12-3-2019/.
> Thank you very much!
>
> Cordially yours,



the paper with cuttle fish has been published

gavriloaie ionel claudiu <ionelclaudiu@yahoo.com>
To: Benny Pratasik

Sun, Jul 7, 2019 at 5:00 AM

Dear Professor Pratasik,

I have operated the necessary corrections and then I uploaded the paper on the journal's website: http://www.bioflux.com.ro/home/volume-12-3-2019/.

We all thank you for publishing again with us!

With best regards,
Bioflux team

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3.6MB

Genetic variations of cuttlefish *Sepia latimanus* (Cephalopoda, Sepiidae) in North Sulawesi waters, Indonesia

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Abstract. This study was aimed at discerning the genetic variations of cuttlefish *Sepia latimanus* in North Sulawesi, using CO1 gene marker. Sampling was conducted in Kalinaung and Likupang, North Minahasa regency, Manado Bay, Manado municipality and Minahasa regency, Poigar, South Minahasa regency, and Basaan, Southeast Minahasa. All samples were put into 95% alcohol and brought to the molecular biology laboratory of Faculty of Fisheries and Marine Sciences for further analysis. Genome DNA extraction of all samples used innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene was amplified applying universal primer pairs LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'TAAACTTCAGGGTGACCAAAAAATCA-3'. Polymerase chain reaction (PCR) was carried out in 35 cycles at 95^{o}C (30 sec), 50^{o}C (30 sec), 72^{o}C (50 sec). The PCR product was visualized in 1% (b/v) agarosa gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). The chromatogram obtained was edited using Geneious v5.6. The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method and BOLDSystems. The phylogenetic tree was built using Neighbor-Joining Method, and genetic similarity was explained by mutation, haplotype and genetic distance. This study found genetic variations in *S. latimanus* of North Sulawesi waters between and within localities. Haplotype similarity between localities made eleven nucleotide sequences only form 9 haplotypes, while haplotype difference within locality could result from genetic mixture. Wide distribution of the species is supported by its adaptability to the environmental conditions.

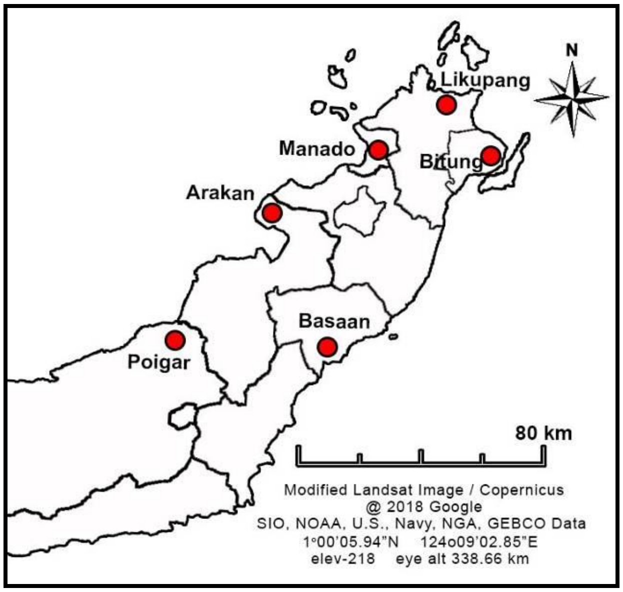
Key Words: CO1 gene marker, phylogenetic tree, haplotype, genetic distance.

Introduction. North Sulawesi has many islands surrounded by coral reef ecosystems that provide many different habitats for various marine organisms either as home or shelter of different life stage. Broadclub cuttlefish, *Sepia latimanus*, is one of marine mollusks taking advantages of the coral reef ecosystem as spawning ground, egg placement site, and shelter for the youngsters (Pratasik et al 2017). Species diversity of cuttlefish is very high with more than a hundred species recorded, and their distribution and abundance are highly affected by the availability of suitable habitats and preys, particularly for small individuals (Neves et al 2009; Pratasik et al 2017). Some species live in the water deeper than 400 m, such as *Sepia australis, S. elegans, S. orbignyana* and *S. hieronis*, and some species occupy shallow waters, such as *S. latimanus*, *S. officinalis,* and *S. pharaonis* (Reid et al 2005). Cuttlefish (and cephalopods in general) are known to have diverse body patterns that can immediately naturally change, being controlled by its chromatophore system (Hanlon et al 2009) and well

known as the most intelligent marine biota with the largest body-brain ratio of all invertebrates (Tricarico et al 2014). These have made many studies have focused on the body pattern alteration-related behavioral aspects (Shohet et al 2006; Barbosa et al 2008; Kelman et al 2008; Allen et al 2009; Chiao et al 2010; Barbosa et al 2011).

Indonesian waters so far hold several species of cuttlefish, such as *Sepia bandensis, S. brevimana, S. kiensis, S. latimanus, S. papuensis, S. pharaonis, S. sulcata, S. senta, Sepiella inermis* and *S. weberi* (Reid et al 2005), but previous study in Manado Bay and Lembeh Strait found only four species (Pratasik et 2016). Low interest in cuttlefish study could result from sample collection problems as a result of no cuttlefish fisheries in Indonesia, and all cuttlefish catches are bycatch of other fisheries. No cuttlefish fisheries in Indonesia, especially North Sulawesi, make cuttlefish catch generally come from traditional fishermen using handline or speargun in the coral reef areas. *S. latimanus* is the main cuttlefish catch in the coral reef ecosystem and has highly market value. *S. latimanus* is the second biggest cuttlefish after *S. apama* with maximum dorsal mantle length of 50 cm and total weight of 10 kg (Reid et al 2005). The species often visit coral reefs for spawning and laying eggs (pers. obs.). They often come in pairs and select certain coral crevices to insert their eggs (Pratasik et al 2017). Cuttlefish are, in general, economic fisheries resources, but the resources have unclear fisheries status, insufficient data (Reid et al 2005), and belong to threatened species (IUCN 2014). The present study focuses on genetic variations using DNA sequence of mitochondrial genome as DNA barcode. CO1 is the most common mitochondrial DNA gene marker used for species identification (Folmer et al 1994) due to belonging to the most conservative gene among the mitochondrial DNA protein coding genes. It has become a standard tool of molecular taxonomy and identification (Ratnasingham & Hebert 2007). Molecular techniques have been well applied for stock discrimination studies in fisheries (Murphy et al 2002) and can provide the basis for better management of whole populations and therefore, sustainable fisheries. This study aims to examine the genetic characteristics of broadclub cuttlefish *S. latimanus* in North Sulawesi waters.

Material and Method. This study was carried out for approximately one-year with 5 months of sample collection, from May to September 2018, using SCUBA dives over different habitat types and obtained from local fishermen. Sample collection was done in Lembeh Strait (Bitung), Basaan (Southeast Minahasa), Manado Bay (Manado), Likupang waters (North Minahasa), Arakan and Poigar (South Minahasa), North Sulawesi (Figure 1).



Modified Landsat Image / Copernicus
@ 2018 Google
SIO, NOAA, U.S., Navy, NGA, GEBCO Data
1°00'05.94"N 124o09'02.85"E
elev-218 eye alt 338.66 km

Figure 1. Map of North Sulawesi with sampling points (marked red).

The first two sites represent the southern part of North Sulawesi waters, while the second four sites represent the northern part of the island, so that these sites have represented the entire North Sulawesi marine waters. In this study, seven cuttlefish specimens were collected from fishermen's catches and brought to the Biotechnology Laboratory of Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado. For DNA extraction preparation, all samples were preserved in 95% ethanol and kept at room temperature.

Extraction, PCR, and sequencing. Genome DNA was extracted using innuPREP DNA Micro Kit (Analytic Jena). The CO1 gene amplification employed universal primer pairs LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al 1994). Polymerase chain reaction (PCR) was performed in 35 cycles at 95°C (30 sec), 50°C (30 sec), 72°C (50 sec), and the PCR product was visualized in 1% (b/v) agarose gel electrophoresis. To maximize the outcome, a bi-directional sequencing was done using Big Dye© terminator chemistry (Perkin Elmer) through First Base CO (Malaysia).

Data analysis. The chromatogram editing employed Geneious v5.6 (Drummond et al 2012). For species confirmation, BLAST (Basic Local Alignment Search Tools) (Altschul et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007) methods were utilized, and then the sequences were compared between localities and with the GenBank data. The phylogenetic tree was built using Neighbor-Joining Method of MEGA X and the heterogeneity index was also estimated (Kumar et al 2018). The analysis of nucleotide base sequence and haplotype variations between *S. latimanus* from different localities used DnaSP Ver.6.12.03 computer program (Rozas et al 2017).

Results and Discussion. During the study, 7 individuals of *S. latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not taken, so that all samples were obtained from fishermen's catches. Sampling in Poigar, South Minahasa, using beach seine and SCUBA dives, did not obtain any sample as well. This condition reveals that sufficient sampling technique is required for cuttlefish studies. In the past, cuttlefish fishing, such as *S. officinalis* (English Channel) and *S. esculanta* (Japan), used trawl, set net, gill net, trammel net and trap with spawning substrate (Pawson 1995; Arkley et al 1996) by taking advantage of their migration to the spawning areas. In North Sulawesi, *S. latimanus* is generally fished using speargun or jig fishing in the coral reef ecosystem (Pratasik et al 2015). Obtaining sufficient number of samples has become constraint in cuttlefish study, particularly in North Sulawesi, due to no cuttlefish fisheries and unclear distribution of the species, so that this study collected only few samples.

Table 1 demonstrates 7 specimens collected from North Sulawesi waters and 4 others are NCBI data from the genbank (Indonesia, Japan, Philippines of Aurora-region 3) used as comparison, in which the genetic heterogeneity gets bigger with geographic distance, except the specimen of Manado Bay. This condition is supported with group positions, in which the cuttlefish collected in North Sulawesi waters, but Manado, are in the same group, while 4 NCBI samples are in another separate group (Figure 2). This finding reconfirms that geographic distance induces the genetic diversity of *S. latimanus*. However, *S. latimanus* of North Sulawesi has revealed that there are genetic variations and similarities among the same or different locations. *S. latimanus* sample from Bitung waters has genetic similarity to that from Arakan waters. Also, one sample of *S. latimanus* from Likupang waters has genetic similarity to that of Basaan waters. On the other hand, both *S. latimanus* samples from Basaan waters are genetically different as happening for both samples from Likupang waters (Figure 2).

Table 1

Genetic distance of cuttlefish *Sepia latimanus* in North Sulawesi waters

No		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	1542916 *Sepia latimanus* (Bitung)										
2	1626051 *Sepia latimanus* (Likupang 1)	0.00435									
3	1626052 *Sepia latimanus* (Likupang 2)	0.00309	0.00371								
4	1626057 *Sepia latimanus* (Manado)	0.06984	0.06983	0.07071							
5	1733049 *Sepia latimanus* (Arakan)	0.00000	0.00435	0.00309	0.06984						
6	3369262 *Sepia latimanus* (Basaan 1)	0.00435	0.00123	0.00247	0.06820	0.00435					
7	3369264 *Sepia latimanus* (Basaan 2)	0.00435	0.00000	0.00371	0.06983	0.00435	0.00123				
8	AB192338.1 *Sepia latimanus* (Japan: Okinawa)	0.05800	0.05627	0.05704	0.06232	0.05800	0.05549	0.05627			
9	AB430406.1 *Sepia latimanus* (Indonesia)	0.05555	0.05384	0.05461	0.06152	0.05555	0.05307	0.05384	0.00434		
10	AY185506.1 *Sepia latimanus* (China)	0.07853	0.07747	0.07747	0.07531	0.07853	0.07581	0.07747	0.07725	0.07739	
11	KF009663.1 *Sepia latimanus* (Philippines: Aurora Region 3)	0.05555	0.05384	0.05461	0.05898	0.05555	0.05307	0.05384	0.00309	0.00247	0.07656

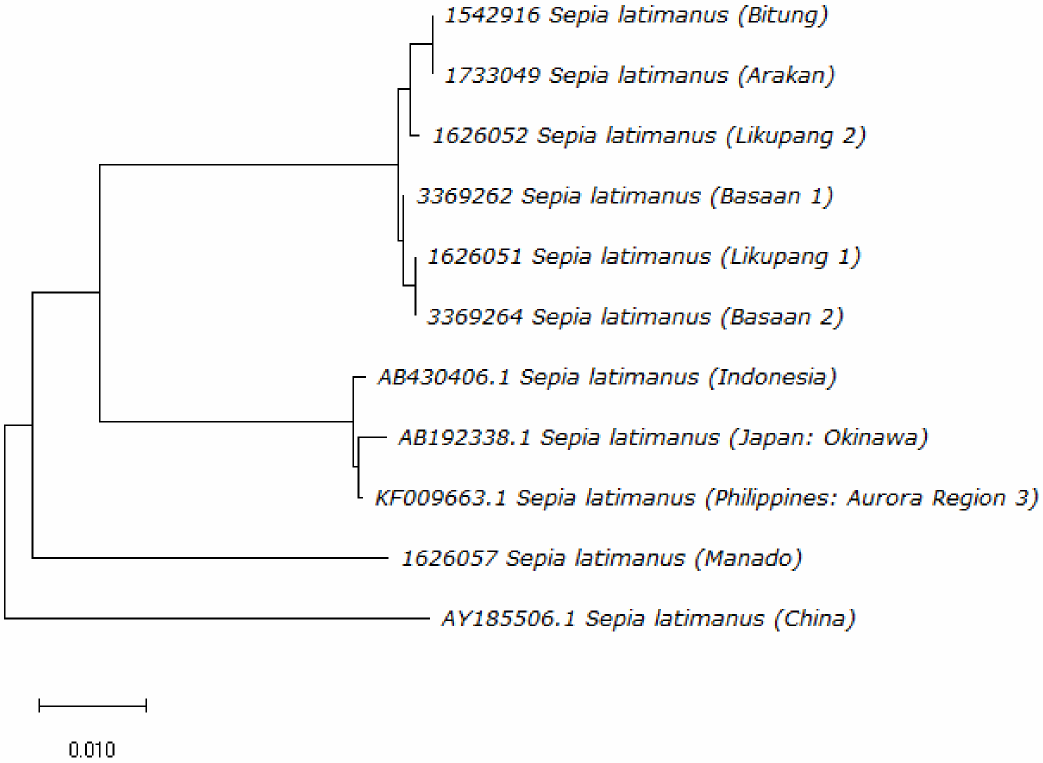
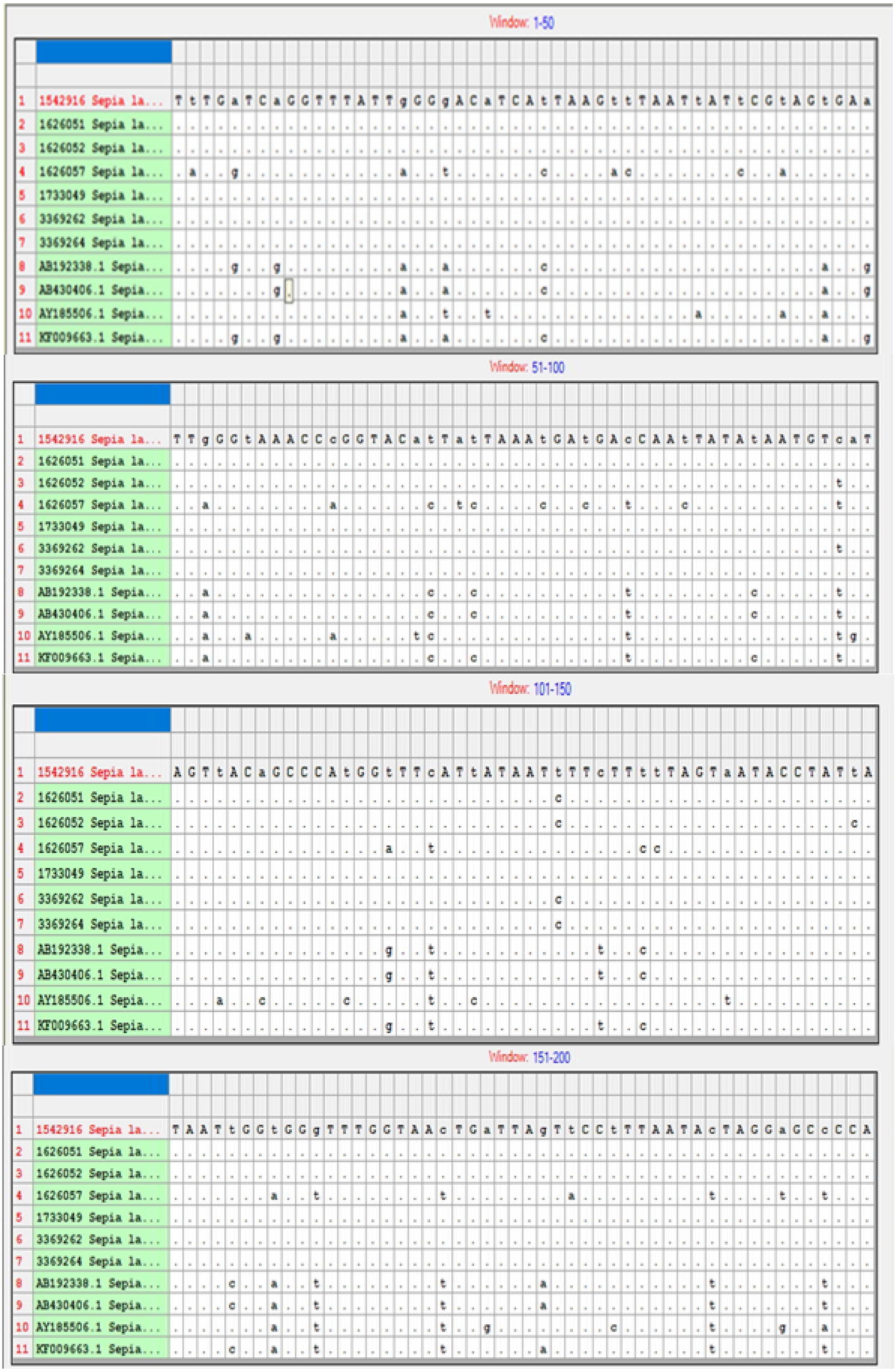


Figure 2. Phylogenetic tree of North Sulawesi *S. latimanus*.

From total numbers of 620 bp, there were 166 polymorphic sites with 186 mutations. Based on variable sites (two variants), there were 73 singleton variable sites with 67 different site positions and 93 parsimony informative sites with 80 site positions. The former was at positions: 2 23 32 33 38 41 56 68 71 77 80 87 99 104 107 113 122 135 140 149 173 179 182 203 212 215 239 254 258 260 263 275 278 279 280 282 293 299 302 332 338 341 347 350 353 371 377 378 384 404 407 410 422 428 431 473 491 497 516 525 526 545 569 587 593 611 618, and the latter at positions 5 8 17 27 44 47 50 53 62 69 72 83 92 98 119 128 131 134 155 158 161 170 177 189 218 224 233 243 251 261 264 266 267 269 272 290 296 317 320 329 336 344 356 359 363 368 374 380 392 393 413 416 435 437 443 452 456 461 470 476 477 485 509 513 515 519 533 539 548 551 566 572 575 578 584 596 602 605 608 617 (Figure 3).

Nucleotide base substitutions occur in the variable sites, and make the specimens of *S. latimanus* reflect the genetic similarity and difference. Therefore, 11 sequences used in the present study only formed 9 haplotypes with haplotype diversity (Hd) of 0.9636, haplotype 1 (Bitung and Arakan), 2 (Likupang-1 and Basaan-2), 3 (Likupang-2), 4 (Manado), 5 (Basaan-1), 6 (Japan: Okinawa), 7 (Indonesia), 8 (China), and 9 (Philippines: Aurora-region 3). Different haplotypes of the same localities show that the collected individuals have heteroplasmic-typed mtDNA. It could be seen from individuals from Basaan-1 and Basaan-2 and Likupang-1 and Likupang-2 form separate haplotypes despite existing in the same locality.



Window: 1-50

1	1542916 Sepia la...	T	t	T	G	a	T	C	a	G	G	T	T	T	A	T	T	g	G	G	g	A	C	a	T	C	A	t	T	A	A	G	t	t	T	A	A	T	t	A	T	t	C	G	t	A	G	t	G	A	a
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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4	1626057 Sepia la...	.	a	.	.	g	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	c	.	.	.	.	a	c	.	.	.	.	.	.	.	c	.	.	a	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	g	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	.	.	.	a	.	.	a	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	g	.	.	g	.	.	.	.	.	.	.	.	a	.	.	a	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	g

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1	1542916 Sepia la...	T	T	g	G	G	t	A	A	A	C	C	c	G	G	T	A	C	a	t	T	a	t	T	A	A	A	t	G	A	t	G	A	c	C	A	A	t	T	A	T	A	t	A	A	T	G	T	c	a	T
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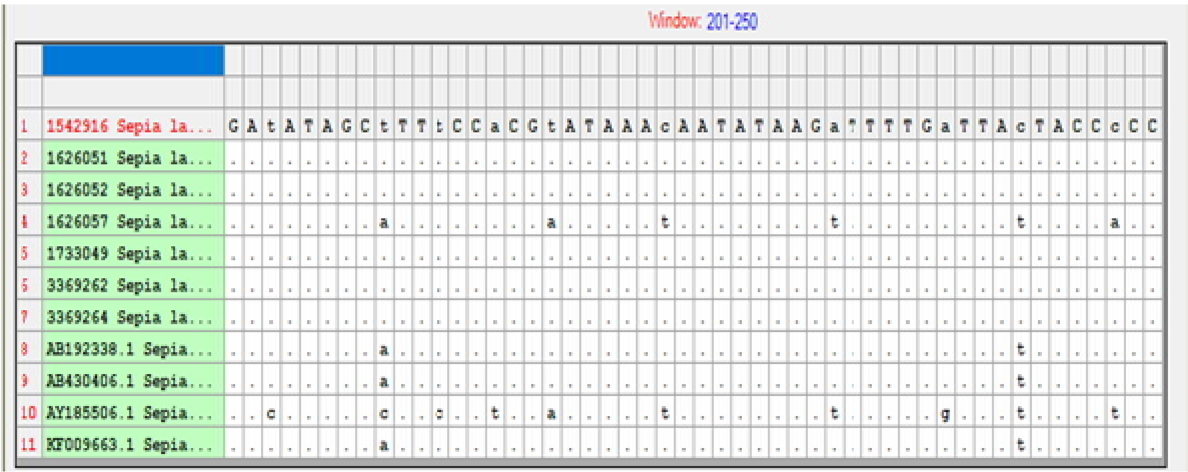
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.
9	AB430406.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.
10	AY185506.1 Sepia...	.	.	a	.	.	a	.	.	.	.	.	a	.	.	.	.	.	t	c	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	g	.
11	KF009663.1 Sepia...	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	c	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	c	.	.	.	.	.	t	.	.

Window: 101-150

1	1542916 Sepia la...	A	G	T	t	A	C	a	G	C	C	C	A	t	G	G	t	T	T	c	A	T	t	A	T	A	A	T	t	T	T	c	T	T	t	t	T	A	G	T	a	A	T	A	C	C	T	A	T	t	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	AY185506.1 Sepia...	.	.	.	a	.	.	c	.	.	.	.	.	c	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	t	.	.	.	.	.	.	.	.	.	.	.	t	.	.	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

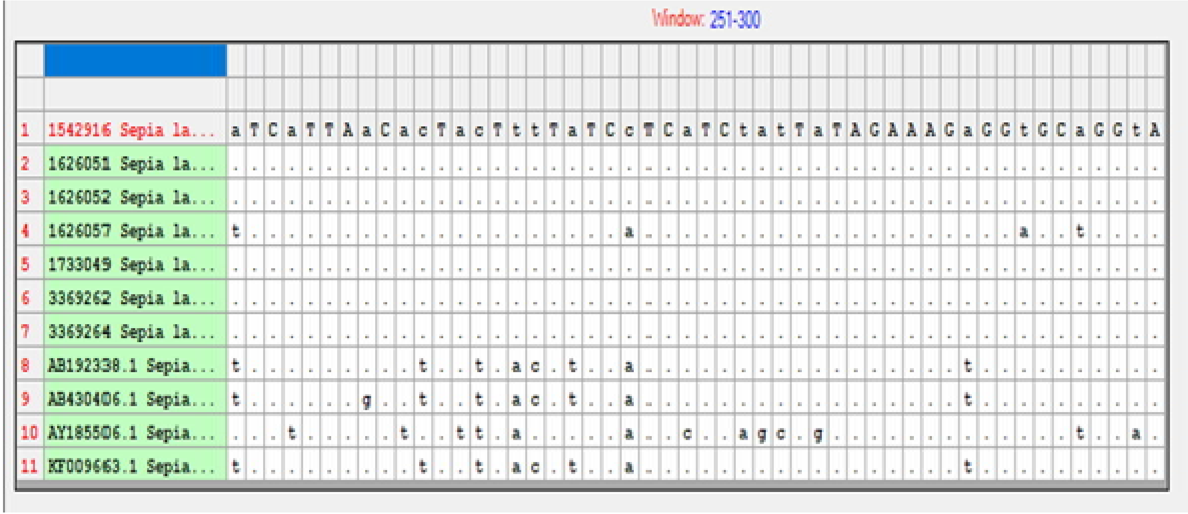
Window: 151-200

1	1542916 Sepia la...	T	A	A	T	t	G	G	t	G	G	g	T	T	T	G	G	T	A	A	c	T	G	a	T	T	A	g	T	t	C	C	t	T	T	A	A	T	A	c	T	A	G	G	a	G	C	c	C	C	A
2	1626051 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	1626052 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	1626057 Sepia la...	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	t	.	.	.	.	t	.	.	t	.	.	.
5	1733049 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	3369262 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	3369264 Sepia la...	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	AB192338.1 Sepia...	.	.	.	.	c	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	t	.	.	.
9	AB430406.1 Sepia...	.	.	.	.	c	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	t	.	.	.
10	AY185506.1 Sepia...	.	.	.	.	.	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	g	.	.	.	.	.	.	.	.	c	.	.	.	.	.	.	t	.	.	.	.	g	.	.	a	.	.	.
11	KF009663.1 Sepia...	.	.	.	.	c	.	.	a	.	.	t	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	t	.	.	.	.	.	.	.	t	.	.	.



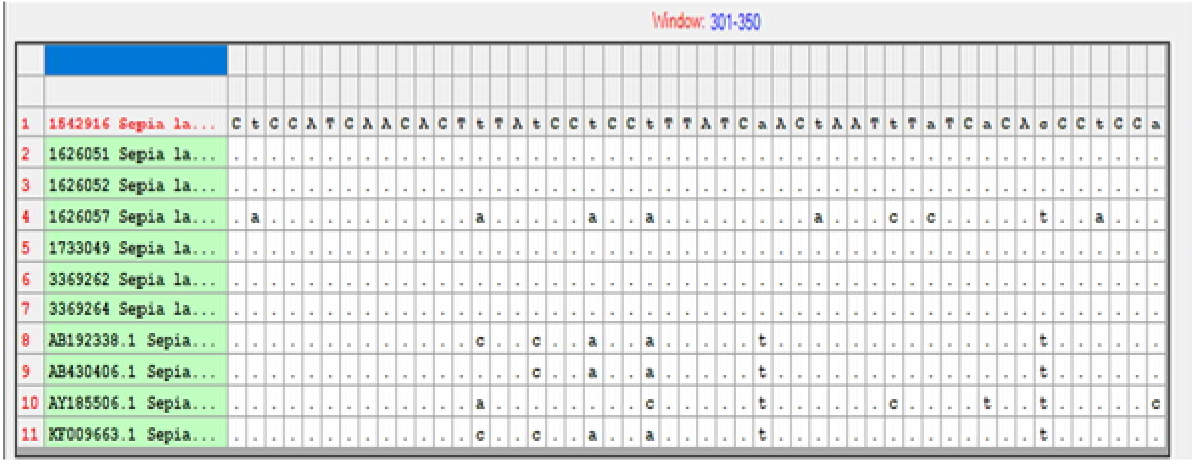
Window: 201-250

1	1542916 Sepia la...	GAtATAGCtTTtCCaCGtATAAAcAATATAAGaTTTTGaTTAcTACCcCC
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	........a........a.....t........t.........t....a..
5	1733049 Sepia la...	....
5	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	........a.................................t.......
9	AB430406.1 Sepia...	........a.................................t.......
10	AY185506.1 Sepia...	..c.....c..c..t..a.....t........t.....g...t....t..
11	KF009663.1 Sepia...	........a.................................t.......



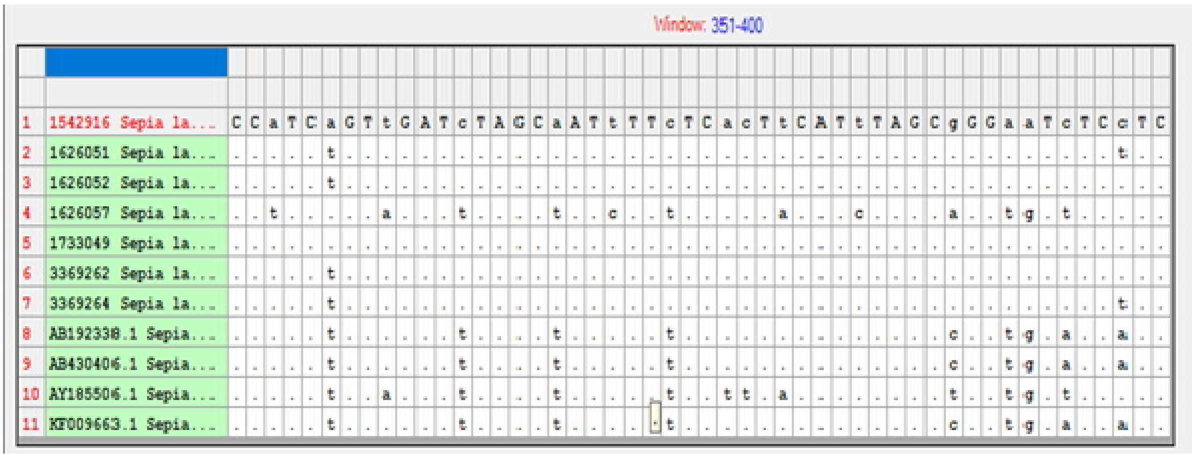
Window: 251-300

1	1542916 Sepia la...	aTCaTTAaCacTacTttTaTCcTCaTCtatTaTAGAAAGaGGtGCaGGtA
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	t....................a....................a..t....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	t.........t..t.ac.t..a.................t..........
9	AB430406.1 Sepia...	t......g..t..t.ac.t..a.................t..........
10	AY185506.1 Sepia...	...t.....t..tt.a.....a..c..agc.g.............t..a.
11	KF009663.1 Sepia...	t.........t..t.ac.t..a.................t..........



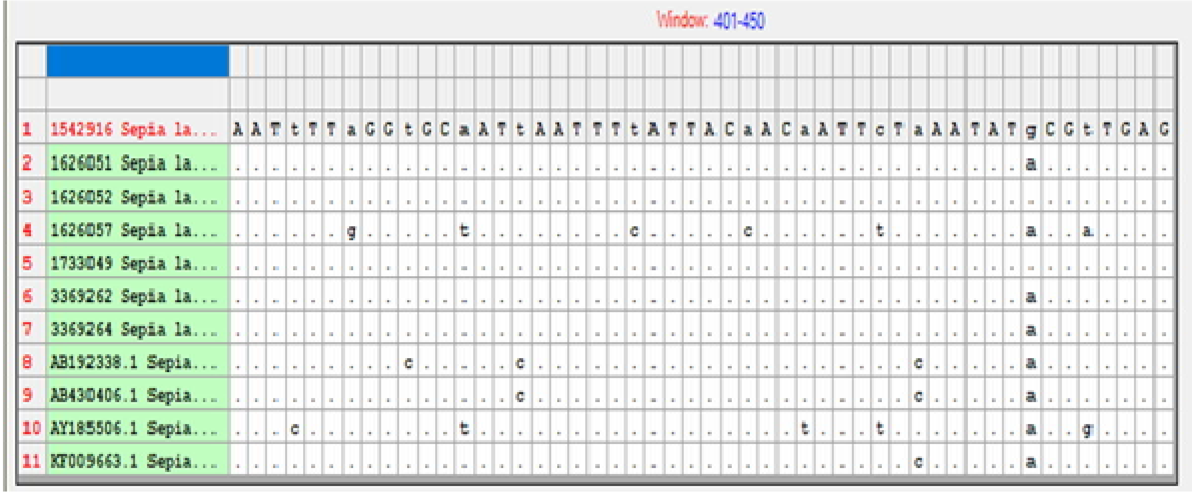
Window: 301-350

1	1542916 Sepia la...	CtCCATCAACACTtTAtCCtCCtTTATCaACtAATtTaTCaCAcCCtCCa
2	1626051 Sepia la...	....
3	1626052 Sepia la...	....
4	1626057 Sepia la...	.a...........a.....a..a........a...c.c.....t..a...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....
7	3369264 Sepia la...	....
8	AB192338.1 Sepia...	.............c..c..a..a.....t..............t......
9	AB430406.1 Sepia...	................c..a..a.....t..............t......
10	AY185506.1 Sepia...	.............a........c.....t......c....t..t.....c
11	KF009663.1 Sepia...	.............c..c..a..a.....t..............t......



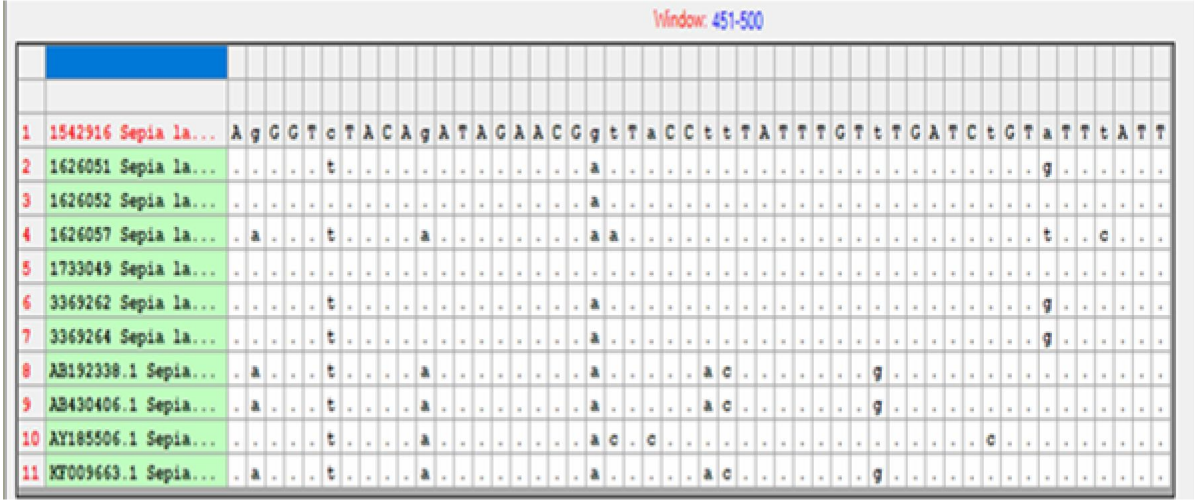
Window: 351-400

1	1542916 Sepia la...	CCaTCaGTtGATcTAGCaATtTTcTCacTtCATtTAGCgGGaaTcTCcTC
2	1626051 Sepia la...	.....t...t..
3	1626052 Sepia la...	.....t..
4	1626057 Sepia la...	..t.....a...t....t..c..t.....a...c....a..tg.t.....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	.....t..
7	3369264 Sepia la...	.....t...t..
8	AB192338.1 Sepia...	.....t......t....t.....t..............c..tg.a..a..
9	AB430406.1 Sepia...	.....t......t....t.....t..............c..tg.a..a..
10	AY185506.1 Sepia...	.....t..a...t....t.....t..tt.a........t..tg.t.....
11	KF009663.1 Sepia...	.....t......t....t.....t..............c..tg.a..a..



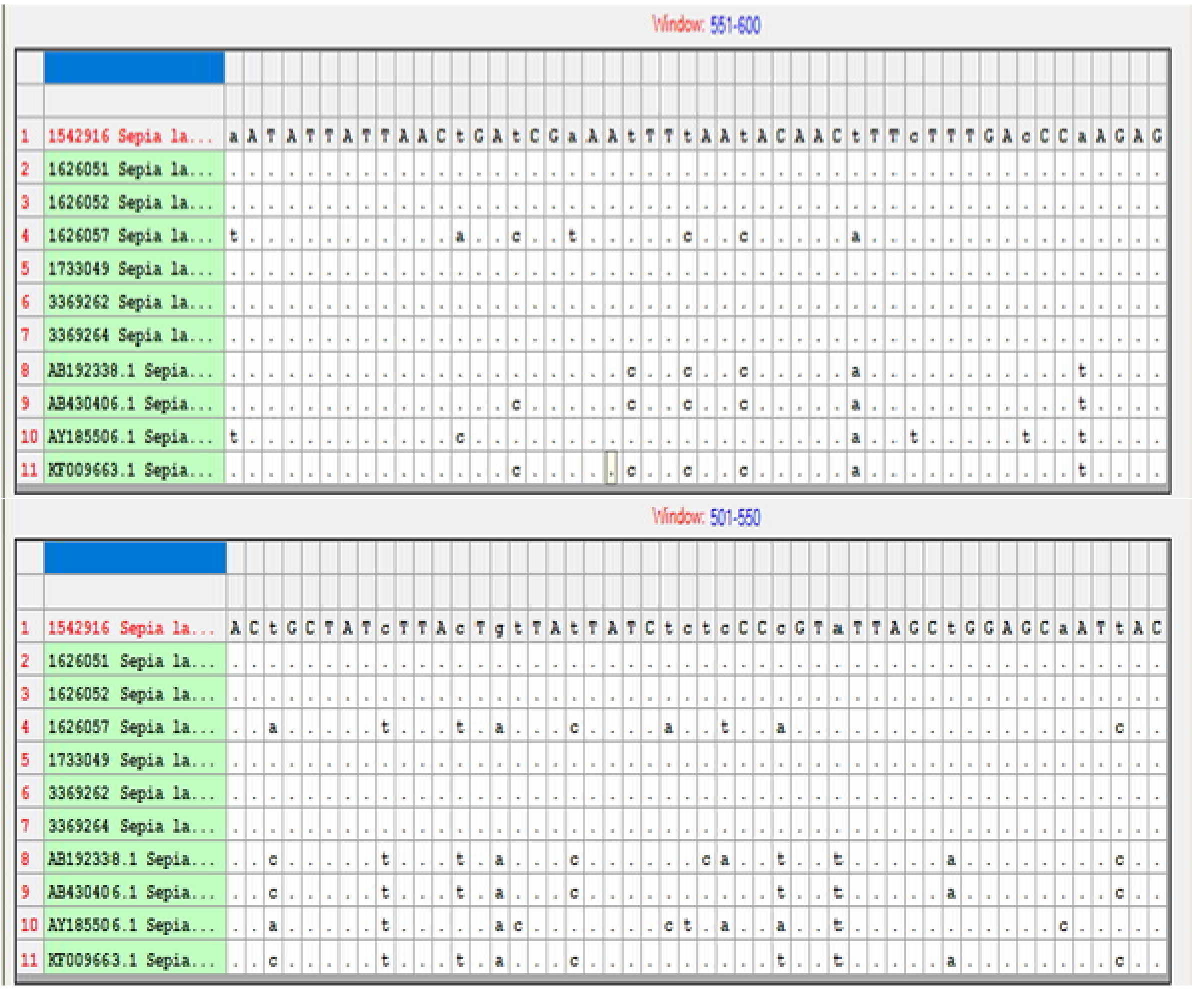
Window: 401-450

1	1542916 Sepia la...	AATtTTaGGtGCaATtAATTTtATTACaACaATTcTaAATATgCGtTGAG
2	1626051 Sepia la...	....a.......
3	1626052 Sepia la...	....
4	1626057 Sepia la...	......g.....t........c.....c......t.......a..a....
5	1733049 Sepia la...	....
6	3369262 Sepia la...	....a.......
7	3369264 Sepia la...	....a.......
8	AB192338.1 Sepia...	.........c.....c....................c.....a.......
9	AB430406.1 Sepia...	...............c....................c.....a.......
10	AY185506.1 Sepia...	...c........t..................t..t.......a..g....
11	KF009663.1 Sepia...	....................................c.....a.......



Window: 451-500

1	1542916 Sepia la...	AgGGTcTACAgATAGAACGgtTaCCttTATTTGTtTGATCtGTaTTtATT
2	1626051 Sepia la...	.....t.............a.......................g......
3	1626052 Sepia la...	...................a..............................
4	1626057 Sepia la...	.a...t....a........aa......................t..c...
5	1733049 Sepia la...	....
6	3369262 Sepia la...	.....t.............a.......................g......
7	3369264 Sepia la...	.....t.............a.......................g......
8	AB192338.1 Sepia...	.a...t....a........a.....ac.......g...............
9	AB430406.1 Sepia...	.a...t....a........a.....ac.......g...............
10	AY185506.1 Sepia...	.....t....a........ac.c.................c.........
11	KF009663.1 Sepia...	.a...t....a........a.....ac.......g...............



Window: 551-600

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1  1542916 Sepia la...   a A T A T T A T T A A C t G A t C G a A A t T T t A A t A C A A C t T T c T T T G A c C C a A G A G
2  1626051 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
3  1626052 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
4  1626057 Sepia la...   t . . . . . . . . . . . a . . c . . t . . . . . c . . c . . . . . a . . . . . . . . . . . . . . . .
5  1733049 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
6  3369262 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
7  3369264 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
8  AB192338.1 Sepia...   . . . . . . . . . . . . . . . . . . . . . c . . c . . c . . . . . a . . . . . . . . . . . t . . . .
9  AB430406.1 Sepia...   . . . . . . . . . . . . . . . c . . . . . c . . c . . c . . . . . a . . . . . . . . . . . t . . . .
10 AY185506.1 Sepia...   t . . . . . . . . . . . c . . . . . . . . . . . . . . . . . . . . a . . t . . . . . t . . t . . . .
11 KF009663.1 Sepia...   . . . . . . . . . . . . . . . c . . . . . c . . c . . c . . . . . a . . . . . . . . . . . t . . . .
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Window: 501-550

```
1  1542916 Sepia la...   A C t G C T A T c T T A c T g t T A t T A T C t c t c C C c G T a T T A G C t G G A G C a A T t A C
2  1626051 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
3  1626052 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
4  1626057 Sepia la...   . . a . . . . . t . . . t . a . . . c . . . . a . . t . . a . . . . . . . . . . . . . . . . . c . .
5  1733049 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
6  3369262 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
7  3369264 Sepia la...   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
8  AB192338.1 Sepia...   . . c . . . . . t . . . t . a . . . c . . . . . . c a . . t . . t . . . . . a . . . . . . . . c . .
9  AB430406.1 Sepia...   . . c . . . . . t . . . t . a . . . c . . . . . . . . . . t . . t . . . . . a . . . . . . . . c . .
10 AY185506.1 Sepia...   . . a . . . . . t . . . . . a c . . . . . . . c t . a . . a . . t . . . . . . . . . . . c . . . . .
11 KF009663.1 Sepia...   . . c . . . . . t . . . t . a . . . c . . . . . . . . . . t . . t . . . . . a . . . . . . . . c . .
```

Figure 3. Nucleotide sequence of *S. latimanus* in North Sulawesi waters (1 = *Sepia latimanus* (Bitung); 2 = *S. latimanus* (Likupang 1); 3 = *S. latimanus* (Likupang 2); 4 = *S. latimanus* (Manado); 5 = *S. latimanus* (Arakan); 6 = *S. latimanus* (Basaan 1); 7 = *S. latimanus* (Basaan 2); 8 = *S. latimanus* (Japan: Okinawa); 9 = *S. latimanus* (Indonesia); 10 = *S. latimanus* (China); 11 = *S. latimanus* (Philippines: Aurora-region 3).

It indicates that *S. latimanus* in North Sulawesi waters has intraspecific population mixture that enables to find different genetic characters at the same locality or same genetic characters at different localities. This condition is also supported by FAO records (Reid et al 2005) that very wide distribution of *S. latimanus* could be a reasonable factor for the population or genetic mixture. This species lives in Indo-Pacific and is distributed in south Mozambik, entire Indian ocean, Malacca strait, Melanesia islands, south China Sea, Philippines Sea, and east China Sea, Taipei province and Japan to south Kyushu, Indonesia to northwest and northeast Australia, Palau, Guam, New Caledonia, Fiji and Cocos Island (Reid et al 2005).

Genetic mixture, as indicated with haplotype similarity between Likupang 1 and Basaan 2 and between Bitung and Arakan, could be influenced by the ability of *S. latimanus* to move from one place to another. Previous studies have indicated that cuttlefish can inhabit different coastal habitats in coral reef ecosystem (Reid et al 2005). Habitats are the resources and conditions present in an area to support survival and reproductive success (Leopold 1933). Cuttlefish are well known as the chameleon of the sea (Hanlon et al 2009) with well-evolved nervous systems enable to search for extensive trophic niches and migrate to exploit different production systems and prey population, and therefore, they can do long distant migration. Our field observations found that juvenile *S. latimanus* occurred under the seagrass, and near branching corals and sponge. Other evidence also shows that fishermen often take advantage of low tide period to catch cuttlefish in seagrass ecosystem, at least in Likupang waters (*field obs*).

S. latimanus from Manado Bay is also in separate group even though its geographic position occurs between Likupang waters and Arakan waters (Figure 2). It could result from that this individual belongs to species complex, a group of closely related organisms that look very similar to the point that the boundaries between them are often unclear. Based on genetic distance, *S. latimanus* from Manado Bay is close to that from Philippines waters (Table 1). Manado bay is the northern part of North Sulawesi with series of coral reef-surrounded small islands northwards to southern Philippines. This area belongs to Coral Triangle area. Closer genetic distance of *S. latimanus* between Manado bay and Philippines Sea than the same species from other Indonesian waters could result from closer geographic distance between Manado bay to Philippines than other location in Indonesian waters where the sample was collected. This finding is supported by previous studies that increasing geographic distance between patches was associated with increasing genetic distance, while increasing habitat continuity between patches was associated with decreasing genetic distance (Alberto et al 2010). Isolation by distance and isolation by environment are important mechanisms in driving the genetic population structure (Fernandez et al 2017). Isolation by distance could be used to predict an increase in genetic differentiation with geographic distance (Ramachandran et al 2005), but genetic structure through time is stable (Pascual et al 2016).

Ocean currents are also an important factor affecting the distribution of marine animals and plants (Hays 2017). The dynamics of the ocean are highly variable and are driven by multiple forcing factors such as solar insolation, winds, tides and freshwater input, and this variability influences the development of distinct water masses of different hydrographic properties in both vertical and horizontal dimensions (McManus et al 2012). All these are related with food chain and habitat distribution that impact the availability of living needs of marine animal's dispersal. Therefore, physical structures and processes are directly related to the swimming and behavioral capabilities of an individual or species, such as *S. latimanus*.

Conclusions. *Sepia latimanus* of North Sulawesi waters had sufficient genetic variations. These occur between and within localities. Genetic similarity was also present between *S. latimanus* from different localities, such as Basaan-2 and Likupang-1 samples and Bitung and Arakan samples. Eleven nucleotide sequences analyzed formed only 9 haplotypes due to the haplotype similarity between localities, and this condition has reflected genetic mixture. Moreover, *S. latimanus* sample of Manado bay had closer kindship to that of NCBI data for Philippines waters. This condition is supported by habitat and food distribution, ocean currents, and the ability of *S. latimanus* to exploit different niches through behavioral adaptation to different environmental condition.

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