## rahoo × +

| NEWS                       | FINANCE        | SPORTS   | ENTERTAINMENT     | LIFE        | SHOPPING     | YAHOO PLUS       | MORE  |
|----------------------------|----------------|--|-------------------|-------------|--------------|------------------|---|
| Find m                     | essages, doci  | uments, phot   | os or people      |             | ~            | Q                |   |
| ← Bac                      | k 🐴 🐗          | +  | Archive           | Move        | 💼 Delete     | Spam 🚥           | * • X   |
| art                        | icle submiss   | sion   |                   |             |              |                  | Yahoo/Sent 🔺  |
| B                          |                | a <b>sik</b> <spjong0<br>aie Ionel Claudi</spjong0<br> | 7@yahoo.com><br>u |             |              | <b>a</b> 0       | Thu, Apr 25, 2019 at 8:18 PM 🔺                              |
| l he<br>Wat<br>Run<br>Sind | ters, Indonesi | new article "G<br>a" to publish                        |                   | hope it cou | ld meet your | requirement. Oth | ollusk, in North Sulawesi<br>er revised papers of<br>ik you |
| 1000                       | etic Vadocx    |  |                   |             |              |                  |   |
| 1.4ME                      | 3              |  |                   | h «h 1      | • •••        |                  |   |
| rch                        |                | Ħ  | i 📄 🍪             | 🚺 🚖         | <b>?</b> (   |                  |   |

mail.yahoo.com/d/folders/2/messages/AH\_7MKNkh\_0bXMGIfwAbWIpALHM?guce\_referrer=aHR0cHM6Ly93d3cuZ29vZ2xILmNvbS8&guce\_

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

<sup>1</sup>Silvester B. Pratasik, <sup>2</sup>Hariyani Sambali, <sup>3</sup>Lefrand. Manoppo, <sup>1</sup>Frans F. Tilaar, <sup>1</sup>Meiske S. Salaki

<sup>1</sup>Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>2</sup>Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup>Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; Indonesia

Correspondence: S.B. Pratasik, spjong07@yahoo.com

**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.

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 eqgs (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

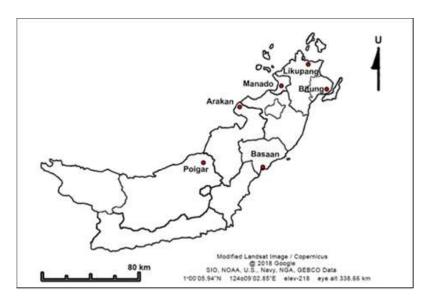


Figure 1. Map of North Sulawesi with sampling points.

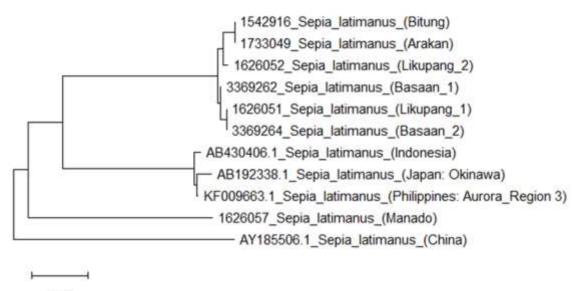
North Sulawesi waters, while the second four sites represent the northern part of the island, so that these sites have represented the entire North Sulawesi marine waters. Sampling activities were carried out for 5 months from May to September 2018. Sampling activities employed SCUBA dives over different 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).



0.010

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 kindship 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.

We would appreciate the Ministry of Research and Technology, Directorate General of Higher Education for financially supporting this study through University Superior Basic Research Grant 2018. Great gratitude is also given to Mr. Andre Kambey and Ms. Inggrid Akerina for field sampling activities.

#### References

- Alberto F., Raimondi P. T., Reed D. C., Coelho N. C., Lebois R., Whitmer A., Serräo E. A., 2010 Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. Ecology, 91(1), 2010, pp. 49–56.
- Allen J. J., Mäthger L. M., Barbosa A., Hanlon R. T., 2009 Cuttlefish use visual cues to control three-dimensional skin papillae for camouflage. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural and Behavioral Physiology 195:547–555.
- Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W., Lipman D. J., 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acids Research 25:3389-3402.
- Arkley, K., M.S. Jacklin, M. Boulter dan J. Tower, 1996. Cuttlefish: a guide to its exploitation in UK waters. The sea fish Industry Authority. Seafish Report no. SR467. 81 pp.
- Barbosa A., Mäthger L. M., Buresch K. C., Kelly J. C., Chubb C., Chiao C., Hanlon R. T., 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive patterns. Vision Research 48:1242–1253.
- Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2011 Cuttlefish use visual cues to determine arm postures for camouflage. Proceedings of the Royal Society B Biological Sciences doi:10.1098/rspb.2011.0196.
- Chiao C.-C., Chubb C., Buresch K. C., Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2010 Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. Journal of Experimental Biology 213:187–199.
- Drummond A. J., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowitz S., Moir M., Stones-Havas S., Sturrock S., Thierer T., Wilson A., 2012 Geneious v5.5.6, http://www.geneious.com
- Fern ´andez L. D.,<sup>†</sup>, Hern ´andez C. E., Schiaffino R. M., Izaguirre I., Lara E., 2017 Geographical distance and local environmental conditions drive the genetic population structure of a freshwater microalga (Bathycoccaceae; Chlorophyta) in Patagonian lakes. FEMS Microbiology Ecology, 2017, Vol. 93, No. 10. 10 pp.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5):294-299.
- Hanlon, R. T., C.-C. Chiao, L.M. Ma<sup>"</sup>thger, A. Barbosa, K.C. Buresch, dan C. Chubb, 2009. Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Phil.Trans. R. Soc. B364, 429 – 437.
- \*\*\* IUCN, 2014 The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 25 September 2014.

- Kelman E. J., Osorio D., Baddeley R. J., 2008 A review of cuttlefish camouflage and object recognition and evidence for depth perception. Journal of Experimental Biology 211:1757–1763.
- Kumar S., Stecher G., Li M., Knyaz C., & Tamura K. 2018 MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
- Leopold A. 1933. Game Management. Charles Scribner's Sons. New York. 481 p.
- McManus M. A., Woodson C. B., 2011. REVIEW Plankton distribution and ocean dispersal. The Journal of Experimental Biology 215, 1008-1016. Doi:10.1242/jeb.059014.
- Murphy J. M., Balgueri ´as, E., Key L. N., Boyle P. R. 2002 Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the Northwest African Coast. Bulletin of Marine Science, 71: 545–553.
- Neves A., Cabral H. N. Sequeira V., Figueiredo I., Moura T., Gordo L. S., 2009 Distribution patterns and reproduction of the cuttlefish, Sepia officinalis in the Sado estuary. (Portugal) Journal of the Marine Biological Association of the United Kingdom 89(3):579– 584.
- \*\*\* NOVA, 2007 Cuttlefish: kings of camouflage. (Television program) NOVA, PBS, April 3, 2007).
- Pascual M., Palero F., García-Merchán V. H., Macpherson E., Robainas-Barcia A., Mestres F., Roda T., Abelló P., 2016. Temporal and spatial genetic differentiation in the crab Liocarcinus depurator across the Atlantic-Mediterranean transition. Scientific RepoRts | 6:29892 | DOI: 10.1038/srep29892. 10 pp.
- Pawson, M.G., 1995. Bio-geographical Identification of English Channel fish and shellfish stocks. CEFAS Fisheries Research Technical Report No 99.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2015 Size at First maturity of cuttlefish, *Sepia latimanus*, from North Sulawesi Waters, Indonesia. Marine Science 5(1): 6-10 DOI: 10.5923/j.ms.20150501.02
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2016 Mitochondrial CO1 genetic markerbased species diversity of cuttlefish (Cephalopod; Mollusk) in Manado Bay and Lembeh Strait, North Sulawesi, Indonesia. AACL Bioflux 9(6):1345-1354.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2017 Egg placement habitat selection of cuttlefish, Sepia latimanus (Sepiidae, Cephalopoda, Mollusca) in North Sulawesi waters, Indonesia. AACL Bioflux 10(6):15141523.
- Ramachandran S., Deshpande O., Roseman C. C., Rosenberg N. A., Feldman M. W., Cavalli-Sforza L. L., 2005 Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. PNAS vol. 102, no. 44. 6 pp.
- Ratnasingham S., Hebert P. D. N., 2007 BOLD: the barcode of life data system. Molecular Ecology Notes 7:355–364.
- Reid A., Jerep P., Roper C. F. E., 2005 Family Sepiidae. In: Cephalopods of the world. An annotated and illustrated catalogue of Cephalopod species known to date. Volume 1.

Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). Jerep P. & Roper C.F.E. pp 54-152. FAO, Rome.

- Rozas J., Ferrer-Matta A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., Sánchez-Gracia A., 2017 DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12): 3299-3302. Doi 10/1093/molbev/msx248.
- Shohet A. J., Baddeley R. J., Anderson J. C., Kelman E. J., Osorio D., 2006 Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage. Journal of Experimental Biology 209:4717–4723.

Authors:

Silvester B. Pratasik, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, email:spjong07@yahoo.com.

Harjany Sambali, Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, email: hariyanisambali@unsrat.ac.id.

Lefrand Manoppo, Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, email: lefrandmanoppo@yahoo.com.

Frans.F. Tilaar, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, email:fftilaar@unsrat.co.id.

Meiske S. Salaki, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, mssalaki@unsrat.co.id.

|     |                                 | _       |         | _       |         | _       | _       | _       | _       | _       |         |
|-----|---------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| No. |                                 | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      |
|     | 1542916_ <i>Sepia_latimanus</i> |         |         |         |         |         |         |         |         |         |         |
| 1   | (Bitung)                        |         |         |         |         |         |         |         |         |         |         |
|     | 1626051_ <i>Sepia_latimanus</i> |         |         |         |         |         |         |         |         |         |         |
| 2   | (Likupang_1)                    | 0.00435 |         |         |         |         |         |         |         |         |         |
|     | 1626052_ <i>Sepia_latimanus</i> |         |         |         |         |         |         |         |         |         |         |
| 3   | (Likupang_2)                    | 0.00309 | 0.00371 |         |         |         |         |         |         |         |         |
|     | 1626057_ <i>Sepia_latimanus</i> |         |         |         |         |         |         |         |         |         |         |
| 4   | (Manado)                        | 0.06984 | 0.06983 | 0.07071 |         |         |         |         |         |         |         |
|     | 1733049_ <i>Sepia_latimanus</i> |         |         |         |         |         |         |         |         |         |         |
| 5   | (Arakan)                        | 0.00000 | 0.00435 | 0.00309 | 0.06984 |         |         |         |         |         |         |
|     | 3369262_Sepia_latimanus         |         |         |         |         |         |         |         |         |         |         |
| 6   | (Basaan_1)                      | 0.00435 | 0.00123 | 0.00247 | 0.06820 | 0.00435 |         |         |         |         |         |
|     | 3369264_ <i>Sepia_latimanus</i> |         |         |         |         |         |         |         |         |         |         |
| 7   | (Basaan_2)                      | 0.00435 | 0.00000 | 0.00371 | 0.06983 | 0.00435 | 0.00123 |         |         |         |         |
|     | AB192338.1_Sepia_latimanus      |         |         |         |         |         |         |         |         |         |         |
| 8   | (Japan: Okinawa)                | 0.05800 | 0.05627 | 0.05704 | 0.06232 | 0.05800 | 0.05549 | 0.05627 |         |         |         |
|     | AB430406.1_Sepia_latimanus      |         |         |         |         |         |         |         |         |         |         |
| 9   | (Indonesia)                     | 0.05555 | 0.05384 | 0.05461 | 0.06152 | 0.05555 | 0.05307 | 0.05384 | 0.00434 |         |         |
|     | AY185506.1_Sepia_latimanus      |         |         |         |         |         |         |         |         |         |         |
| 10  | (China)                         | 0.07853 | 0.07747 | 0.07747 | 0.07531 | 0.07853 | 0.07581 | 0.07747 | 0.07725 | 0.07739 |         |
|     | KF009663.1_Sepia_latimanus      |         |         |         |         |         |         |         |         |         |         |
| 11  | (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 1. Genetic distance of cuttlefish *Sepia latimanus* in North Sulawesi waters.

|  |     |                |          |            |   |       |   |   |     |       |   |          |     |       |        |    |          | ١   | Nin  | dow      | 14    | 50    |           |     |       |   |    |      |     |   |   |   |      |     |                        |     |      |          |        |    |
|--|-----|----------------|----------|------------|---|-------|---|---|-----|-------|---|----------|-----|-------|--------|----|----------|-----|------|----------|-------|-------|-----------|-----|-------|---|----|------|-----|---|---|---|------|-----|------------------------|-----|------|----------|--------|----|
|  |     |                |          |            |   |       |   |   |     |       |   |          |     |       |        |    |          |     |      |          |       |       |           |     |       |   |    |      |     |   |   |   |      |     |                        |     |      |          |        |    |
|  |     |                |          |            |   |       |   |   |     |       |   |          |     |       |        |    |          |     |      |          |       |       |           |     |       |   |    |      |     |   |   |   |      |     |                        |     |      |          |        |    |
| 1 1542916 Sepia la   | T   | t 1            | r g      | a          | T | C a   | G | G | T   | Т     | A | T        | ΤĢ  | g G   | G      | g  | A C      | a   | T    | C        | A t   | : Т   | A         | A   | ; t   | t | T. | A A  | V T | t | A | T | t C  | G   | t                      | Α   | Gt   | t G      | A      | a  |
| 2 1626051 Sepia la   |     |                | • •      |            |   |       |   |   |     |       |   |          |     |       |        |    |          |     |      |          |       | •     |           |     | •     |   |    |      |     |   |   |   |      |     |                        | •   | •    |          |        |    |
| 3 1626052 Sepia la   | ·   | <u>.</u>       | <u>.</u> |            |   |       |   | • |     |       |   |          |     |       |        | •  | <u>.</u> |     |      | •        |       | •     |           | •   | ·     |   |    | •    | •   |   | • |   |      |     |                        | -   | ÷    |          |        |    |
| 4 1626057 Sepia la   |     | <b>a</b> .     | .  .     | g          |   |       |   |   | •   |       |   |          | . ; | a .   |        | t  | • •      |     | •    |          | . c   | : .   |           | •   | a     | С |    | •    | •   |   | • | • | с.   |     | a                      | •   | •    |          |        | •  |
| 5 1733049 Sepia la   | ·   | <u>.</u>       | ·        | •          | • |       |   | • | •   |       | • | •        | •   | • •   | •      | •  | <u>.</u> |     | ·    | •        | • •   | •     | •         | •   | ·     | ŀ | •  | •    | •   | • | • | • |      | ŀ   | ŀ                      | -   | ÷    | <u>.</u> | •      | •  |
| 6 3369262 Sepia la   | •   | •              | · ŀ·     | •          | • | • •   | • | • | •   | .   . | • | •        | •   | • •   | •      | •  | • •      | •   | •    | •        | • •   | •     | •         | •   | ·     | ŀ | •  | •    | •   | • | • | • |      |     | ŀ                      | •   | ·    |          | •      | •  |
| 7 3369264 Sepia la   | ·   | •              | ·  ·     | •          | • | •   • | • | • | ·   | •  •  | • | •        | •   | • •   | •      | •  | • •      | •   | ·    | •        | • •   | •     | •         | •   | ŀ     | ŀ | •  | •    | •   | • | · | • | • •  |     | ŀ                      | ·   | ÷    | • •      | •      | ·  |
| 8 AB192338.1 Sepia   |     | •              | ·  ·     | g          | • | . g   |   | • | ·   | •   • | • | ·        | -   | a .   |        | a  | • •      | •   | ·    | •        | . c   | -     | •         | •   | ·     | ŀ | •  | •    | •   | ŀ | · | • | • •  | •   | ŀ                      | ·   | -    | a.       | -      | g  |
| 9 AB430406.1 Sepia   |     | •              | ·        | ŀ          | • | . g   | Ŀ | • | ·   | • •   | • | •        | . : | +     | -      | a  | • •      | +-  | ŀ    | $\vdash$ | . c   |       | •         | •   | •     | • | •  | •    | •   | • | · | • | • •  | •   | $\left  \cdot \right $ | ·   | -    | a.       | •      | g  |
| 10 AY185506.1 Sepia  | ŀ   | •              | ÷        | ·          | • | •  •  | • | • | ·   | •  •  | • | •        | . 8 | +     | -      | t  | • •      | t   | ·    | •        | • •   | •     | •         | •   | ŀ     | ŀ | •  | •    | •   | a | · | · | • •  | ŀ   | a                      | ÷   | -    | a.       | •      | ·  |
| 11 KF009663.1 Sepia  | •   | •              |          | g          | • | . g   | • | • | •   |       | • | •        | . a | a .   | •      | a  |          | •   | •    | •        | . 0   |       | •         | •   | •     | • | •  | •    | •   | • | • | • |      | •   | •                      | •   | . a  | a.       | •      | g  |
|  | _   |                |          | _          |   |       |   |   |     |       |   |          |     |       |        |    |          | 1   | Nin  | dow      | 51    | -100  | 0         |     |       |   |    |      |     |   |   |   |      |     |                        |     |      |          |        |    |
|  |     |                |          |            |   |       |   |   |     |       |   |          |     |       |        |    |          |     |      |          |       |       |           |     |       |   |    |      |     |   |   |   |      |     |                        |     |      |          |        |    |
| 1 1542916 Sepia la   | -   | Tg             | g G      | G          | t | A A   | A | С | C   | c G   | G | T        | A ( | C a   | t      | T  | a t      | T   | A    | A        | A t   | G     | A         | t   | ; A   | c | C  | A    | 1 t | T | A | T | A t  | A   | A                      | T   | G 1  | ſc       | a      | T  |
| 2 1626051 Sepia la   | Ŀ   |                | ·   ·    | ·          | • | .   . |   | • | •   | .   . | • |          | -   | •   • | •      | •  | ·   ·    |     | •    | •        | .  .  | ŀ     |           | •   |       | • | •  | •    |     |   | • | • | •  • | ŀ   | ·                      | ÷   | •    | ·   ·    |        | •  |
| 3 1626052 Sepia la   | ·   | •              | ·  ·     | ŀ          | • | •   • | • | • | ·   | •   • | • | •        | -   | •   • | •      | •  | · ·      |     | ·    | •        | •  •  | ·     | ŀ         | -   | ·     | • | •  | •    | ŀ   | ŀ | • | • | •  • | ŀ   | ŀ                      | ÷   | ÷    | . t      | +-     | ŀ  |
| 4 1626057 Sepia la   | ·   | . a            | a.       | ŀ          | • | •   • | • | • | •   | a .   | • | •        | -   | •   • | С      | •  | t c      |     | ·    | •        | . 0   |       | ŀ         | C   | ·     | t | •  | •    | C   | • | · | • | •  • | ŀ   | ŀ                      | ÷   | ÷    | . t      | -      | ŀ  |
| 5 1733049 Sepia la<br>6 3369262 Sepia la   | ŀ   | •              | ÷        | ŀ          | • | •  •  | • | • | ·   | •  •  | • | •        | -   | •  •  | •      | •  | • •      | +   | ·    | •        | • •   | ·     | ŀ         | •   | ÷     | ŀ | ·  | •    | ŀ   | ŀ | • | • | •  • | ŀ   | ŀ                      | ÷   | ÷    | <br>. t  | ·      | ŀ  |
| 7 3369264 Sepia la   | ÷   | - -            | ·   ·    | ÷          | • | • •   | • | • | ·   | •   • | • | •        | -   | • •   | •      | •  | ·   ·    | •   | •    | •        | •     | ·   · | •         |     | ·   · | ŀ | •  | •    | +   | • | • | • | • •  | ÷   | •                      | ·   | +    |          |        | ŀ  |
| 8 AB192338.1 Sepia   |     |                | а.       |            |   |       |   |   |     |       |   |          |     |       | c      |    | . c      |     |      |          |       |       |           |     |       | t |    |      |     |   |   |   |      |     |                        |     |      | . t      |        |    |
| 9 AB430406.1 Sepia   |     | . a            | a .      |            |   |       |   |   |     |       |   |          |     |       | с      |    | . c      |     |      |          |       |       |           |     |       | t |    |      |     |   |   |   | . c  |     |                        |     |      | . t      |        |    |
| 10 AY185506.1 Sepia  |     | . a            | a .      |            | a |       |   |   | . 1 | а.    |   |          |     | . t   | с      |    |          |     |      |          |       |       |           |     |       | t |    |      |     |   |   |   |      |     |                        |     |      | . t      | g      |    |
| 11 KF009663.1 Sepia  |     | . a            | a .      |            |   |       |   |   |     |       |   |          |     |       | с      |    | . c      | : . |      |          |       |       |           |     |       | t |    |      |     |   |   |   | . c  | : . |                        |     |      | . t      |        |    |
|  |     |                | _        |            |   | _     | _ |   | _   | _     |   |          |     |       |        |    | _        | ١   | Vinc | dow:     | 10    | 1-18  | 50        |     |       | _ |    |      | _   | _ |   | _ | _    | _   |                        |     |      | _        |        |    |
| 1 1542916 Sepia la   | Δ   | 6.1            | π +      | - A        | C |       |   |   |     | A +   | 6 | C        | + 1 | րդր   |        | Δ. | π +      | - A | Ψ    | 2        | λ Π   | • +   | π         | π.  | , т   |   | +  | + 1  |     | C | π | - | ΔΨ   | - A | C                      | C 1 | т 2  | <u></u>  | +      | Δ  |
| 2 1626051 Sepia la   |     |                | -        | -          |   |       | - |   |     |       |   | 3        |     | -     | -      |    |          | -   | •    |          |       |       |           |     | -     |   |    | - 1  | -   | 3 | • | - |      | -   |                        | -   | -    |          |        | ** |
| 3 1626052 Sepia la   | Ĥ   | i ti           | +        | +-         |   | +     | · | • | +   | +     | + | •        | •   | +     | ŀ      | •  | +        | +   | •    | •        |       | c     | $\square$ | •   |       |   | •  |      | +   | • | • | • |      | ÷   | $\left  \cdot \right $ | +   | +    | -        | ·<br>c | •  |
| 4 1626057 Sepia la   | Ĥ   | ·   ·          |          | +          | • | •     | • | • | •   | .   . |   | •        | •   | +     | ·<br>t | •  | +        |     | •    | •        | •   • |       | •         | •   | •     | • | •  |      |     | • | • | • | •    | ÷   | ++                     | •   | +    |          | -      | •  |
| 5 1733049 Sepia la   |     | •••            | +•       | ÷          | • | •   • | • | • | •   | •   • | • | •        | a . | •     |        | •  | +        | •   | •    | •        | •   • | •     | •         | •   | •     | • | C  | • .  | •   | • | • | • | •  • | ŀ   | $\left  \cdot \right $ | +   | +    | •        | •      | •  |
| -  | ť   | • •            | +•       | ŀ          | · | • • • | • | · | •   | +•    | • | ·        | •   | •     | ·      | •  | • •      | •   | ·    | •        | •   • | •     | ·         | +   | •     | • | •  | • •  | •   | • | • | • | •  • | ŀ   | $\left  \cdot \right $ | +   | +    | •        | ·      | •  |
| 6 3369262 Sepia la   | Ŀ   | • •            | +        | ŀ          | • | •   • | • | • |     | •   • | • | ·        | •   | ·     | ŀ      | •  | ÷        | ŀ   | ŀ    | •        | •   • | c     | $\square$ | •   | +     | · | •  | • •  | •   | • | • | • | • •  | ŀ   | $\left  \cdot \right $ | +   | +    |          | ŀ      | •  |
| 7 3369264 Sepia la   | ·   | • •            | +•       | •          | • | • •   | • | • | •   | • •   | • | ·        | •   | •     | +      | •  | ÷        | ŀ   | ŀ    | •        | • •   | C     | ŀ         | • • | +-    | • | •  | • •  | •   | • | • | • | • •  | ŀ   | ·                      | +   | +    | •        | ·      | •  |
| 8 AB192338.1 Sepia   | Ŀ   | • •            | • •      | ·          | • | •  •  | • | • | •   | •   • | • |          | g.  | •     | +      | •  | ÷        | •   | •    | •        | •   • | •     | ·         | -   | ; .   | • | C  | • •  | •   | • | • | • | •  • | ·   | $ \cdot $              | •   | ·+·  | • •      | •      | •  |
|  | 4 1 | ( <b>.</b> ] . |          |            | • | • •   | • | • | •   | ·  ·  | • | •        | g.  | · ·   | t      | •  | • •      | •   | ·    | •        | •   • | •     | ·         | . t | ; .   | • | С  | •  • | •   | • | • | • | • •  | ŀ   | ·                      | •   | ···  | • •      | •      | •  |
| 9 AB430406.1 Sepia   | ÷   | $\vdash$       | -        |            |   |       |   |   | - I |       |   |          |     |       | 1      |    |          |     |      |          |       |       |           |     |       |   |    |      |     |   |   |   |      |     |                        |     |      |          |        |    |
| <ul> <li>9 AB430406.1 Sepia</li> <li>10 AY185506.1 Sepia</li> <li>11 KF009663.1 Sepia</li> </ul> | •   |                | . a      | <b>.</b> . |   |       |   |   | •   | . c   |   | $\vdash$ |     | •     | -      | _  | . c      | _   | •    |          | •   • | •     | •         | • • |       | • | •  | · ·  |     | • |   | t | •  • |     | •                      | •   | •  • | •        | ·      | •  |

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

|    |                  | - |   |     |     |   |   | _   |     |   | _ |   |   |   | _ |     | _     | _ |   |   | _   |   |   | _   | _   |   |           |   | _ |     |   |   |   | _   | _   | - | - |   |   | _   | -   |   |   |
|----|------------------|---|---|-----|-----|---|---|-----|-----|---|---|---|---|---|---|-----|-------|---|---|---|-----|---|---|-----|-----|---|-----------|---|---|-----|---|---|---|-----|-----|---|---|---|---|-----|-----|---|---|
|    |                  | - |   | +   | _   | - |   | _   | _   | + | - | - |   |   | - | _   | +     | - |   | _ | _   | + |   | +   | +   | + | $\square$ |   | + | _   | - |   |   | +   | -   | + | - |   | _ | _   | +   |   | - |
| 1  | 1542916 Sepia la | т | A | A 1 | r t | G | G | t ( | ; G | g | Т | Т | Т | G | G | T 2 | A A   | c | Т | G | a T | т | A | g ! | r t | С | С         | t | т | T A | A | Т | A | c ' | т А | G | G | a | G | Co  | ; C | С | 2 |
| 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.  |   |   |     |     |   |           | с |   |     |   |   |   | t   |     |   |   | g |   | . a | ι.  |   |   |
| 11 | KF009663.1 Sepia |   |   |     | . c |   |   | a.  |     | t |   |   |   |   |   |     |       | t |   |   |     |   |   | a   |     |   |           |   |   |     |   |   |   | t   |     |   |   |   |   | . t | ; . |   |   |

|    |                  |   |   |   |   |     |     |   |   |     |       |     |   |   |   |     |     |   |   |   | W | indo | w: 1 | 201- | -250 | )  |   |   |     |     |   |   |   |     |    |     |   |   |   |     |     |   |   |
|----|------------------|---|---|---|---|-----|-----|---|---|-----|-------|-----|---|---|---|-----|-----|---|---|---|---|------|------|------|------|----|---|---|-----|-----|---|---|---|-----|----|-----|---|---|---|-----|-----|---|---|
|    |                  |   |   |   |   |     |     |   |   |     |       |     |   |   |   |     |     |   |   |   |   |      |      |      |      |    |   |   |     |     |   |   |   |     |    |     |   |   |   |     |     |   |   |
| 1  | 1542916 Sepia la | G | A | t | A | т 2 | A G | c | t | T 1 | r t   | : C | С | a | С | G   | t A | Т | A | A | A | c A  | A    | Т    | A    | ТА | A | G | a 1 | r T | Т | T | G | a ' | тт | ' A | c | т | A | C ( | : c | С | С |
| 2  | 1626051 Sepia la |   |   |   |   |     |     |   |   |     |       |     |   |   |   |     |     |   |   |   |   |      |      |      |      |    |   |   |     |     |   |   |   |     |    |     |   |   |   |     |     |   |   |
| 3  | 1626052 Sepia la |   |   |   |   |     |     |   |   |     |       |     |   |   |   |     |     |   |   |   |   |      |      |      |      |    |   |   |     |     |   |   |   |     |    |     |   |   |   |     |     |   |   |
| 4  | 1626057 Sepia la |   |   |   |   |     |     |   | a |     |       |     |   |   |   | . i | 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 |   |   | с |   |     |     |   | с |     |       | : . |   | t |   | . i | a.  |   |   |   |   | t.   |      |      |      |    |   |   | t.  |     |   |   |   | g   |    |     | t |   |   |     | t   |   |   |
| 11 | KF009663.1 Sepia |   |   |   |   |     |     |   | a |     |       |     |   |   |   |     |     |   |   |   |   |      |      |      |      |    |   |   |     |     |   |   |   |     |    |     | t |   |   |     |     |   |   |

|   |                  |   |     |       |     |   |   |   |   |     |       |     |   |   |   |   |       |     |     |   | W | lind | ow: | 25    | 1-30 | )() |   |   |      |   |   |   |       |   |   |   |   |   |   |     |     |   |   |   |
|---|------------------|---|-----|-------|-----|---|---|---|---|-----|-------|-----|---|---|---|---|-------|-----|-----|---|---|------|-----|-------|------|-----|---|---|------|---|---|---|-------|---|---|---|---|---|---|-----|-----|---|---|---|
|   |                  |   |     |       |     |   |   |   |   |     |       |     |   |   |   |   |       |     |     |   |   |      |     |       |      |     |   |   |      |   |   |   |       |   |   |   |   |   |   |     |     |   |   | - |
|   | 1542916 Sepia la | a | T ( | C a   | T   | T | A | a | С | a d | : T   | ! a | c | Т | t | t | T a   | 1 T | ' C | с | Т | C    | a 1 | r c   | t    | a   | t | Т | a T  | A | G | A | A A   | G | a | G | G | t | G | C a | ı G | G | t | A |
|   | 1626051 Sepia la |   |     |       |     |   |   |   |   |     | .   . |     |   |   |   |   |       |     |     |   |   |      |     |       |      |     |   |   |      |   |   |   |       |   |   |   |   |   |   |     |     |   |   |   |
|   | 1626052 Sepia la |   |     | .   . |     |   |   |   |   |     | .   . |     |   |   |   |   | .   . |     |     |   |   |      |     | .   . |      |     |   |   | .  . |   |   |   | .   . |   |   |   |   |   |   |     |     |   |   |   |
|   | 1626057 Sepia la | t |     |       |     |   |   |   |   |     |       |     |   |   |   |   |       |     |     | a |   |      |     |       |      |     |   |   |      |   |   |   |       |   |   |   |   | a |   | . t | : . |   |   |   |
|   | 1733049 Sepia la |   |     |       |     |   |   |   |   |     |       |     |   |   |   |   |       |     |     |   |   |      |     |       |      |     |   |   |      |   |   |   |       |   |   |   |   |   |   |     |     |   |   |   |
|   | 3369262 Sepia la |   |     |       |     |   |   |   |   |     |       |     |   |   |   |   |       |     |     |   |   |      |     |       |      |     |   |   |      |   |   |   |       |   |   |   |   |   |   |     |     |   |   |   |
|   | 3369264 Sepia la |   |     |       |     |   |   |   |   |     |       |     |   |   |   |   |       |     |     |   |   |      |     |       |      |     |   |   |      |   |   |   |       |   |   |   |   |   |   |     |     |   |   |   |
|   | AB192338.1 Sepia | t |     |       |     |   |   |   |   | . t | ι.    |     | t |   | a | с | . t   | : . |     | a |   |      |     |       |      |     |   |   |      |   |   |   |       |   | t |   |   |   |   |     |     |   |   |   |
|   | AB430406.1 Sepia | t |     |       |     |   |   | g |   | . t | ι.    |     | t |   | a | с | . t   | : . |     | a |   |      |     |       |      |     |   |   |      |   |   |   |       |   | t |   |   |   |   |     |     |   |   |   |
| 0 | AY185506.1 Sepia |   |     | . t   | ; . |   |   |   |   | t.  |       | t   | t |   | a |   |       |     |     | a |   |      | c.  |       | a    | g   | с |   | g .  |   |   |   |       |   |   |   |   |   |   | . t | ; . |   | a |   |
| 1 | KF009663.1 Sepia | t |     |       |     |   |   |   |   | . t | ε.    |     | t |   | a | с | . t   |     |     | a |   |      |     |       |      |     |   |   |      |   |   |   |       |   | t |   |   |   |   |     |     |   |   |   |

|    |          |         |     |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   | V | Vind | ow: | 301 | -35 | 0   |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   |           |   |   |
|----|----------|---------|-----|---|-----|-----|-----|---|---|-----|---|---|---|-----|-----|-----|---|---|---|-----|---|---|------|-----|-----|-----|-----|-----|---|---|-----|-----|-----|---|---|---|---|-----|-----|---|---|---|-----------|---|---|
|    |          |         |     |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   |   |      |     |     |     |     |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   | $\square$ |   | _ |
| 1  | 1542916  | Sepia   | la  | С | t ( | G C | ; A | Т | G | A A | C | A | G | T   | t 1 | r A | t | C | C | t C | C | t | T    | r 2 | A T | С   | a j | A G | t | A | A ! | T t | ; T | a | T | C | a | C 1 | A c | G | C | t | G         | 3 | a |
| 2  | 1626051  | Sepia   | la  |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   |   |      |     |     |     |     |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   |           |   |   |
| 3  | 1626052  | Sepia   | la  |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   |   |      |     |     |     |     |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   |           |   |   |
| 4  | 1626057  | Sepia   | la  |   | a   |     |     |   |   |     |   |   |   | . 4 | a . |     |   |   |   | a . |   | a |      |     |     |     |     |     | a |   |     | . c |     | с |   |   |   |     | . t |   |   | a |           |   |   |
| 5  | 1733049  | Sepia   | la  |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   |   |      |     |     |     |     |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   |           |   |   |
| 6  | 3369262  | Sepia   | la  |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   |   |      |     |     |     |     |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   |           |   |   |
| 7  | 3369264  | Sepia   | la  |   |     |     |     |   |   |     |   |   |   |     |     |     |   |   |   |     |   |   |      |     |     |     |     |     |   |   |     |     |     |   |   |   |   |     |     |   |   |   |           |   |   |
| 8  | AB19233  | .1 Seg  | oia |   |     |     |     |   |   |     |   |   |   | . ( | c . |     | с |   |   | a . |   | a |      |     |     |     | t   |     |   |   |     |     |     |   |   |   |   |     | . t |   |   |   |           |   |   |
| 9  | AB43040  | 5.1 Seg |     |   |     |     |     |   |   |     |   |   |   |     |     |     | с |   |   | a . |   | a |      |     |     |     | t   |     |   |   |     |     |     |   |   |   |   |     | . t |   |   |   |           |   |   |
|    | AY18550  |         |     |   |     |     |     |   |   |     |   |   |   |     | a . |     |   |   |   |     |   | с |      |     |     |     | t   |     |   |   |     | . c |     |   |   |   | t |     | . t |   |   |   |           |   | c |
| 11 | KF009663 | 3.1 Seg | oia |   |     |     |     |   |   |     |   |   |   | . ( | c . |     | с |   |   | a . |   | a |      |     |     |     | t   |     |   |   |     |     |     |   |   |   |   |     | . t |   |   |   |           |   |   |

|    |                  |   |   |   |   |   |   |     |     |     |   |   |   |   |   |   |     |   |   |   |     | Wii | ndo | N: S | 51- | 400 |     |     |   |   |     |       |     |   |   |   |   |     |     |     |   |   |     |   |
|----|------------------|---|---|---|---|---|---|-----|-----|-----|---|---|---|---|---|---|-----|---|---|---|-----|-----|-----|------|-----|-----|-----|-----|---|---|-----|-------|-----|---|---|---|---|-----|-----|-----|---|---|-----|---|
|    |                  |   |   |   |   |   |   |     |     |     |   |   |   |   |   |   |     |   |   |   |     |     |     |      |     |     |     |     |   |   |     |       |     |   |   |   |   |     |     |     |   |   |     |   |
| L  | 1542916 Sepia la | C | C | a | Т | C | a | G 1 | r 1 | t G | A | T | c | Т | A | G | C a | A | Т | t | T 1 | l 0 | T   | C    | a   | c ' | r t | ; C | A | т | t 1 | r A   | A G | C | g | G | G | a   | a T | [ 0 | T | C | c ' | T |
| 2  | 1626051 Sepia la |   |   |   |   |   | t |     |     |     |   |   |   |   |   |   |     |   |   |   |     |     |     |      |     |     |     |     |   |   |     |       |     |   |   |   |   |     |     |     |   |   | t   |   |
| 3  | 1626052 Sepia la |   |   |   |   |   | t |     |     |     |   |   |   |   |   |   |     |   |   |   |     |     |     |      |     |     |     |     |   |   |     |       |     |   |   |   |   |     |     |     |   |   |     |   |
|    | 1626057 Sepia la |   |   | t |   |   |   | -   |     | a . |   |   | t |   |   |   | . t |   |   | с |     | . t |     |      |     |     | . a | ι.  |   |   | c.  |       |     |   | a |   |   | t   | g.  | . t |   |   |     |   |
| 5  | 1733049 Sepia la |   |   |   |   |   |   | -   |     |     |   |   |   |   |   |   |     |   |   |   |     |     |     |      |     |     |     |     |   |   |     |       |     |   |   |   |   |     |     |     |   |   |     |   |
| 5  | 3369262 Sepia la |   |   |   |   |   | t |     |     |     |   |   |   |   |   |   |     |   |   |   |     |     |     |      |     |     |     |     |   |   |     | .   . |     |   |   |   |   |     |     |     |   |   |     |   |
| 1  | 3369264 Sepia la |   |   |   |   |   | t |     |     |     |   |   |   |   |   |   |     |   |   |   |     |     |     |      |     |     |     |     |   |   |     | .   . |     |   |   |   |   |     |     |     |   |   | t   |   |
| 3  | AB192338.1 Sepia |   |   |   |   |   | t | -   |     |     |   |   | t |   |   |   | . t |   |   |   |     | . t |     |      |     |     |     |     |   |   |     |       |     |   | с |   |   | t   | g.  | . a |   |   | a   |   |
| 9  | AB430406.1 Sepia |   |   |   |   |   | t | -   |     |     |   |   | t |   |   |   | . t |   |   |   |     | . t |     |      |     |     |     |     |   |   |     |       |     |   | с |   |   | t   | g.  | . a |   |   | a   |   |
| 10 | AY185506.1 Sepia |   |   |   |   |   | t |     | . a | a . |   |   | t |   |   |   | . t |   |   |   |     |     |     |      | t   | t   | . a | ι.  |   |   |     | .   . |     |   | t |   |   | t   | g.  | . t |   |   |     |   |
| 1  | KF009663.1 Sepia |   |   |   |   |   | t |     |     |     |   |   | t |   |   |   | . t |   |   |   |     | · t |     |      |     |     |     |     |   |   |     |       |     |   | с |   |   | t / | g.  | . a |   |   | a   |   |

|   | 1542916 Sepia la | A | A | Т | t | T | r a | ı G        | G | t | G | C a | a A | Т | t | A | A 1 | r 1   | Т | t | A ' | r T | A | C | a A | C | a | A ' | T T | ' c | T | a | A | A 1 | r A   | Т | g | C | G | t T | G | A | G |
|---|------------------|---|---|---|---|---|-----|------------|---|---|---|-----|-----|---|---|---|-----|-------|---|---|-----|-----|---|---|-----|---|---|-----|-----|-----|---|---|---|-----|-------|---|---|---|---|-----|---|---|---|
|   | 1626051 Sepia la |   |   |   |   |   |     |            |   |   |   |     |     |   |   |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   |   |   |     |       |   | a |   |   |     |   |   |   |
|   | 1626052 Sepia la |   |   |   |   |   |     |            |   |   |   |     |     |   |   |   |     | .   . |   |   |     |     |   |   |     |   |   |     |     |     |   |   |   |     | .   . |   |   |   |   |     |   |   |   |
|   | 1626057 Sepia la |   |   |   |   |   | . 9 | <b>j</b> . |   |   |   | . 1 | t . |   |   |   |     | .   . |   | с |     |     |   |   | с.  |   |   |     |     | t   |   |   |   |     | .   . |   | a |   |   | a . |   |   |   |
|   | 1733049 Sepia la |   |   |   |   |   |     |            |   |   |   |     |     |   |   |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   |   |   |     |       |   |   |   |   |     |   |   |   |
|   | 3369262 Sepia la |   |   |   |   |   |     |            |   |   |   |     |     |   |   |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   |   |   |     |       |   | a |   |   |     |   |   |   |
|   | 3369264 Sepia la |   |   |   |   |   |     |            |   |   |   |     |     |   |   |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   |   |   |     |       |   | a |   |   |     |   |   |   |
|   | AB192338.1 Sepia |   |   |   |   |   |     |            |   | с |   |     |     |   | с |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   | с |   |     |       |   | a |   |   |     |   |   |   |
|   | AB430406.1 Sepia |   |   |   |   |   |     |            |   |   |   |     |     |   | с |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   | с |   |     |       |   | a |   |   |     |   |   |   |
| ) | AY185506.1 Sepia |   |   |   | с |   |     |            |   |   |   | . 1 | t . |   |   |   |     |       |   |   |     |     |   |   |     |   | t |     |     | t   |   |   |   |     |       |   | a |   |   | g.  |   |   |   |
| 1 | KF009663.1 Sepia |   |   |   |   |   |     |            |   |   |   |     |     |   |   |   |     |       |   |   |     |     |   |   |     |   |   |     |     |     |   | c |   |     |       |   | a |   |   |     |   |   |   |

|    |                  |   |   |   |    |   |   |   |       |     |   |   |   |   |   |     |     |   |   |   | W | ind | OW: | 451 | -500 | ) |       |   |   |   |   |   |       |   |   |   |   |   |   |   |     |     |    |    |
|----|------------------|---|---|---|----|---|---|---|-------|-----|---|---|---|---|---|-----|-----|---|---|---|---|-----|-----|-----|------|---|-------|---|---|---|---|---|-------|---|---|---|---|---|---|---|-----|-----|----|----|
|    |                  | Γ |   |   | Τ  |   |   |   | Τ     |     | Γ |   |   |   |   |     |     | Γ |   |   |   |     |     |     |      |   | Τ     |   |   |   |   |   | Τ     |   |   |   |   |   |   | Τ | Τ   | Τ   |    |    |
|    |                  |   |   |   |    |   |   |   |       |     |   |   |   |   |   |     |     |   |   |   |   |     |     |     |      |   |       |   |   |   |   |   |       |   |   |   |   |   |   |   |     |     |    |    |
|    | 1542916 Sepia la | A | g | G | GT | с | T | A | C   2 | ٩   | A | T | A | G | A | A C | : G | g | t | Т | a | c   | C   | t   | T    | A | r   1 | T | G | T | t | T | GA    | T | C | t | G | Т | a | T | T t | t A | T  | T  |
| 2  | 1626051 Sepia la |   |   |   |    | t |   |   |       |     |   |   |   |   |   |     |     | a |   |   |   |     |     |     |      |   | .   . |   |   |   |   |   | .   . |   |   |   |   |   | g |   |     |     |    |    |
| )  | 1626052 Sepia la |   |   |   |    |   |   |   |       |     |   |   |   |   |   |     |     | a |   |   |   |     |     |     |      |   |       |   |   |   |   |   |       |   |   |   |   |   |   |   |     |     |    |    |
|    | 1626057 Sepia la |   | a |   |    | t |   |   |       | . a |   |   |   |   |   |     |     | a | a |   |   |     |     |     |      |   |       |   |   |   |   |   |       |   |   |   |   |   | t |   | . 0 | ; . |    |    |
| 5  | 1733049 Sepia la |   |   |   |    |   |   |   |       |     |   |   |   |   |   |     |     |   |   |   |   |     |     |     |      |   |       |   |   |   |   |   |       |   |   |   |   |   |   |   |     |     |    |    |
| ;  | 3369262 Sepia la |   |   |   |    | t |   |   |       |     |   |   |   |   |   |     |     | a |   |   |   |     |     |     |      |   |       |   |   |   |   |   |       |   |   |   |   |   | g |   |     |     |    |    |
|    | 3369264 Sepia la |   |   |   |    | t |   |   |       |     |   |   |   |   |   |     |     | a |   |   |   |     |     |     |      |   |       |   |   |   |   |   |       |   |   |   |   |   | g |   |     |     |    |    |
| )  | AB192338.1 Sepia |   | a |   |    | t |   |   |       | . a |   |   |   |   |   |     |     | a |   |   |   |     | . a | c   |      |   |       |   |   |   | g |   |       |   |   |   |   |   |   |   |     |     |    |    |
| )  | AB430406.1 Sepia |   | a |   |    | t |   |   |       | . a |   |   |   |   |   |     |     | a |   |   |   |     | . a | c   |      |   |       |   |   |   | g |   |       |   |   |   |   |   |   |   |     |     |    |    |
| .0 | AY185506.1 Sepia |   |   |   |    | t |   |   |       | . a |   |   |   |   |   |     |     | a | с |   | с |     |     |     |      |   |       |   |   |   |   |   |       |   |   | с |   |   |   |   |     |     |    |    |
| 11 | KF009663.1 Sepia |   | a |   |    | t |   |   |       | . a |   |   |   |   |   |     |     | a |   |   |   |     | . a | c   |      |   |       |   |   |   | g |   |       |   |   |   |   |   |   |   |     |     | Γ. | Γ. |

|   |                  |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       | Wir | ndov | W: | 501 | -55( | 0 |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
|---|------------------|---|---|-----|-----|---|---|---|---|-------|---|-----|-------|---|---|---|---|---|-----|-----|-------|-----|------|----|-----|------|---|-----|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|-----|---|---|
|   |                  |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       | Τ   |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     | Τ   | Τ |   |
|   |                  |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       |     |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
| L | 1542916 Sepia la | A | С | t   | ; C | Т | A | T | c | T   T | 1 | A   | 2     | g | t | T | A | t | T . | A 7 | r   C | ; t | c    | t  | С   | С    | C | 0   | ; 1 | ! a | T | Т | A | G | С | t | G | G | A | G | С | a | A | T   | t Z | A | С |
| 2 | 1626051 Sepia la |   |   |     |     |   |   |   |   |       |   |     | .   . |   |   |   |   |   |     |     | .   . |     |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
|   | 1626052 Sepia la |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       |     |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
|   | 1626057 Sepia la |   |   | a . |     |   |   |   | t |       |   | . t | : .   | a |   |   |   | с |     |     |       | a   |      |    | t   |      |   | a.  |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   | . ( | c.  |   |   |
| 5 | 1733049 Sepia la |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       |     |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
| 5 | 3369262 Sepia la |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       |     |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
|   | 3369264 Sepia la |   |   |     |     |   |   |   |   |       |   |     |       |   |   |   |   |   |     |     |       |     |      |    |     |      |   |     |     |     |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |   |   |
| 3 | AB192338.1 Sepia |   |   | с.  |     |   |   |   | t |       |   | . t | ; .   | a |   |   |   | с |     |     |       |     |      | с  | a   |      |   | t.  |     | t   |   |   |   |   |   | a |   |   |   |   |   |   |   | . ( | с.  |   |   |
| ) | AB430406.1 Sepia |   |   | с.  |     |   |   |   | t |       |   | . t | ; .   | a |   |   |   | с |     |     |       |     |      |    |     |      |   | t.  |     | t   |   |   |   |   |   | a |   |   |   |   |   |   |   | . ( | c.  |   |   |
| 0 | AY185506.1 Sepia |   |   | а.  |     |   |   |   | t |       |   |     |       | a | с |   |   |   |     |     |       | c   | t    |    | a   |      |   | a . |     | t   |   |   |   |   |   |   |   |   |   |   |   | с |   |     |     |   |   |
| 1 | KF009663.1 Sepia |   |   | c.  |     |   |   |   | t |       |   | . t |       | a |   |   |   | с |     |     |       |     |      |    |     |      |   | t.  |     | t   |   |   |   |   |   | a |   |   |   |   |   |   |   | . ( | c   | T |   |

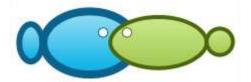
|    |                  |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   | W | ind | W:  | 551 | -600 | )   |   |   |   |     |       |     |   |   |   |    |     |     |   |   |   |     |     |
|----|------------------|---|---|----|-----|-----|-----|---|---|---|-----|-----|----|---|---|---|-----|-----|---|---|---|-----|-----|-----|------|-----|---|---|---|-----|-------|-----|---|---|---|----|-----|-----|---|---|---|-----|-----|
|    |                  |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   |   |     |     |     |      |     |   |   |   |     |       |     |   |   |   |    | Ι   |     |   |   |   |     |     |
| 1  | 1542916 Sepia la | a | A | T. | A 1 | r 1 | r A | Т | T | A | A ( | C t | G  | A | t | C | Ga  | a A | A | t | T | T I | t A | A   | t    | A C | A | A | C | t 1 | r 1   | ' c | Т | T | T | Gj | A ( | ; C | c | a | A | G 2 | 1 0 |
| 2  | 1626051 Sepia la |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   |   |     |     |     |      |     |   |   |   |     |       |     |   |   |   |    |     |     |   |   |   |     |     |
| 3  | 1626052 Sepia la |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   |   |     |     |     |      |     |   |   |   |     |       |     |   |   |   |    |     |     |   |   |   |     |     |
| 1  | 1626057 Sepia la | t |   |    |     |     |     |   |   |   |     | . a | ι. |   | с |   | . 1 | έ.  |   |   |   | . ( |     |     | с    |     |   |   |   | a.  | .   . |     |   |   |   |    |     |     |   |   |   |     |     |
| 5  | 1733049 Sepia la |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   |   |     |     |     |      |     |   |   |   |     | .   . |     |   |   |   |    |     |     |   |   |   |     |     |
| 6  | 3369262 Sepia la |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   | .   |     |   |   |   |     |     |     |      |     |   |   |   |     | .   . |     |   |   |   |    |     |     |   |   |   |     |     |
| 7  | 3369264 Sepia la |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   |   |     |     |     |      |     |   |   |   |     |       |     |   |   |   |    |     |     |   |   |   |     |     |
| в  | AB192338.1 Sepia |   |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   | с |   | . ( |     |     | с    |     |   |   |   | a.  |       |     |   |   |   |    |     |     |   | t |   |     |     |
| 9  | AB430406.1 Sepia |   |   |    |     |     |     |   |   |   |     |     |    |   | с |   |     |     |   | с |   | . ( |     |     | с    |     |   |   |   | a.  |       |     |   |   |   |    |     |     |   | t |   |     |     |
| 10 | AY185506.1 Sepia | t |   |    |     |     |     |   |   |   |     |     |    |   |   |   |     |     |   |   |   |     |     |     |      |     |   |   |   | a.  |       | t   |   |   |   |    | . t | ; . |   | t |   |     |     |
| 11 | KF009663.1 Sepia |   |   |    |     |     |     |   |   |   |     |     |    |   | с |   |     |     |   | с |   | . ( |     |     | с    |     |   |   |   | a.  |       |     |   |   |   |    |     |     |   | t |   |     |     |

|    |                  | _ | - | - | - | - | - |   |   |   |   |   |   |   | <u> </u> |   | - |   | - |   |   |
|----|------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|----------|---|---|---|---|---|---|
| 1  | 1542916 Sepia la | G | t | G | G | t | G | G | a | G | A | с | С | С | т        | A | т | с | t | т | А |
| 2  | 1626051 Sepia la | - |   | - | - | - | - | - | - | - | - | - | - | - | -        | - | - | - | - |   |   |
| 3  | 1626052 Sepia la | - | - | - |   | - | - |   | - | - | - | - | - | - | -        | - | - | - |   |   |   |
| 4  | 1626057 Sepia la | - | - | - | - | - | - | - | - | - | - | - | - | - | -        | - | - | t | с | - |   |
| 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: Auroraregion 3).

| NS FINANCE  | SPORTS   | ENTERTAINMENT   | LIFE                     | SHOPPING      | YAHOO PLUS        | MORE                  |          |
|---|--|---|--------------------------|---------------|-------------------|-----------------------|----------|
| ind messages, c   | documents, phot  | os or people  |                          | ~             | ٩                 |                       |          |
| 🕂 Back 🔦 🔇  | * *  | M Archive   | Move                     | 🔟 Delete      | 😵 Spam \cdots     |                       | A ¥ 3    |
| • Re: article s   | ubmission  |   |                          |               |                   | Yahoo/                | 'inbox 🖈 |
|   | <b>aie ionel claudiu</b> «<br>inv Pratasik   | <ionelclaudiu@yahoo.co< td=""><td>om&gt;</td><td></td><td>🖶 Fri</td><td>, Apr 26, 2019 at 1:4</td><td>42 AM 🔺</td></ionelclaudiu@yahoo.co<> | om>                      |               | 🖶 Fri             | , Apr 26, 2019 at 1:4 | 42 AM 🔺  |
| 12 92 12  |  |   |                          |               |                   |                       |          |
|   | 4  |   |                          |               |                   |                       |          |
| Dear Dr. Pra  | itasik,  |   |                          |               |                   |                       |          |
| I received yo   | our paper. More  | soon. But not soon  |                          |               | se I am on some   | e Internaitonal       |          |
| I received yo   | our paper. More  | soon. But not soon<br>d then I will have a  |                          |               | se I am on some   | e Internaitonal       |          |
| l received yo<br>Conference<br>Thank you!   | our paper. More<br>these days, and   |   |                          |               | se I am on some   | e Internaitonal       |          |
| I received yo<br>Conference   | our paper. More<br>these days, and<br>urs,   |   |                          |               | se I am on some   | e Internaitonal       |          |
| l received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav                                 | our paper. More<br>these days, and<br>urs,<br>riloaie  |   | small vaca               | ition.        |                   |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case                 | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of E  | d then I will have a s  | small vaca<br>are ready, | ition.        |                   |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case                 | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of E  | d then I will have a s<br>Dangeubun etc. etc.   | small vaca<br>are ready, | ition.        |                   |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case<br>and I can wo | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of E<br>ork on the pape                       | d then I will have a s<br>Dangeubun etc. etc.   | small vaca<br>are ready, | , you can ser | nd them. I have t |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case<br>and I can wo | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of E<br>ork on the pape                       | d then I will have a s<br>Dangeubun etc. etc.<br>rs from time to time   | small vaca<br>are ready, | , you can ser | nd them. I have t |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case<br>and I can wo | our paper. More<br>these days, and<br>urs,<br>riloaie<br>the papers of E<br>ork on the pape<br>April 25, 2019 3:18 | d then I will have a s<br>Dangeubun etc. etc.<br>rs from time to time   | small vaca<br>are ready, | , you can ser | nd them. I have t |                       |          |

| NS FINANCE  | SPORTS   | ENTERTAINMENT   | LIFE                     | SHOPPING      | YAHOO PLUS        | MORE                  |          |
|---|--|---|--------------------------|---------------|-------------------|-----------------------|----------|
| ind messages, c   | documents, phot  | os or people  |                          | ~             | ٩                 |                       |          |
| 🕂 Back 🔦 🔇  | * +  | M Archive   | Move                     | 🔟 Delete      | 😵 Spam \cdots     |                       | A ¥ 3    |
| • Re: article s   | ubmission  |   |                          |               |                   | Yahoo/                | 'inbox 🖈 |
|   | <b>aie ionel claudiu</b> «<br>inv Pratasik   | <ionelclaudiu@yahoo.co< td=""><td>om&gt;</td><td></td><td>🖶 Fri</td><td>, Apr 26, 2019 at 1:4</td><td>42 AM 🔺</td></ionelclaudiu@yahoo.co<> | om>                      |               | 🖶 Fri             | , Apr 26, 2019 at 1:4 | 42 AM 🔺  |
| 12 92 12  |  |   |                          |               |                   |                       |          |
|   | 4  |   |                          |               |                   |                       |          |
| Dear Dr. Pra  | itasik,  |   |                          |               |                   |                       |          |
| I received yo   | our paper. More  | soon. But not soon  |                          |               | se I am on some   | e Internaitonal       |          |
| I received yo   | our paper. More  | soon. But not soon<br>d then I will have a  |                          |               | se I am on some   | e Internaitonal       |          |
| l received yo<br>Conference<br>Thank you!   | our paper. More<br>these days, and   |   |                          |               | se I am on some   | e Internaitonal       |          |
| I received yo<br>Conference   | our paper. More<br>these days, and<br>urs,   |   |                          |               | se I am on some   | e Internaitonal       |          |
| l received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav                                 | our paper. More<br>these days, and<br>urs,<br>riloaie  |   | small vaca               | ition.        |                   |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case                 | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of D  | d then I will have a s  | small vaca<br>are ready, | ition.        |                   |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case                 | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of D  | d then I will have a s<br>Dangeubun etc. etc.   | small vaca<br>are ready, | ition.        |                   |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case<br>and I can wo | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of E<br>ork on the pape                       | d then I will have a s<br>Dangeubun etc. etc.   | small vaca<br>are ready, | , you can ser | nd them. I have t |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case<br>and I can wo | our paper. More<br>these days, and<br>urs,<br>rriloaie<br>the papers of E<br>ork on the pape                       | d then I will have a s<br>Dangeubun etc. etc.<br>rs from time to time   | small vaca<br>are ready, | , you can ser | nd them. I have t |                       |          |
| I received yo<br>Conference<br>Thank you!<br>Cordialyl you<br>Claudiu Gav<br>P.S. In case<br>and I can wo | our paper. More<br>these days, and<br>urs,<br>riloaie<br>the papers of E<br>ork on the pape<br>April 25, 2019 3:18 | d then I will have a s<br>Dangeubun etc. etc.<br>rs from time to time   | small vaca<br>are ready, | , you can ser | nd them. I have t |                       |          |



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

<sup>1</sup>Silvester B. Pratasik, <sup>2</sup>Hariyani Sambali, <sup>3</sup>Lefrand. Manoppo, <sup>1</sup>Frans F. Tilaar, <sup>1</sup>Meiske S. Salaki

<sup>1</sup> Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>2</sup> Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Corresponding author: S. B. Pratasik, spjong07@yahoo.com

**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 eqgs (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.

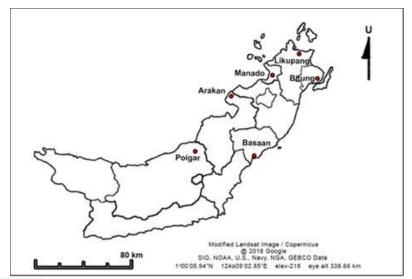


Figure [indra7]1. Map of North Sulawesi with sampling points (marked red).

**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°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). [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]).

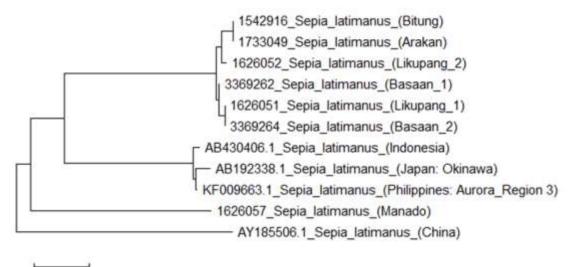




Figure [indra13]2. Phylogenetic tree of North Sulawesi *S. latimanus*.

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 reefsurrounded 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.

**Acknowledgements**. We would like to appreciate the Ministry of Research and Technology, Directorate General of Higher Education for financially support of this study through

University Superior Basic Research Grant 2018. Great gratitude is also given to Mr. Andre Kambey and Ms. Inggrid Akerina for field sampling activities. **References** [indra22]

- Alberto F., Raimondi P. T., Reed D. C., Coelho N. C., Lebois R., Whitmer A., Serräo E. A., 2010 Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. Ecology, 91(1), 2010, pp. 49–56.
- Allen J. J., Mäthger L. M., Barbosa A., Hanlon R. T., 2009 Cuttlefish use visual cues to control three-dimensional skin papillae for camouflage. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural and Behavioral Physiology 195:547– 555.
- Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W., Lipman D. J., 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acids Research 25:3389-3402.
- Arkley, K., M.S. Jacklin, M. Boulter dan J. Tower, 1996. Cuttlefish: a guide to its exploitation in UK waters. The sea fish Industry Authority. Seafish Report no. SR467. 81 pp.
- Barbosa A., Mäthger L. M., Buresch K. C., Kelly J. C., Chubb C., Chiao C., Hanlon R. T., 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive patterns. Vision Research 48:1242–1253.
- Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2011 Cuttlefish use visual cues to determine arm postures for camouflage. Proceedings of the Royal Society B Biological Sciences doi:10.1098/rspb.2011.0196[indra23].
- Chiao C.-C., Chubb C., Buresch K. C., Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2010 Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. Journal of Experimental Biology 213:187–199.
- Drummond A. J., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowitz S., Moir M., Stones-Havas S., Sturrock S., Thierer T., Wilson A., 2012 Geneious v5.5.6, http://www.geneious.com.
- Fernandez L. D.,<sup>+</sup>, Hernandez C. E., Schiaffino R. M., Izaguirre I., Lara E., 2017 Geographical distance and local environmental conditions drive the genetic population structure of a freshwater microalga (Bathycoccaceae; Chlorophyta) in Patagonian lakes. FEMS Microbiology Ecology, 2017, Vol. 93, No. 10. 10 pp.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5):294-299.
- Hanlon, R. T., C.-C. Chiao, L.M. Ma<sup>°</sup>thger, A. Barbosa, K.C. Buresch, dan C. Chubb, 2009. Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Phil.Trans. R. Soc. [indra24]B364, 429 – 437.
- IUCN, 2014 The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 25 September 2014.
- Kelman E. J., Osorio D., Baddeley R. J., 2008 A review of cuttlefish camouflage and object recognition and evidence for depth perception. Journal of Experimental Biology 211:1757–1763.
- Kumar S., Stecher G., Li M., Knyaz C., & Tamura K. 2018 MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
- Leopold A. 1933. Game Management. Charles Scribner's Sons. New York. 481 p.
- McManus M. A., Woodson C. B., 2011. REVIEW Plankton distribution and ocean dispersal. The Journal of Experimental Biology 215, 1008-1016.
- Murphy J. M., Balgueri ´as, E., Key L. N., Boyle P. R. 2002 Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the Northwest African Coast. Bulletin of Marine Science, 71: 545–553.

- Neves A., Cabral H. N. Sequeira V., Figueiredo I., Moura T., Gordo L. S., 2009 Distribution patterns and reproduction of the cuttlefish, Sepia officinalis in the Sado estuary. (Portugal) Journal of the Marine Biological Association of the United Kingdom 89(3):579–584.
- NOVA, 2007 Cuttlefish: kings of camouflage. (Television program) NOVA, PBS, April 3, 2007).[indra25]
- Pascual M., Palero F., García-Merchán V. H., Macpherson E., Robainas-Barcia A., Mestres F., Roda T., Abelló P., 2016. Temporal and spatial genetic differentiation in the crab Liocarcinus depurator across the Atlantic-Mediterranean transition. Scientific RepoRts 6:29892
- Pawson, M.G., 1995. Bio-geographical Identification of English Channel fish and shellfish stocks. CEFAS Fisheries Research Technical Report No 99. [indra26]
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2015 Size at First maturity of cuttlefish, *Sepia latimanus*, from North Sulawesi Waters, Indonesia. Marine Science 5(1):6-10.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2016 Mitochondrial CO1 genetic markerbased species diversity of cuttlefish (Cephalopod; Mollusk) in Manado Bay and Lembeh Strait, North Sulawesi, Indonesia. AACL Bioflux 9(6):1345-1354.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2017 Egg placement habitat selection of cuttlefish, Sepia latimanus (Sepiidae, Cephalopoda, Mollusca) in North Sulawesi waters, Indonesia. AACL Bioflux 10(6):15141523.
- Ramachandran S., Deshpande O., Roseman C. C., Rosenberg N. A., Feldman M. W., Cavalli-Sforza L. L., 2005 Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. PNAS [indra27]vol. 102, no. 44. 6 pp[indra28].
- Ratnasingham S., Hebert P. D. N., 2007 BOLD: the barcode of life data system. Molecular Ecology Notes 7:355–364.
- Reid A., Jerep P., Roper C. F. E., 2005 Family Sepiidae. In: Cephalopods of the world. An annotated and illustrated catalogue of Cephalopod species known to date. Volume 1. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). Jerep P. & Roper C.F.E. pp 54-152. FAO, Rome.
- Rozas J., Ferrer-Matta A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., Sánchez-Gracia A., 2017 DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12): 3299-3302.
- Shohet A. J., Baddeley R. J., Anderson J. C., Kelman E. J., Osorio D., 2006 Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage. Journal of Experimental Biology 209:4717–4723.

Received: 25 April 2019. Accepted: 31 May 2019. Published online: xx June 2019. Authors:

Silvester B. Pratasik, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: spjong07@yahoo.com

Harjany Sambali, Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: hariyanisambali@unsrat.ac.id Lefrand Manoppo, Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: lefrandmanoppo@yahoo.com

Frans F. Tilaar, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: fftilaar@unsrat.co.id Meiske S. Salaki, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: mssalaki@unsrat.co.id This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Pratasik S. B., Sambali H., Manoppo L., Tilaar F. F., Salaki M. S., 2019 Genetic variations of cuttlefish *Sepia latimanus*, cephalopod, mollusk, in North Sulawesi waters, Indonesia. AACL Bioflux 12(3):xxx-xxx.

## Appendix 1

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

|    |                  |   |   |     |     |     |   |   |     |       |   |   |   |   |   |     |     |   |   |   | W   | ndo | w. 1 | -50 |    |   |    |   |     |     |   |   |    |     |     |     |   |   |   |     |   |   |   |
|----|------------------|---|---|-----|-----|-----|---|---|-----|-------|---|---|---|---|---|-----|-----|---|---|---|-----|-----|------|-----|----|---|----|---|-----|-----|---|---|----|-----|-----|-----|---|---|---|-----|---|---|---|
| 1  |                  |   |   |     |     |     |   | 1 |     |       |   |   |   |   | 1 |     | -   |   |   |   |     |     |      |     |    |   |    |   |     | 1   |   |   |    |     |     |     |   |   |   |     |   |   |   |
| 1  | 1542916 Sepia la | 7 | t | T ( | ; a | τ   | с | a | G ( | 5 7   | T | Ŧ | λ | T | T | g ( | 3 0 | g | A | С | a ' | r c | λ    | t   | 73 | A | G  | t | t 1 | r A | A | Ŧ | t. | A 1 | 1 2 | C   | G | t | A | Gt  | G | A |   |
| ź  | 1626051 Sepia la |   |   |     |     |     |   | - |     |       |   |   | - |   |   |     |     |   |   |   |     |     |      |     |    | - | 4  | 4 |     |     |   |   |    |     |     |     |   | - | - |     |   |   |   |
|    | 1626052 Sepia la |   | 1 | j:  |     |     | + |   |     |       |   | + | + | ÷ |   |     |     |   |   |   |     | 4   | +    |     | 14 |   | ¥. | 4 |     |     | 4 |   |    |     | 1   | +   |   | - | + |     | - |   | - |
|    | 1626057 Sepia la |   | a |     | . 9 | r . |   | + |     |       |   | , |   | , |   | a . |     | t |   | - |     |     |      | c   |    | - | ,  | a | c   |     |   |   |    |     | c   | ; . |   | a |   |     | - |   |   |
| 5  | 1733049 Sepia la |   |   |     | Ţ.  |     |   |   |     | Ι.    |   |   |   |   |   |     | Ι.  |   |   | + |     |     |      |     |    | - |    | - |     | Ι.  |   |   |    |     |     |     |   |   |   |     |   |   |   |
|    | 3369262 Sepia la |   |   |     | Π.  |     |   | - |     | . J., |   |   | - |   |   |     |     |   |   |   |     |     |      |     |    | - |    | 4 |     |     |   |   |    |     |     |     |   |   | - |     |   |   |   |
| ,  | 3369264 Sepia la | 1 | 1 | j.  |     |     | 4 |   |     |       |   | 4 | - | ÷ |   |     |     | 4 | 4 |   |     | 4   |      |     | 14 |   | ų, | 4 |     |     | 1 |   |    |     | 1   |     |   |   | 4 |     |   |   |   |
| i. | AB192338.1 Sepia |   |   | ,   | . 9 |     |   | g |     |       |   | , |   | , |   | a . | . , | a |   | - |     |     | -    | c   |    |   | ,  | - |     | . , |   |   | -  |     | Ι.  | -   |   |   |   | . a | - |   | 9 |
|    | AB430406.1 Sepia |   |   |     | Τ.  |     |   | 9 |     | Ι.    |   |   |   |   |   | a   | Į,  | a |   |   |     |     |      | c   |    | - |    | - |     | Ι.  |   |   |    |     |     |     |   |   |   | . a |   |   | g |
| 0  | AY185506.1 Sepia |   |   |     | 1.  |     |   | - |     |       |   |   | - |   |   | a   |     | t |   | + | t   |     |      |     |    |   |    | 4 |     |     |   |   | a  |     |     |     |   | a | - | . a |   |   |   |
| 11 | KF009663.1 Sepia |   |   |     |     |     |   | a |     |       |   | 4 |   |   |   | a   |     | a |   |   |     |     |      | c   | 4  |   |    | - |     |     |   |   |    |     |     | 1   |   |   | 4 | . a |   |   | a |

|    |                  |    |   |   |     |     |      |   |    |     |   |     |    |       |    |    |   |   |     |     | 8   | Min | dow | 1.5 | 1-1 | 0   |     |     |   |   |     |    |   |    |   |   |     |     |   |   |   |     |     |     |
|----|------------------|----|---|---|-----|-----|------|---|----|-----|---|-----|----|-------|----|----|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|---|-----|----|---|----|---|---|-----|-----|---|---|---|-----|-----|-----|
|    |                  |    |   |   |     | -   |      | - |    |     | - |     | -  | -     | 1  |    |   |   |     |     | -   |     |     |     |     |     | -   |     |   | - |     | -  | ŀ |    |   |   | +   | I   |   |   |   |     | -   | ļ   |
|    | 1542916 Sepia la | T  | T | g | G   | 3 1 | : A  | λ | λ  | с   | с | c ( | GO | 1     | A  | с  | a | t | 7 : | a f | : 7 | A   | A   | λ   | t   | G 2 | ( e | G   | A | c | C 2 | A  | t | Ŧ  | λ | 7 | A 1 | : 3 | A | Ŧ | G | Ŧ   | c 7 | . 7 |
| 2  | 1626051 Sepia la |    |   | - |     |     |      |   | +  |     | - |     |    |       |    |    | + | + |     |     |     | 1.  | +   |     | +   |     |     | -   |   |   |     |    |   |    |   |   |     |     |   | + |   |     |     |     |
|    | 1626052 Sepia la |    |   |   |     |     |      |   |    |     | - | -   |    |       |    | -  |   |   |     |     |     |     | +   |     |     |     |     | +   |   |   |     |    |   |    |   |   |     |     |   |   |   | +   | ŧ.  |     |
|    | 1626057 Sepia la |    |   | a |     |     | 1.   |   | -  |     | - | a   |    |       |    | -  |   | c | . 1 | t e | e . |     | ÷   |     | c   |     | c   | ÷   |   | t |     |    | c |    |   |   |     |     | ÷ |   | - |     | ŧ.  |     |
| 5  | 1733049 Sepia la | ų, |   | 1 | 1 1 |     | . 14 |   | 12 | 4   | - | 1   |    |       | 4  | -  | 4 | 4 |     |     |     | 4   |     |     | -   |     | 4   | 1   |   | 4 |     | 4  |   |    | 4 | 2 |     |     |   |   | 4 |     |     |     |
| 5  | 3369262 Sepia la |    |   |   |     |     |      |   |    |     | - |     |    | .   . |    |    |   | + | -   |     |     |     |     |     |     |     |     |     |   |   |     |    |   | ,  |   |   | , , |     |   |   |   | . 1 | ŧ.  |     |
|    | 3369264 Sepia la | ٠. |   |   |     |     |      |   |    | • : | - |     |    |       |    | -  |   |   | -   |     |     |     | +   |     |     |     |     |     |   | • |     |    |   |    |   | - |     |     |   |   | - | +.  |     |     |
| 8  | AB192338.1 Sepia |    |   | a |     |     | 19   |   | -  |     |   |     |    |       |    | -  |   | c |     | . 0 | a . | 1   | ÷   |     |     |     |     | ÷   |   | ŧ |     |    |   | ÷  |   |   | . c | : . | ÷ |   | - | +   | ŧ.  |     |
| 9  | AB430406.1 Sepia | 4  | 2 | a | ¢ s |     | 1    |   | 1  | 4   | 2 | 1   |    |       |    | -  | 1 | c |     |     | z . |     |     | -   |     |     |     | 1   | 1 | t |     |    |   |    | 2 |   |     | : . |   |   | 1 | . 1 | t.  |     |
| 10 | AY185506.1 Sepia |    |   | a |     | 4   | λ,   |   |    |     | - | a   |    |       | Ι. |    | t | c |     |     |     |     |     | -   |     |     |     |     |   | t |     |    |   |    |   |   | , , |     |   |   |   |     | tę  | , . |
| 1  | KF009663.1 Sepia |    |   | a | UT. | T   | Ι.   |   |    |     |   |     |    | Τ.    | 1  | 1. |   | c |     |     |     | Π.  |     |     |     |     | 1.  | [.] |   | e | 1   | Ι. |   | 1. |   |   |     |     |   |   |   |     | ŧ . | T   |

|    |          |          |   |   | _   |     | _ |   |   | _    |       | -<br>T |   |   |   |   |      | Г |   | _   | _   | //// | dow | : 10 | 1-19 | 0 | _   |     |   |   | -    | _     |   |   | _   | _   | Т   | _ |   | _ | _   |   | Ē  |
|----|----------|----------|---|---|-----|-----|---|---|---|------|-------|--------|---|---|---|---|------|---|---|-----|-----|------|-----|------|------|---|-----|-----|---|---|------|-------|---|---|-----|-----|-----|---|---|---|-----|---|----|
| 1  | 1542916  | Sepia la | A | G | T 1 | : A | С | a | G | C (  | - c   | : A    | t | G | G | t | T T  | c | A | T   | t A | T    | A   | A 1  | r t  | T | 7 0 | . 1 | T | t | t 1  | r A   | G | Ŧ | a i | A T | 1 A | C | С | T | A T | t | 2  |
| 2  | 1626051  | Sepia la |   | - |     | -   |   |   | - | .  . | .   . |        | - |   |   | - | .  . | - |   | -   |     | -    |     | -    | c    |   |     |     | - |   | -  - | .   . | - |   | -   |     | -   |   | - | - |     | - |    |
| 3  | 1626052  | Sepia la |   |   |     | -   |   |   | - |      |       |        | - |   |   | - |      | - |   | -   |     | -    |     |      | c    |   |     |     | - |   |      |       | - |   | -   |     | -   |   |   | - |     | с |    |
|    | 1626057  | Sepia la |   |   |     | -   |   |   | - |      |       |        |   |   |   | a |      | t |   |     |     |      |     |      |      |   |     |     |   | с | с.   |       |   |   | -   |     | -   |   |   |   |     | - |    |
| 5  | 1733049  | Sepia la |   |   |     | -   |   |   |   |      |       |        |   |   |   |   |      | - |   |     |     | -    |     |      |      |   |     |     |   |   |      |       |   |   | -   |     | -   |   |   | - |     | - |    |
| 6  | 3369262  | Sepia la |   |   |     |     |   |   |   |      |       |        |   |   |   |   |      | - |   | -   |     | -    |     |      | c    |   |     |     |   |   |      |       |   |   | -   |     | -   |   |   |   |     | - |    |
| 1  | 3369264  | Sepia la |   |   |     |     |   |   | - |      |       |        | - |   |   | - |      | - |   | -   |     | -    |     |      | c    |   |     |     |   |   |      |       | - |   | -   |     |     |   |   |   |     | - |    |
| в  | AB192338 | 1 Sepia  |   |   |     |     |   |   |   |      |       |        | - |   |   | g |      | t |   | -   |     | -    |     |      |      |   | . t |     |   | с |      |       |   |   | -   |     | -   |   |   |   |     | - |    |
| 9  | AB430406 | 1 Sepia  |   |   |     |     |   |   | - |      |       |        | - |   |   | g |      | t |   |     |     | -    |     |      |      |   | . t | : . |   | с |      |       |   |   |     |     |     |   |   |   |     |   |    |
| 10 | AY185506 | 1 Sepia  |   |   | . : | ۱.  |   | с |   |      |       |        | с |   |   |   |      | t |   | - 1 | с.  |      |     |      |      |   |     |     |   |   |      |       |   |   | t   |     |     |   |   |   |     |   |    |
| 11 | KF009663 | 1 Sepia  |   |   |     |     |   |   |   |      | 1     |        |   |   |   | g |      | t |   |     |     |      |     |      |      |   | . t |     |   | с |      |       |   |   |     |     |     |   |   |   |     |   | Γ. |

| -  | _                     |          |    |   | _   | _    |    |   | _   | _  | _ | _  | _ | _ | _   | _  | _  |   | _  | _   | 1  | /lin | dow | 15  | 1-2 | 00 | _ | _   | _   | _  | _  |   | _   |    | _  | _   | _  | _   | _   | _   | _  | _ | _   |
|----|-----------------------|----------|----|---|-----|------|----|---|-----|----|---|----|---|---|-----|----|----|---|----|-----|----|------|-----|-----|-----|----|---|-----|-----|----|----|---|-----|----|----|-----|----|-----|-----|-----|----|---|-----|
| 1  | 1542916               | Sepia la | T  | A | A : | r t  | G  | G | t G | G  | 0 | 7  | T | T | : 0 |    | A  | à | c  | T   | Ga | T    | 7   | A   | . 7 | 't | с | C   | . 7 | 7  | A  | 2 | T 2 |    | Ŧ  | A   | G  | Gi  | a   | c c | c  | с | C 1 |
| 2  | and the second second | Sepia la |    |   | Ĩ.  | 1.   | 1. |   | T.  | Ι. | Ĩ | 1  | Ţ | 1 |     | Ī. | Ī. |   |    |     | T  | Ī.   | -   | Ţ   | T.  | T. |   |     |     | Ī. | Ī. |   | Τ.  | T. | Ľ  | Ī.  |    | T   | Ι.  | T.  | Ī. |   | 1   |
| 3  | 1626052               | Sepia la |    |   |     |      | -  | + |     |    |   | +  | + |   | Ţ.  |    | 1  |   |    |     |    | +    | -   |     |     |    | + | -   |     |    | Ļ  |   |     |    |    | +   |    |     |     | T.  |    |   |     |
| 4  | 1626057               | Sepia la | 14 |   |     |      | -  | 4 | a   | +  | t | 4  | ÷ | - |     |    |    | - | t. |     |    |      | -   |     |     | a  | + |     |     |    | 4  |   |     | t  |    | 4   | -  | . 1 | t . |     | t  |   |     |
| 5  | 1733049               | Sepia la |    |   |     |      |    | + |     |    | - |    | - |   |     | -  |    |   |    |     |    | +    | 4   |     |     |    | + |     |     |    | +  |   |     |    |    | ÷   |    |     |     |     |    |   |     |
| 6  | 3369262               | Sepia la |    |   |     |      |    |   |     |    |   | -  |   | - | 1.  |    |    | - |    |     |    |      | -   |     | .]. |    |   |     |     | Į. |    |   |     |    | 1  | ļ,  |    |     |     | ].  |    |   |     |
| 7  | 3369264               | Sepia la |    |   |     | 1.00 |    | ÷ |     |    | × | а. |   |   |     |    |    |   |    |     | 14 |      | ×   | •   |     |    |   |     |     |    | 4  | * |     |    | 4  | (e) | 2  |     |     | a   |    |   |     |
| 8  | AB192336              | 1 Sepia  |    |   |     | . c  | +  | 4 | a . | +  | t | 4  | + |   |     |    | 4  | 4 | t  |     |    |      | 4   | . 4 | a . | +  | - |     |     |    | 4  | - |     | t  | 4  | 4   | 4  |     |     | 1.  | t  |   |     |
| 9  | AB430406              | .1 Sepia |    |   |     | . 0  | +  | + | a . |    | t |    |   |   |     |    |    | - | ŧ. |     |    |      |     | . 4 | a . |    |   |     |     | -  |    |   |     | t  |    |     |    |     |     |     | t  |   |     |
| 10 | AY185506              | .1 Sepia |    |   |     |      |    | + | Α.  |    | t |    |   |   |     |    |    | - | t  | + . | g  |      | -   |     |     | +  |   | . 1 |     |    | +  |   |     | t  | 1. | Ļ,  |    | . 1 | g . |     | a  |   |     |
| 11 | KF009663              | .1 Sepia |    |   |     | c    |    |   | a . | 1. | t |    |   |   | Ι.  |    |    |   | t  |     |    |      |     |     | λ.  |    |   |     |     |    | 4  |   |     | t  |    |     | 20 |     |     | 4   | t  |   |     |

|   |                  | _ | _ |    | _   | _     | _ | _ | _ | _ | _ | _ | _ | _ | _ | _    |     |     |     |       |     | 1 | Vin | low: | 20    | 1-25 | 50 | _ | _ | _   | _     | _     | _ | _ | _ | _ | _ | _ |   |   |     |       | _   | _ |   |
|---|------------------|---|---|----|-----|-------|---|---|---|---|---|---|---|---|---|------|-----|-----|-----|-------|-----|---|-----|------|-------|------|----|---|---|-----|-------|-------|---|---|---|---|---|---|---|---|-----|-------|-----|---|---|
|   |                  |   |   |    |     |       |   |   |   |   | + |   | + | + | + | +    |     |     |     |       |     |   |     |      | +     |      |    |   | + |     |       |       |   |   |   |   |   |   |   | - | +   | -     |     |   |   |
| L | 1542916 Sepia la | G | A | ti | A 1 | 1     | G | С | t | Ŧ | T | t | C |   | a |      | 6 1 | t 1 | A 1 | 2     | i A | A | С   | A    | 1     | A    | T  | A | A | G   | a 1   | 1     | T | T | G | a | T | T | A | С | T 2 | 1 0   | : C | С | С |
| 2 | 1626051 Sepia la |   |   |    |     | .   . |   |   |   |   | - | - | - |   |   | -  - |     |     |     | .   . |     |   | -   | -    | .   . |      | -  |   | . |     | .   . | .   . | - |   |   | - |   | - | - |   |     | .   . |     |   |   |
|   | 1626052 Sepia la |   |   |    |     |       |   |   |   |   |   |   |   |   |   |      |     |     |     |       |     |   |     | -    |       |      |    |   |   |     |       |       | - |   |   |   |   | - |   |   |     |       |     | - |   |
|   | 1626057 Sepia la |   |   |    |     |       |   |   | a |   |   |   |   |   |   | -    |     | a . |     |       |     |   | t   | -    |       |      | -  |   |   | . 1 | t.    |       |   |   |   |   |   | - |   | t |     |       |     | a |   |
|   | 1733049 Sepia la |   |   |    |     |       |   |   |   |   |   |   |   |   |   | -    |     |     |     |       |     |   |     | -    |       |      | -  |   |   |     |       |       |   |   |   |   |   | - |   |   |     |       |     |   |   |
|   | 3369262 Sepia la |   |   |    |     |       |   |   |   |   |   |   |   |   |   | -    |     |     |     |       |     |   |     | -    |       |      | -  |   |   |     |       |       |   |   |   |   |   | - |   |   |     |       |     |   |   |
|   | 3369264 Sepia la |   |   |    |     |       |   |   |   |   |   |   |   |   |   | -    |     |     |     |       |     |   |     | -    |       |      | -  |   |   |     |       |       |   |   |   |   |   | - |   |   |     |       |     |   |   |
|   | AB192338.1 Sepia |   |   |    |     |       |   |   | a |   |   |   |   |   |   | -    |     |     |     |       |     |   |     | -    |       |      | -  |   |   |     |       |       |   |   |   |   |   | - |   | t |     |       |     |   |   |
|   | AB430406.1 Sepia |   |   |    |     |       |   |   | a |   |   |   |   |   |   | -    |     |     |     |       |     |   |     | -    |       |      | -  |   |   |     |       |       |   |   |   |   |   | - |   | t |     |       |     |   |   |
| 0 | AY185506.1 Sepia |   |   | с  |     |       |   |   | с |   | - | c |   | - | ŧ | -    |     | a   |     |       |     |   | t   | -    |       |      | -  |   | - | . 1 | t.    |       | - |   |   | g |   | - |   | t |     |       |     | t |   |
| 1 | KF009663.1 Sepia |   |   |    |     |       |   |   | a |   |   |   |   |   |   | _    |     |     |     | Τ.    | Τ.  |   |     |      |       |      |    |   |   |     |       |       |   |   |   |   |   |   |   | t |     |       |     |   |   |

|    |                  |   |     |     |   |   |    |     |            |   |     |     |    |   |   |    |   |   |   |   |     | 1          | rin, | daw | 2 | 51- | 300 |     |    |   |   |   |     |     |     |   |    |   |   |   |    |   |     |     |     |     |
|----|------------------|---|-----|-----|---|---|----|-----|------------|---|-----|-----|----|---|---|----|---|---|---|---|-----|------------|------|-----|---|-----|-----|-----|----|---|---|---|-----|-----|-----|---|----|---|---|---|----|---|-----|-----|-----|-----|
|    |                  |   |     |     |   |   | -  |     | +          |   |     | -   |    |   | - |    |   | _ |   |   | +   | +          | -    |     |   | -   | +   |     |    |   |   |   | +   | +   | +   | - |    |   |   |   |    | - |     | -   |     | Ŧ   |
| 1  | 1542916 Sepia la | a | Ŧ   | С   | a | Ŧ | ۴. | k a | a (        | a | . 0 | ę   | a  | c | 7 | t  | t | 7 | a | Ŧ | C 0 | : 7        | С    | a   | Ŧ | С   | t a | ı t | Ŧ  | a | 7 | A | G 1 | A 3 | A A | G | a  | G | G | t | G  | С | a   | G   | G 1 | t 3 |
| 2  | 1626051 Sepia la |   |     |     |   | + |    |     |            |   |     |     |    |   |   |    | - |   | ų |   |     | Į.         |      |     |   |     |     |     |    |   |   |   |     |     | Į.  |   | +  | - | + | + |    | - |     | -   |     | Į.  |
| 3  | 1626052 Sepia la | 4 | a   | 4   |   | + | -  | + - |            |   |     | -   | ÷  |   | - | -  | ÷ | + | ÷ |   |     |            | ÷    | ÷   |   | -   |     |     | ÷  | a | - |   | ÷   | -   |     | - | +  | + | - | + |    | - | + 1 | +   |     |     |
| 4  | 1626057 Sepia la | t |     | 111 | - | ÷ |    | 2   |            | 1 | 4   | 1.4 | 1  | 4 | - | 10 |   | 2 | ÷ | 4 | . 3 | 1          | 1    | -   | 4 | -   | -   | -   |    | 4 | 1 | 4 | 4   |     | 4   | 1 | 10 | ÷ | - | a | 4  | - | t   | +   |     |     |
| 5  | 1733049 Sepia la |   |     |     |   |   |    |     |            |   |     |     | Į. |   |   | -  |   |   |   |   |     |            |      |     |   | -   |     |     | -  |   |   | - |     |     |     |   | -  |   |   | - |    |   |     |     |     |     |
| 6  | 3369262 Sepia la |   |     |     |   | + |    | Ι.  |            |   | Ι.  |     | Į. |   |   |    | ÷ |   | Ļ |   |     | Ι.         |      | ļ.  |   |     | .], |     | ļ. |   |   |   |     | Į.  | Į.  |   |    | - | - | ÷ |    | - |     |     | Ι.  | Ι.  |
| 1  | 3369264 Sepia la |   |     | -   |   | + |    | +   |            |   |     |     |    |   | - | +  | + | + | + |   |     |            |      | ł   |   | -   |     |     | +  | 4 | - |   | -   |     |     |   | +  | + |   | + |    | - |     | + - |     |     |
| 8  | AB192338.1 Sepia | t | 114 | 1   | 4 | ÷ |    |     |            |   | t   |     |    | t | 1 | a  | c |   | t |   | . 3 | 4          |      | ÷   | 4 |     |     |     | +  | 4 |   |   | 4   |     |     |   | t  | + |   | 4 | a. |   |     | -   |     | 1   |
| 9  | AB430406.1 Sepia | t |     |     |   |   |    | . 9 | <b>j</b> . |   | t   |     |    | t |   | a  | c |   | t |   |     | <b>i</b> . |      | -   |   |     |     |     | -  |   | - |   |     |     |     |   | t  | + |   | - |    |   |     |     |     |     |
| 10 | AY185506.1 Sepia |   |     |     | t | + | 1  |     |            |   |     |     | t  | t |   | a  | + |   |   |   |     |            |      | c   | + |     | a g | t c |    | g |   |   |     |     |     |   |    |   |   |   |    |   | t   |     |     | a . |
| 11 | KF009663.1 Sepia | t | 4   |     |   | 4 | -  |     |            |   | t   |     | ļ, | t |   | a  | c |   | t |   |     | 1          |      |     |   |     |     |     |    |   |   |   | 4   |     |     |   | t  | 4 |   |   |    |   |     | 2   |     |     |

| _  |                  |     |   |   |     |     |   |   |     | _ |     |     |   |     | _ | _ |   |     |     | _ | N | Inde | WF: | 301 | -350 | 1   |    |    |    |       |   |     | - |     |     |    |   |   |     |   |   | _   |
|----|------------------|-----|---|---|-----|-----|---|---|-----|---|-----|-----|---|-----|---|---|---|-----|-----|---|---|------|-----|-----|------|-----|----|----|----|-------|---|-----|---|-----|-----|----|---|---|-----|---|---|-----|
| 1  | 1542916 Sepia la | С   | t | G | G 2 | 1 7 | G | A | A   | C | A ( | GI  | t | T   | λ | t | c | C t | c   | c | t |      | 7 A | T   | с    | a 2 | G  | t  | λ. | A T   | t | 7   | 2 | 7 0 | C a | C  | A | c | G   | t | G | G   |
| 2  | 1626051 Sepia la |     | - |   |     | -   |   | - |     | - |     | ÷ . |   | 1.  |   | + | + |     | -   | - | - |      |     | -   | _    |     |    | 4  |    |       |   |     | + |     |     | Τ. |   | + |     | T | - | -   |
| 3  | 1626052 Sepia la | 1.4 | - |   | + - | 4   |   | - | +   | - | -   | 2   |   | +   |   | + | + |     | +   |   | + |      |     | -   | -    |     | 14 | +  |    |       |   | 1.1 |   |     |     |    |   |   | + 1 |   |   |     |
| 4  | 1626057 Sepia la |     | a |   |     |     |   | - |     | - |     |     | a |     |   |   |   | . a | ι.  | - | a |      |     |     |      |     |    | a  |    |       | c |     | c |     |     |    |   | t |     | a |   |     |
| 5  | 1733049 Sepia la |     | - | + |     |     |   | - |     | - | -   |     |   | ļ., |   | - |   |     | -   |   |   |      |     | -   |      |     |    | +- |    |       | - |     |   |     |     |    |   | - | -   |   |   |     |
| 6  | 3369262 Sepia la |     | - |   | -   | +   |   | - | +   | - | -   | + 9 | - | ÷   | - | 4 | ÷ |     | -   | ÷ | + |      |     | -   | -    |     |    | ÷  |    |       |   |     | + |     |     |    | • | ÷ |     |   | - | ÷., |
| 7  | 3369264 Sepia la | 4   | - | - | -   | -   | 4 | - | 1.4 | - |     |     |   | +   |   |   | + |     | +   |   |   |      |     | -   | -    |     |    | +  |    |       |   | 4   | ÷ |     |     |    | 1 | - | 4   | 1 | - |     |
| 8  | AB192338.1 Sepia |     | - |   |     |     |   | - |     | - |     |     | c |     |   | с |   | . a | ı . | - | a |      |     | -   | -    | ŧ.  |    |    |    |       |   |     |   |     |     |    |   | t |     |   |   |     |
| 9  | AB430406.1 Sepia |     | + | + |     |     |   | - | +   | - |     |     |   | +   | - | c |   | . a | i . | - | a |      |     | -   |      | ŧ . |    | +  | -  |       | - |     |   |     |     |    |   | t | +   |   |   |     |
| 10 | AY185506.1 Sepia |     | - | + |     | 4   |   | - | +   | - | -   | + + | a | +   | - | + | + |     | +   | a | c | + -  | . 4 | +   | -    | t . |    | +  |    | + - + | c |     | + |     | , t |    | ÷ | t |     |   | 4 | 1   |
| 11 | KF009663 1 Sepia | 1   |   |   |     | 4   |   |   |     | - |     |     | c |     |   | с | - | . a |     |   | a |      |     |     |      | ŧ . |    | +  |    |       |   | ÷   | - |     |     |    |   | t |     |   |   |     |

|    |            |        | - |    |   |       |   |   |     | -<br>T |     |    |   | _   |      | Т     | -   |    |   | -   |    | //in | dow | 35  | 1-4( | 00 |   |   |       |     |    |   |   |     |   |   | Τ   | Т    | Т   | Г  |   | Т   | Т   |
|----|------------|--------|---|----|---|-------|---|---|-----|--------|-----|----|---|-----|------|-------|-----|----|---|-----|----|------|-----|-----|------|----|---|---|-------|-----|----|---|---|-----|---|---|-----|------|-----|----|---|-----|-----|
| 1  | 1542916 Se | pia la | C | С  | a | 7 0   | a | G | T   | t (    | ; A | T  | с | T . | A (  | 3 0   | a a | A  | T | t 1 | T  | с    | T   | C a | 1 C  | T  | t | C | A T   | t t | T  | A | G | ; g | G | G | a   | a 1  | t c | T  | С | c 1 | r ( |
| 2  | 1626051 Se | pia la |   | -  | - | .   . | t | - | -   |        |     | -  | - | -   | -  - | .   . |     |    | - |     |    | -    | -   |     |      | -  | - | - | .   . | -   | -  |   | - |     |   |   | -   | -  - |     |    | - | t.  |     |
| 3  | 1626052 Se | pia la |   |    | - |       | t | - |     |        |     | -  | - |     |      | .   . |     |    | - |     |    |      | -   |     |      | -  | - |   |       | -   | -  |   |   |     |   |   | -   |      |     |    |   |     |     |
| 4  | 1626057 Se | pia la |   |    | t |       |   | - | . ; | a      |     | -  | t |     |      |       | t   |    | - | с.  |    | t    | -   |     |      | -  | a |   |       | c   | -  |   |   | a   |   |   | t   | g .  | t   |    |   |     |     |
| 5  | 1733049 Se | pia la |   |    | - |       |   |   |     |        |     | -  |   |     |      |       |     |    |   |     |    |      |     |     |      |    | - |   |       |     |    |   |   |     |   |   |     |      |     |    |   |     |     |
| 6  | 3369262 Se | pia la |   |    | - |       | t | - |     |        |     |    |   |     |      |       |     |    | - |     |    |      |     |     |      |    | - |   |       |     |    |   |   |     |   |   |     |      |     |    |   |     |     |
| 7  | 3369264 Se | pia 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  |   | 1. | 1 |       | t | 1 |     |        |     | 1. | t |     |      | Ť.    | t   | Γ. |   |     | Ţ. | t    |     |     |      |    |   |   |       | 1   | 1. |   |   | c   |   |   | t / | a .  | a   | t. |   | a   | Ť   |

| _  |                  |     |     |   |     |   |   |   |   |   |   |     |     |    |   |    |    |    |    |     |     | Vie | do | N. 4 | Q1- | 450 | Ē.  |   |     |   |     |   |     |   |    |    |       |            |   |    |     |     |   |
|----|------------------|-----|-----|---|-----|---|---|---|---|---|---|-----|-----|----|---|----|----|----|----|-----|-----|-----|----|------|-----|-----|-----|---|-----|---|-----|---|-----|---|----|----|-------|------------|---|----|-----|-----|---|
| 3  |                  |     |     |   |     |   |   |   |   |   |   |     |     |    |   |    |    |    |    |     |     |     |    |      |     |     |     |   |     |   |     |   |     |   |    |    |       |            |   |    |     |     |   |
| 1  | 1542916 Sepia la | A   | A   | ī | t 1 | 1 | s | G | G | t | G | Ca  | a 2 | 1  | t | A  | A  | T  | ī  | T   | t 2 | 17  | T  | Å    | С   | a l | 1 0 | a | A   | 1 | 7 0 | 1 | a   | A | A  | 14 | g     | C          | G | ٤. | T ( | 3 3 | 6 |
| 2  | 1626051 Sepia la | -   | +   | • |     |   |   | + | - |   | - |     |     | 4  |   |    |    |    |    | ÷   |     | -   |    | -    |     |     |     | - |     | - |     |   | +   | • | -  | -  | a     | +          | + |    | -   |     |   |
| 3  | 1626052 Sepia la | ÷   | + 1 | - | ÷   | - | ÷ | + | + | - | + |     |     |    | - |    | ÷  | à. | -  | ÷   |     |     | ÷  | -    |     | + - |     |   | ÷   | - |     |   | ÷   | ÷ | +  | -  | - +   | ÷          | + | -  | -   |     |   |
| 4  | 1626057 Sepia la | 1.4 | +   |   |     |   | g |   |   | 4 | + | . 1 | :   |    | 1 |    | 4  | 4  | 14 | + + | c . |     | 1  | 4    |     | c.  |     |   | 1.1 | - | . t |   | 1.1 | 4 | 14 | -  | a     | ·:+        | + | a  | -   |     |   |
| 5  | 1733049 Sepia la |     |     |   |     | - | - |   | - | - |   |     |     |    |   | ļ. | Ι. |    |    |     |     |     |    |      |     |     |     |   | -   | - |     |   |     |   | -  |    | <br>  |            |   |    |     |     |   |
| 6  | 3369262 Sepia la | 4   | +   |   |     |   |   | + | - | - | - |     |     | Į. |   | ļ. | ļ, | +  |    |     |     |     | Į. |      |     |     |     | - |     | - |     |   | 1   |   | -  |    | а     | +          | Ļ |    | ļ   |     |   |
| 7  | 3369264 Sepia la | ÷3  | + 3 | + | ÷ . | - | + |   | + | - | ÷ |     |     |    | - |    |    | a, |    | ÷   | -   |     |    | -    | •   | 4   |     |   | ÷   | - |     |   | +   | ÷ |    | -  | a     | <u>+</u> + | + | +  | ÷   |     |   |
| 8  | AB192338.1 Sepia | 4   | +   |   |     | - | 4 |   | ÷ | c | 4 |     |     |    | c |    | 4  | 4  | ÷  | +   |     |     | +  | -    |     | 4   |     |   | 14  |   |     |   | с   | 4 | 4  |    | a     | 1          | + | 4  | -   |     |   |
| 9  | AB430406.1 Sepia |     |     | + |     |   | - |   | - | - | + |     |     |    | c | -  |    |    |    |     |     |     |    |      |     |     |     |   | -   |   |     |   | c   |   | -  |    | <br>a |            |   |    |     |     |   |
| 10 | AY185506.1 Sepia | -   | +   |   | c . |   |   |   |   |   | - | . 1 |     | Į. |   |    | ļ. |    |    |     |     |     | Į. |      |     |     |     | t |     |   | . 1 |   |     |   | ÷  |    | a     |            | 1 | g  |     |     |   |
| 11 | KF009663.1 Sepia | 40  |     | - |     |   |   |   |   | 4 | - |     |     | Į. |   |    | 1. | 4  |    | +   |     |     | 1. |      |     | -   |     |   |     |   |     |   | c   |   |    |    | <br>a | 1.         |   |    |     |     |   |

| _  |                  | _ | _ |   | _   |   |   | _ | _ | _   | _   | _   | _ | _  | _ | _ | _ | _   | _   |    | W | indi | W, | 451 | -500 | )   | _ | _ | _  | _   | _          | _ | _ | _ |     |     | _   |   | _  |     | _   |     |    |
|----|------------------|---|---|---|-----|---|---|---|---|-----|-----|-----|---|----|---|---|---|-----|-----|----|---|------|----|-----|------|-----|---|---|----|-----|------------|---|---|---|-----|-----|-----|---|----|-----|-----|-----|----|
|    |                  |   |   | - | +   | + |   |   |   | -   | -   |     |   |    |   |   |   |     | +   |    |   | +    |    | -   |      |     |   |   | -  |     | +          |   |   |   | +   | +   |     |   |    |     |     | -   | ł  |
| 1  | 1542916 Sepia la | λ | g | G | G 1 | 0 | Ŧ | A | С | A   | 1 1 | 1 7 | λ | G  | λ | A | С | Gg  | t   | 7  | a | c    | t  | t   | Ŧ    | λ 1 | T | 7 | G  | 7 1 | : 7        | G | A | 7 | C 1 | t ( | ; T | a | 7  | 7   | t A | 1 7 | 1  |
| 2  | 1626051 Sepia la |   |   |   |     | t | 4 |   | - | ÷   |     |     |   |    |   |   |   | . 4 |     | +  |   |      |    |     | ÷    |     |   |   |    |     |            | ÷ |   |   |     |     |     | g |    | -   |     |     |    |
| 3  | 1626052 Sepia la |   | ÷ |   | - 1 |   |   | ÷ |   | ÷   |     |     | - | +  |   | ÷ | ÷ | . 4 | i . | 4  | ÷ |      |    | +   |      |     | ÷ | 4 | +  |     |            | ÷ | + | - | 4   |     |     |   | +  |     |     |     |    |
| 4  | 1626057 Sepia la | 4 | a | , |     | t |   |   |   | , i | a . |     |   | Ι, | - |   |   | . a | a   |    |   |      |    | ,   | ,    |     |   |   | Ŧ  |     |            | , |   | - |     | , , |     | t | ., | - ( | с,  |     |    |
| 5  | 1733049 Sepia la | - | + |   |     |   |   |   |   | -   | Į,  |     |   | I. | - |   | - |     |     | 1. | • |      |    | +   | ,    |     |   |   |    | Į,  |            |   |   | - |     |     |     | + |    |     |     |     | Į. |
| 6  | 3369262 Sepia la |   |   |   |     | t | 4 |   | - |     | Į.  |     |   |    | - | 4 |   | . 4 |     | +  |   |      |    |     | 4    |     |   |   |    |     |            | + | - |   |     |     |     | g |    | -   |     |     |    |
| 7  | 3369264 Sepia la | - | ÷ |   | 4   | t | 4 | ÷ |   | ÷   |     |     | - | +  | + | ÷ | ÷ | . a | i . | 4  | + |      |    | +   | +    |     | ÷ | 4 | +  |     |            | ÷ | + | - | 4   |     |     | 9 | +  |     |     |     |    |
| 8  | AB192338.1 Sepia | 4 | a | , |     | t |   |   |   | , i | R . |     |   | Ι, | - | , |   | . a | i . | +  | , |      | a  | c   | ,    |     | , | , |    | . 4 | <b>;</b> . | , |   |   | , , | , , |     |   |    |     |     |     |    |
| 9  | AB430406.1 Sepia | 4 | ā |   |     | t |   |   |   |     | 8   |     |   | I. |   |   | - | . 4 | i . |    | • |      | a  | c   | ,    |     | , |   | •] |     | r .        |   |   |   |     |     |     | + |    |     |     |     | Į, |
| 10 | AY1855D6.1 Sepia |   |   |   |     | t | 4 |   |   |     | A . |     |   |    |   |   |   | . 4 | c   |    | c |      |    |     | ÷    |     |   |   |    |     |            | + |   |   | . ( | 2   |     |   |    |     |     |     | I. |
| 11 | KF009663.1 Sepia | - | ā |   |     | t |   |   |   | -   | A . |     |   | 4  |   | 4 | : |     | 1   | 4  | + |      | a  | c   |      |     |   | 4 | -  |     | J .        | + | + |   | 4   |     |     |   | +  |     |     |     | 5  |

|   |                  |   |   | _   | -   |     |   | _ |   | _ | _  | _ | -   |     | _   | _   |    | _ |   | _ | _   | Wir | 1do | WC 5 | i01- | 550 | _   |   | _ |   | -   | _   | _   | _   | _ |   | _ | _ | _ | _   | _   | _ |   | _ |
|---|------------------|---|---|-----|-----|-----|---|---|---|---|----|---|-----|-----|-----|-----|----|---|---|---|-----|-----|-----|------|------|-----|-----|---|---|---|-----|-----|-----|-----|---|---|---|---|---|-----|-----|---|---|---|
| 1 | 1542916 Sepia la | A | С | t ( | 5 C | : T | A | T | с | 7 | T. | A | c 1 | r g | j t | : 1 | A  | t | Ŧ | A | 7 0 | ; t | c   | t    | с    | С   | ; c | G | Ŧ | a | 7 1 | 7 2 | 4 0 | ; c | t | G | G | A | G | C a | a 2 | T | t | A |
| 2 | 1626051 Sepia la | - |   |     |     |     | - |   |   |   |    |   |     |     |     |     |    | - |   |   |     |     | -   |      |      |     |     | - |   |   | -   |     |     |     |   |   |   |   |   |     |     |   |   |   |
|   | 1626052 Sepia la | - |   |     |     |     | - |   |   |   |    |   |     |     |     |     |    | - |   |   |     |     |     |      |      | -   |     | - |   |   | -   |     |     |     |   |   |   |   |   |     |     |   | - |   |
|   | 1626057 Sepia la |   |   | a   |     |     | - |   | t |   |    | - | t   | . a | a . |     |    | с |   |   |     | a   |     |      | t    |     | a   | - |   |   | -   |     |     |     |   |   |   |   | - |     |     |   | с | - |
| 5 | 1733049 Sepia la | - |   | -   |     |     | - |   |   |   |    |   |     |     |     |     |    | - |   |   |     |     |     |      |      |     |     | - |   |   | -   |     |     |     |   |   |   |   | - |     |     |   | - |   |
| ; | 3369262 Sepia la |   |   |     |     |     | - |   |   |   |    |   |     |     |     |     |    | - |   |   |     |     |     |      |      |     |     | - |   |   | -   |     |     |     |   |   |   |   | - |     |     |   |   |   |
|   | 3369264 Sepia la |   |   |     |     |     |   |   |   |   |    |   |     |     |     |     |    | - |   |   |     |     |     |      |      |     |     | - |   |   | -   |     |     |     |   |   |   |   | - |     |     |   |   |   |
|   | AB192338.1 Sepia |   |   | c . |     |     |   |   | t |   |    | - | t   | . a | 1.  |     |    | с |   |   |     |     |     | с    | a    |     | t   | - |   | t | -   |     |     |     | a |   |   |   | - |     |     |   | с |   |
| • | AB430406.1 Sepia |   |   | c . |     |     |   |   | t |   |    | - | t   | . a | 1.  |     |    | с |   |   |     |     |     |      |      |     | t   | - |   | t |     |     |     |     | a |   |   |   | - |     |     |   | с |   |
| 0 | AY185506.1 Sepia | - |   | a   |     |     |   |   | t |   |    |   |     | . a | 1 0 | : . |    | - |   |   |     | c   | t   |      | a    |     | a   | - |   | t | _   |     |     |     |   |   |   |   |   |     |     |   |   |   |
| 1 | KF009663.1 Sepia |   |   | с   |     |     |   |   | t |   |    |   | t   | . a |     | 1.  | 1. | c |   |   | T.  |     |     |      |      |     | t   |   |   | t |     |     |     |     | a |   |   |   | T |     | T.  |   | c |   |

|    |                  |   |    |   |   |   |     |    |   |   |    |     |     |     |     |     |       |   |    |   |   |   | W   | nd  | DMT. | 551 | -600 |   |       |    |   |    |   |     |   |   |     |       |   |     |     |   |   |     |    |    |
|----|------------------|---|----|---|---|---|-----|----|---|---|----|-----|-----|-----|-----|-----|-------|---|----|---|---|---|-----|-----|------|-----|------|---|-------|----|---|----|---|-----|---|---|-----|-------|---|-----|-----|---|---|-----|----|----|
|    |                  |   |    |   |   |   |     |    |   | - |    | -   | +   | +   |     | +   | +     |   |    |   |   |   |     |     | +    |     |      |   | +     | +  |   |    |   |     |   |   | +   |       |   |     |     |   |   |     |    | ł  |
| 1  | 1542916 Sepia la | a | A  | Ŧ | A | Ŧ | T   | A  | Ŧ | Ŧ | h. | A I | c   | t ( | ; ; | L t | : 0   | G | a  | A | A | t | T 1 | 7   | t A  | A   | t    | A | : 1   | A  | c | t  | Ŧ | Ŧ   | c | Ŧ | T I | 1 0   | A | c   | С   | С | a | A C | A  | Į, |
| 2  | 1626051 Sepia la | ÷ | 4  |   | 4 | ÷ | 14  | ÷  | 4 |   | 4  | +   |     | 4   |     |     |       |   | +  | 4 |   |   | +   |     |      |     | 4    | 4 |       |    |   | 4  | 4 | 4.1 | - |   |     |       |   | +   |     |   | 4 | -   |    | 4  |
| 3  | 1626052 Sepia la |   |    |   |   |   |     |    |   |   |    |     |     |     |     |     |       |   |    |   |   |   |     |     |      |     |      | , |       |    |   |    | , |     |   |   |     |       |   |     |     | - |   |     |    | Į. |
| ŧ. | 1626057 Sepia la | t |    |   | + | + |     | ÷  |   |   |    | -   | . [ | a . |     |     |       |   | t  |   |   |   | +   |     |      |     | c    |   |       |    |   | a  | + |     | ł |   |     |       |   | ÷   |     | + |   |     |    |    |
| 5  | 1733049 Sepia la | 4 |    | - |   | ÷ | +   | ÷  |   | - |    | +   |     |     |     |     |       |   | ÷  |   |   |   | +   |     |      | -   |      | + |       |    | - |    | 4 |     | + |   | -   |       |   | +   |     | - |   |     | +  | -  |
| 6  | 3369262 Sepia la |   | 4  |   | 1 | + | 1   | ÷  | 4 | - | -  | +   |     |     |     |     |       |   |    |   | - | 4 | +   |     | : .  | 1   | 4    | + |       |    | - | 10 | + | -   | - | - |     |       |   | +   | 4   | 1 |   |     |    |    |
| 7  | 3369264 Sepia la |   |    |   |   |   |     |    |   | - |    |     | ļ   |     |     |     | .   . |   | ļ, |   |   |   |     | Į.  |      |     |      |   | ļ.    | l. |   |    |   |     |   |   |     | .   . |   | ļ., |     | - |   |     | Ι. |    |
| 8  | AB192338.1 Sepia |   | Į. |   |   | + |     |    |   |   |    |     | .]  |     |     |     | ].    |   | ļ. |   |   | c | -   | ł   | e .  |     | c    | - |       |    |   | а  | + |     | 4 |   |     |       |   |     |     | - | t | -   | Į. | Į. |
| 9  | AB430406.1 Sepia | 4 |    | - |   | + | ÷   | +  | 4 | - | -  | +   |     |     |     | . 0 |       |   | ÷  |   | - | c | ÷   | . ( | c .  | -   | с    | ÷ |       |    | - | a  | ÷ |     | + |   | -   |       |   | +   |     | - | t | -   | +  | 4  |
| 10 | AY185506.1 Sepia | t | 4  |   | 3 | ÷ | 1.4 | ÷. | 4 | - | 2  | +   | . 1 | c . |     |     |       |   | +  | 4 |   |   | 4   |     |      | -   |      | 4 |       |    |   | a  | ÷ |     | t | 4 | -   |       |   | t   | 1.4 | - | t |     | +  |    |
| 11 | KF009663 1 Sepia |   |    |   |   |   |     |    |   |   |    |     |     |     |     |     |       |   |    |   |   | c |     |     |      |     | c    |   | . [ . |    |   | a  |   |     |   |   |     |       |   | 1.  |     |   | t |     |    | 1  |

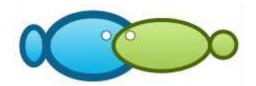
|    |                  |    | _   |     |      | _  |      |     |   |      |            |    |      |    |    |   |   |    |   | _ |              |
|----|------------------|----|-----|-----|------|----|------|-----|---|------|------------|----|------|----|----|---|---|----|---|---|--------------|
|    |                  |    |     | -   | -    | -  | -    | -   |   | -    |            | -  | -    |    |    | - |   | _  | - | - | -            |
| 1  | 1542916 Sepia la | G  | t   | G   | G    | t  | G    | G   | a | G    | A          | с  | с    | с  | т  | A | т | с  | t | т | A            |
| 2  | 1626051 Sepia la |    | -   |     |      |    |      |     | - | +    |            | +  |      | -  | +  | - | - |    | - | - | -            |
| 3  | 1626052 Sepia la | -  | -   | :23 | 1963 | 40 | - 23 | -   | - | ÷    | 3¥         |    | -    | -  | ÷  | 4 | - | 2¥ | - | - | :43          |
| 4  | 1626057 Sepia la | +  |     | -   |      | 40 | -    | 1.2 |   | 84 I | ÷4         | ÷. | -    | 48 | 18 |   |   | t  | с |   | 4            |
| 5  | 1733049 Sepia la | 14 |     |     | -    | -  |      |     | - |      |            |    |      | -  |    |   | - |    | - | - |              |
| 6  | 3369262 Sepia la | Į. | -   |     |      | •  | +    |     |   |      |            |    |      |    | +  |   | - | -  | - | - | -            |
| 7  | 3369264 Sepia la | -  | - 2 | ÷.  | (#)  | 40 | +3   |     |   | 4    | 8 <b>4</b> | ÷. | -    | 43 | ÷  |   | - | 24 |   | × | ; <b>4</b> 3 |
| 8  | AB192338.1 Sepia |    | a   | 1   | 4    | a  | 122  | 1.0 | t | 4    | -          | 84 | 43   | 48 | 41 |   |   | t  | - | - | 4            |
| 9  | AB430406.1 Sepia | +  | a   |     |      | a  | -    |     | t |      | -          |    |      | -  | -  | - | - | t  | - | - | -            |
| 10 | AY185506.1 Sepia |    | a   |     | -    |    | +    |     | t |      | -          | t  | -    |    | +  |   | - | t  |   |   | -            |
| 11 | KF009663.1 Sepia | 4  | a   |     | 40   | a  | -    |     | t | -    | -          | \$ | - 24 | 40 | +  |   |   | 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).

rahoo 🗙 🕂

| ws   | FINANCE   | SPORTS  | ENTERTAINMENT   | LIFE                           | SHOPPING        | YAHOO PLU                            | IS MORE   |   |
|--|---|---|---|--------------------------------|-----------------|--------------------------------------|---|---|
| Find me  | essages, doci   | uments, phot  | os or people  |                                | ~               | Q                                    |   |   |
| ← Back   | k 🔦 🔦   | +   | M Archive   | Move                           | 🖬 Delete        | 😵 Spam                               | ••••  | • • ×                                       |
| • arti   | i <mark>cle revised</mark>  |   |   |                                |                 |                                      |   | Yahoo/Sent 🔺                                |
| I her<br>comp<br>subs<br>also<br>to im<br>Sinc | To: Gavriloa<br>r Dr. Gavriloaie<br>ewith send you<br>pleted. Append<br>stitutions and co | the revised ar<br>ices are moved<br>onsequently the<br>status of Dang<br>c you for your c | ticle (attached). All com<br>d to the text. Figure 3 is<br>e genetic difference. I h<br>eubun's article. She ha | a series of n<br>ope that this | ucleotide seque | ence as specie<br>ulfil your require | tions. Method i<br>s identity to sh<br>ements. Last b | ow the position of<br>ut not least, I would |
| Prata<br>1.3MB                                 | asik et adoc  |   |   |                                |                 |                                      |   |   |
|  |   |   |   | h «h 1                         | ••••            |                                      |   |   |
| h  |   | Ĭ   | i 🔒 📦   | N 🗢                            | <b></b>         | 8                                    |   |   |

mail.yahoo.com/d/folders/2/messages/AGtCqQtYSCBfXQltQwgiCGZ6LsM?guce\_referrer=aHR0cHM6Ly93d3cuZ29vZ2xlLmNvbS8&guce\_ref



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

<sup>1</sup>Silvester B. Pratasik, <sup>2</sup>Hariyani Sambali, <sup>3</sup>Lefrand. Manoppo, <sup>1</sup>Frans F. Tilaar, <sup>1</sup>Meiske S. Salaki

<sup>1</sup> Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>2</sup> Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia: Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia. Corresponding author: S. B. Pratasik, spjong07@yahoo.com

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 LC01490: 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).

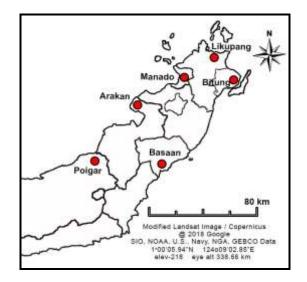


Figure 1. Map of North Sulawesi with sampling points.

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

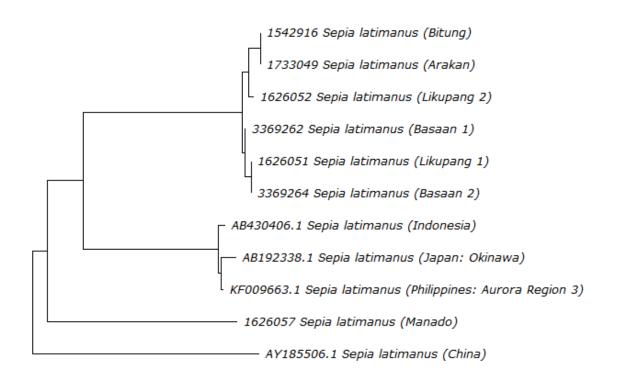
2 3 5 6 7 8 9 No 1 4 10 1 1542916 Sepia latimanus (Bitung) 1626051 Sepia latimanus 2 0.00435 (Likupang 1) 1626052 Sepia latimanus 3 0.00309 0.00371 (Likupang 2) 1626057 Sepia latimanus 0.06984 4 0.06983 0.07071 (Manado) 5 1733049 Sepia latimanus 0.00000 0.00435 0.00309 0.06984 (Arakan) 3369262 Sepia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

Heterogeneity index of cuttlefish Sepia latimanus in North Sulawesi waters.

#### Table 1

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 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).



0.010

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

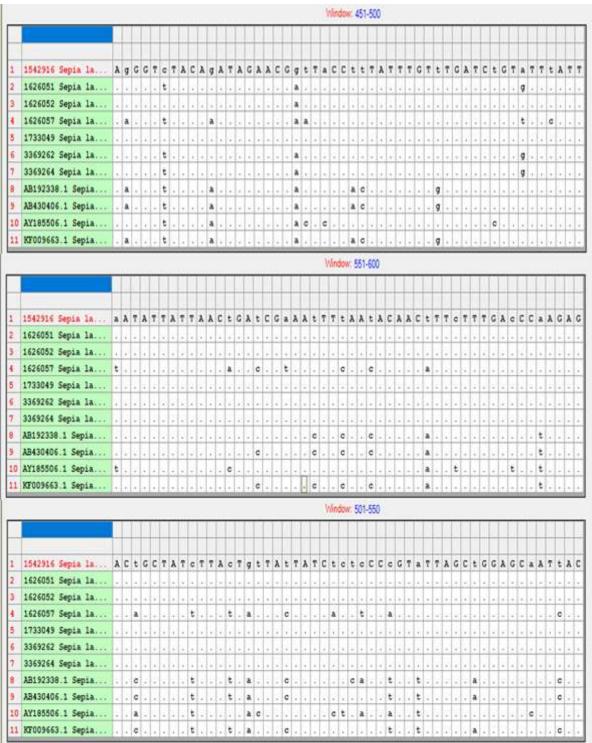
From total numbers of 620 bp, there were 166 polymorphic sites with 186 mutations. Based on variable sites (two variants), there were 73 singleton variable sites with 67 different site positions and 93 parsimony informative sites with 80 site positions. The former was at positions: 2 23 32 33 38 41 56 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 98 119 128 131 134 155 158 161 170 177 189 218 224 233 243 251 261 264 266 267 269 272 290 296 317 320 329 336 344 356 359 363 368 374 380 392 393 413 416 435 437 443 452 456 461 470 476 477 485 509 513 515 519 533 539 548 551 566 572 578 584 596 602 605 608 617. 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).

|   | -   |    |    |    |    |    |   |     | _ |       |     |    |    |    |    |   | _ | _        |    |     |       | 1  | \hn  | dow | 1  | 50   |    | _  | _   |    |     |     |    |    |    |    | _ |   |    |   |     |     |     |     |     |   |
|---|---|----|----|----|----|----|---|-----|---|-------|-----|----|----|----|----|---|---|----------|----|-----|-------|----|------|-----|----|------|----|----|-----|----|-----|-----|----|----|----|----|---|---|----|---|-----|-----|-----|-----|-----|---|
|   | E.  |    |    |    | -  | -  | _ |     | _ | +     | -   | +  | -  | -  | -  |   |   | -        | -  | +   |       | -  | -    |     | -  |      | -  |    |     | -  | -   | -   | -  |    | -  |    |   |   | -  | - | +   | +   | +   | -   | -   | - |
|   | 1542916 Sepia la  |    |    | -  | c  |    | 7 | c   |   | c (   |     |    |    |    | -  | - |   | c        | c  |     |       |    | -    | c   |    |      |    | 1  | c   |    |     |     |    |    |    | 1  |   |   |    | c |     |     | 5 6 | G   |     |   |
| - | 1626051 Sepia la  | ť  | Ť  | f  | ř  | ñ  | Ĥ | -   | 7 |       | Ť   | Ť  | ť  | f  | T. | Ť |   | Ť        | Ť  |     | Ť     | T  | f    | ñ   | 1  | T    | f  | Ť. | Ì   | î  | Ť   | 1   | 1  | Ê  | ĥ  | ñ  | î | 1 | 1  | Ť | Ť   | Ť   | 1   | Ť   | ť   | Ť |
| 1 | 1626052 Sepia la  | ť  | ŕ  | ŕ  | ŕ  | t  | - | +   | + | +     | Ť   | ť  | ť  | ŕ  | f  | - | - | -        | +  | +   | Ť     | t  | ŕ    | -   | ÷  | ť    | ť  | H  | i   | ÷  | ÷   | +   | t  | ÷  | ÷  | +  | - | 1 | t  | t | Ť   | Ŧ   | Ŧ   | t   | ť   | t |
|   | 1626057 Sepia la  | ť  |    | t  | f  | a  | - | -   | Ť | Ť     | Ŧ   | Ť  | ť  | ŕ  | ŕ  | ÷ |   | -        | Ť  |     | Ť     | t  | ŕ    | -   | +  | e .  | ť  | Ĥ  | 1   | a  | c . | +   | f  | f  | -  | ÷  | - | c | +  | 1 | +   | Ť   | Ŧ   | t   | f   | t |
|   | 1733049 Sepia la  |    | f  | f  | -  | 4  | - | -   | 1 | Ť     | +   | Ť  | t  | ŕ  | ÷  | ŕ | 1 | -        | +  | +   | Ť     | ÷  | ÷    |     | Ť  |      | ť  | H  | ÷   | -  | -   | t   | t  | ÷  | ÷  | -  | - | Ť | +  | Ť | t   | +   | +   | t   | f   | t |
|   | 3369262 Sepia la  | ť  | ŕ  | ŕ  | ÷  | ŕ  |   | -   | 1 | 1     | 1   | 1  | ť  | f  | -  |   | - | -        | Ť  | +   | Ť     | Ť  | ÷    |     | +  | Ť    | ť  | H  |     | 1  | Ť   | +   | Ť  | Ť  | -  | Ť  | - | 1 | 1  | Ť | +   | +   | Ť   | Ť   | Ť   | t |
| - | 3369264 Sepia la  | ť  | ť  | ŕ  | ÷  | ŕ  | - | -   | + | +     | Ť   | Ť  | ť  | ŀ  | t  | - | - | -        | t  | 1   | Ť     | ť  | ÷    | H   | +  | +    | t  | H  | 1   | +  | +   | -   | ť  | 1ª |    | -  | - | ÷ | 1  | + | +   | +   | 1   | t   | ť   | † |
|   | AB192338.1 Sepia  | ť  | ŕ  | ÷  | ÷  | g  | - | -   | - | +     | +   | ÷  | ÷  | ŀ  | ÷  |   | ÷ | 4        | ÷  | A . | ÷     | ÷  | ÷    | ÷   | +  | e .  | ÷  | H  | 1   | +  | +   | ÷   | ÷  | 1  | -  | -  | - | ÷ | 4  | + | ÷   | +   | t   | ÷   | t   | 1 |
|   | AB430406.1 Sepia  | ť  | ÷  | ľ  |    | A  |   | -   | a | if    | +   | ť  | ť  | ŀ  | ÷  |   | a | ÷        | ÷  | A . | ÷     | ÷  | -    |     | ÷  | e .  | t  | ÷  | -   | -  | +   | t   | ÷  | ľ  | -  | ÷  | 1 | ÷ | ή  | + | +   | +   | ÷,  | ÷   | t   | + |
| • | AY185506.1 Sepia  | 1  | ÷  | 1  | ŕ  | ŕ  | * | +   | 4 | 4     | ÷   | ÷  | ť  | ŀ  | ÷  | • |   | ÷        | ÷  |     | ÷     | t  | •    | -   | 1  |      | ť  | ÷  | ÷   | 1  | +   | ÷   | ÷  | Ť  | -  | -  | Ť | ÷ | +  | * | 1   | ÷   | ÷,  | ÷   | ÷   | + |
| 1 |   | ť  | +  | ŀ  | *  | -  | + | *   | 1 | +     | ł   | ť  | ÷  | ŀ  | ÷  | • | a | ÷        | ÷  | t . | 1     | 10 | F    | -   | -  | -    | ť  | ÷  | ÷   | +  | +   | ÷   | ÷  | ÷  | a  | -  | * | + | +  | + | a   | ÷   | . 4 | -   | ť   | + |
|   | KF009663.1 Sepia  | Ŀ  | 1  | 1. | Ŀ  | 9  | 1 |     | g | : ] : |     | 14 | ÷  | Ŀ  | ÷  | - | a | 1        |    | a . | 1.1.4 | 1. | ÷    | -   | ni | ¢,   | ÷  | 1. | ÷   | ÷. | -   | 1   | 1. | Ŀ  | 1  |    | 1 | - | -  | 1 | 1   |     | . a | 1   | Ŀ   |   |
|   |   | _  |    |    |    | _  |   |     |   |       |     | _  |    |    |    | _ |   |          | _  |     | _     | 1  | tin. | dow | 5  | 1-10 | 0  |    |     | _  | _   |     |    |    |    | _  |   |   |    | _ | _   |     |     |     | _   |   |
|   | 1   | L, | 1  | L  | Ľ  |    |   |     | _ | 1     |     | 1  | 1  | L  | 1  |   |   | _        | 1  |     | 1     | 1  | L    |     | 4  | 4    | 1  |    | _   | 1  | 1   | 4   | 1  | 1  | 1  |    | 1 |   | _  | 1 | -   | 4   | 4   | 1   | 4   | 1 |
|   | THE REPORT OF THE PARTY OF THE | Į. | 1  | -  | Ļ  | Ļ  |   | _   | _ | -     | -   | 4  | -  | Ļ  | 1  |   | _ | 4        | -  | +   | -     |    | Ļ    |     | -  | -    | +  |    | _   |    | +   | -   | +  | Ļ  | Ļ  | -  |   |   | -  | - | +   | +   | -   | 4   | Ļ   |   |
|   | 1542916 Sepia la  | 7  | T  | đ  | G  | G  | t | λ   | λ | A (   | 0   | c  | C  | G  | 1  | λ | C | <b>A</b> | t  | 74  | 4 5   |    | λ    | λ   | ٨  | tQ   | À  | t  | G   | λ  | c ( | 4   | A  | t  | 7  | λ  | T | λ | t  | λ | Y,  | 1   | GT  | 1 0 | 7 a | 8 |
|   | 1626051 Sepia 1a  | ÷  | ÷  | ÷  | ÷  | ŀ  |   | •   | • | ÷     | -   | ÷  | ÷  | ŀ  | ÷  | - |   | ÷        | -  | ÷   | ÷     | ÷  | ŀ    | -   | 4  | ÷    | ÷  | ŀ  |     | -  | ÷   | ÷   | ÷  | ŀ  | ÷  | ÷  | + | • | 4  | 4 | +   | +   | ÷   | ÷   |     | 1 |
|   | 1626052 Sepia 1a  | +  | ÷  | 1  | ÷  | +  | - | -   | * | +     | +   | +  | ÷  | ÷  | ÷  | * | 4 | 4        | 4  | 4   |       | +  | ŀ    | -   | +  | ÷    | ÷  | 1  | •   | *  |     | +   | -  | Ê  | ÷  | -  | • | - | ÷  | 1 | -   | +   | ÷   |     | +   | - |
|   | 1626057 Sepia la<br>1733049 Sepia la  | ŀ  | •  | -  | -  | •  | - | +   | 4 | ÷     | +   | 1  | -  | ŕ  | ÷  | • | * | ť        | c  | +   | te    | -  | ŀ    | •   | *  | ¢ .  | t  | C  | •   | -  | -   | ł   | 1  | c  | ŀ  | +  | + | - | 1  | 1 | -   | +   | ÷÷  | Ŧ   | -   | 1 |
|   |   | ŀ  | ÷  | ť  | ť  | ŕ  |   | ÷   | 4 | ÷     | +   | ť  | f  | ť  | t  | 1 |   | ÷        | 1  | ÷   | +     | +  | ŕ    | -   | 1  | ÷    | ť  | f  | •   | -  | ÷   | ÷   | ÷  | ť  | t  | ÷  | + |   | i  | i | 1   | +   | ÷   | ÷,  |     | - |
|   | 3369264 Sepia 1a  |    | t  | ť  | t  | Ċ  | İ | Ì   | Ť | Ť     | Ť.  | t  | t  | ť  | ť  | Ċ | Ť | t        | t  | Ť   | Ť     |    | t    | Ľ.  | Ť  | Ť    | t  | Ċ  | Ì   | Ì  | Ť   | Ť   | Ť. | ť  | t  | Ė  | Ť | İ | İ  | t | Ť   | t   | Ť   | t   | Ť   | Ì |
| Ī | AB192338.1 Sepia  | t. | t  | a  | t  | İ. | İ | Ì   | Ì | Ť.    | Ť.  | Ť. | t. | t. | t  | Ū |   | Ċ.       | c  | Ť   |       |    | t    | Ť   | Ì  |      | Ť. | i. | Ì   | Ì  | t . | Ť.  | Ť. | t  | t  | Ċ. | Û | Ì | a  | Ì |     | Ì   | T   |     |     |   |
|   | AB430406.1 Sepia  | 1  | 1. | a  | t. | 1  |   |     |   | T     |     | T. | 1. | 1. | Ť. |   |   | 1        | c  | T   |       | 1. | 1.   | 1.1 | 1  | 1    | T. | 1  |     | 1  | t . | T.  | 1  | t. | 1. | 1  |   |   | c  | 1 |     | 1   |     |     | T.  |   |
|   | AY185506.1 Sepia  |    |    | a  |    |    | A | -   | ÷ |       |     | a  |    |    | 1. |   | 4 | t        | c  |     |       |    |      |     | ÷  |      |    |    |     | 2  | t . |     |    |    |    |    |   |   |    |   | -   |     |     |     | t 9 | ; |
| 1 | KF009663.1 Sepia  |    |    | a  |    |    |   |     |   |       |     |    |    |    |    |   |   |          | c  |     | . 9   |    |      |     |    |      |    | 4  |     |    | ŧ.  |     |    |    |    | +  |   |   | c  |   | -   |     |     | . 1 |     |   |
|   |   |    |    |    |    |    |   |     |   |       |     |    |    | Ī  |    |   |   |          |    |     |       | 1  | Vin  | dow | 1  | )1-1 | 50 | }  |     |    |     |     |    |    |    |    |   |   |    |   | _   |     |     |     |     | ľ |
|   |   |    |    |    |    |    |   |     |   |       | 1   |    |    |    |    |   |   |          |    |     |       |    |      |     |    | 1    | 1  |    |     |    |     |     |    |    |    |    |   |   |    |   |     |     | T   | T   | 1   |   |
|   | 1542916 Sepia la  | À  | G  | 7  | t  | A  | c | a   | G | c 0   | : 0 | A  | e  | G  | G  | t | 7 | T        | ci | A 1 | r t   | A  | Ŧ    | A   | A  | r t  | T  | Ŧ  | c   | 7  | 7 1 | : t | T  | A  | C  | 7  | a | A | T. | A | c ( | c 1 | 7 3 | 1 7 |     |   |
| ŝ | 1626051 Sepia la  |    | 1. |    |    |    |   |     |   |       | T.  |    | Ī. | Γ. |    |   | Ţ | 1        | 1  | T.  | Τ.    | 1. | -    |     |    |      | I. |    |     | 1  | Ţ   |     | T. | Ι. |    |    |   |   |    | 1 |     |     | T   | T   | T   |   |
|   | 1626052 Sepia la  |    |    |    |    |    |   | 1   | Ţ |       | t   |    | Ī. | t  |    |   |   |          | Ī  |     | T.    | 1  |      |     |    |      | İ. |    |     | T  | t   | 1.  | L  | t  |    |    |   |   |    | Ţ | T   | t   | t   | t   |     |   |
|   | 1626057 Sepia la  |    |    | Ċ. |    |    |   |     | Ì | t     | t   |    | İ. | Ē  |    | a |   |          | t  |     | t     |    |      |     | Ì  |      | t  |    |     |    |     |     |    | Ē  |    |    |   |   |    | Ì |     |     |     | t   | T   |   |
|   | 1733049 Sepia la  | f  | Ê  | ÷  | ĥ  |    | - | 1   | 1 | 1     | Ť   | f  | f  | f  |    |   | 1 | 1        | 1  |     | t     | f  | Ē    | H   |    | -    | f  | Ĥ  |     | 1  | 1   | -   | f  | f  | -  |    |   | 1 |    | Ť |     | -   | +   | t   | ť   | 1 |
| 1 |   | ÷  | i. | ÷  | -  | *  | - | +   | + | ÷     | Ŧ   | ÷  | ŕ  | ÷  | +  | * | * | +        | +  | ÷   | ÷     | ÷  | ŕ    | -   | -  | ÷    | ł  | P. | *   | 1  | +   | +   | +  | ÷  | •  | ÷  | - | * | *  | + | +   | +   | Ŧ   | ÷   | ÷   | - |
|   | 3369262 Sepia la  |    | -  |    |    |    | _ | - 1 |   |       | -   | _  |    | -  |    |   |   |          |    |     |       | -  | -    |     |    | 100  |    |    |     |    |     | _   | _  |    |    |    | _ | _ |    | - |     | -   |     |     | ÷   | 1 |
| 1 | 3369264 Sepia la  | -  |    |    |    | _  | _ |     |   | _     |     |    | -  |    |    |   |   | -        |    |     | _     |    | -    | *   | -  | _    | 1  |    |     | -  | -   | 11  |    |    |    |    | - |   | -  | - | -   | +   | 42  | +   | +   | - |
| • | AB192338.1 Sepia  | _  |    | +  | +  | •  | * | •   | • | • •   | +   |    | ŀ  | •  | •  | g | * | -        | _  |     |       |    |      |     | -  | • •  |    |    | - 1 |    | . ( | -   | _  | _  |    |    | - | + | •  |   | •   | •   | +   | -   | +   | - |
|   | AB430406.1 Sepia  |    |    | ÷  |    | +  |   | -   |   |       |     |    | 1  |    |    | g |   |          | t  | •   |       |    |      | •   | ,  |      |    | -  | t   |    | . 4 |     |    |    |    |    | ÷ | 1 |    |   | •   | •   |     |     |     |   |
| 1 |   | -  | -  | _  |    | _  |   |     |   |       |     |    |    |    |    |   |   |          |    |     |       |    |      |     |    |      |    |    |     |    |     |     |    |    |    |    |   |   |    |   |     |     |     |     |     |   |
|   | AY185506 1 Sepia  |    |    | 4  | a  | +  |   | ¢   |   |       |     |    | ¢  |    |    |   |   |          | t  |     | c     |    | -    | -   |    |      |    |    |     |    |     |     |    |    |    |    | ŧ |   |    |   |     |     |     |     |     |   |

| -  |                                      | _ |    | -   |     | -   | -    |         | -   |       | -    | _  |       | -  | -                                       | -  |       | -     | - | -     | Ym    | 140  |     | 51-2 | -   |     |                   |            |             |     | -            |    |     | -  | -  | _     | -    | -          | -   | -   | _     | _   | -   |   |
|----|--------------------------------------|---|----|-----|-----|-----|------|---------|-----|-------|------|----|-------|----|---|----|-------|-------|---|-------|-------|------|-----|------|-----|-----|-------------------|------------|-------------|-----|--------------|----|-----|----|----|-------|------|------------|-----|-----|-------|-----|-----|---|
|    |                                      |   | -  | -   | +   | -   | -    |         |     | +     | +    |    |       | +  | +                                       | +  | -     |       | - | +     | +     | +    |     | -    | +   | -   |                   | -          | -           | +   | +            | -  | -   | -  | -  | +     | +    | -          | H   | H   | -     | -   | L   |   |
|    | TEAMER PLATE IN                      |   |    |     |     |     |      |         |     |       |      |    |       |    |   |    |       | 1     |   |       |       |      |     |      |     |     |                   |            |             |     |              |    |     |    | -  |       |      |            |     |     |       |     |     |   |
| -  | 1542916 Sepia la                     | T | A  | A 1 | 1   | 0   | 6    | -       | 6   | G g   | -    | T  | T     | 6  | 61                                      | -  |       | 0     | T | GI    | 17    | 1    | •   | g 1  | +   | -   | 5                 | 2          | 4           | -   | A            | T  | A   | C  | -  | A 19  | -    | 1 4        | -   | 10  | •     | C   | -   | i |
|    | 1626051 Sepia la                     | - | -  | * * | +   | +   | ŀ    | -       | + 1 | -     | +    | ÷  | -     | +  | +                                       | +  | ÷     | +     | - | ÷     | ÷     | ÷    | ÷   | *    | ÷   | ÷   | ÷                 | -          | +           | +   | +            | ŀ  | -   | +  | -  | +     | ÷    | +          | ÷   | -   | +     | ÷   | ŀ   |   |
| -  | 1626052 Sepia la                     | - | 1  |     | +   | 1   | -    | -       | *   |       | ł    | -  | 4     | +  | ÷                                       | ÷  | f     | •     | * | 1     | ÷     | ť    |     | 4    | ÷   | 1   | +                 | 1          | 1           | -   | -            | ť  |     | -  | 1  | 1     | ÷    | ÷          | ÷   | ÷   | ÷     | -   | ł   |   |
|    | 1626057 Sepia la<br>1733049 Sepia la |   | -  | * * | ť   | +   |      | A       | +   | 1     | +    |    | •     | +  | ÷                                       | ÷  | ŀ     | -     | • | +     | ÷     | ÷    |     | 4    | 1   | -   | +                 | •          | 1           | -   |              | ŕ  | •   | t  | 1  | -     | ÷    | t          | ÷   | ÷   | t     |     | ŀ   |   |
| -  | 3369262 Sepia la                     | + | ÷  | +   | +   | ÷   | ŕ    | ÷       | ÷   | ÷     | ÷    | 1  | H     | +  | ť                                       | t  | ŀ     | ÷     | 1 | +     | ÷     | ť    | ÷   | -    | ÷   | t   | ÷                 | 1          | ÷           | ÷   | 1ª           | ŕ  | ÷   | +  | ÷  | ÷     | ÷    | ÷          | t   | t   | ÷     | ÷   | ť   |   |
| -  | 3369264 Sepia la                     | 1 | -  | +   | +   | ÷   | ť    | H       | -   | +     | ÷    | 1  | ÷     | +  | +                                       | +  | ť     | H     | - | +     | +     | t    | +   | 4    | ť   | 17  | ++-               | -          | ÷           | ÷   | ÷            | f  | H   | -  | ÷  | +     | Ŧ    | ÷          | f   | ÷   | ÷     | -   | f   |   |
|    | AB192338.1 Sepia                     | Ê | 1  | Ť   |     |     | Ť    | a       | i   |       |      | ÷  | H     | 1  | Ť                                       | Ť  | t     | ŧ     |   | Ť     |       | t    |     |      | 1   | t   | Ċ                 |            | t           | Ť   |              | t  | Ċ   | ŧ  | Ť  | t     | Ť    | Ť          | ť   | t   | t     | Ê   | ť   |   |
| -  | AB430406.1 Sepia                     |   | Ť  | t   | -   |     | t    | A       | Ť   |       | ++   |    | Ľ.    | 1  | t                                       | t  | Ľ.    | e     | Ì | t     | Ŧ.    | t    | Ċ   | a .  | t   | Ť.  | Ē                 | Ľ.         | t           | t   | t            | Ē  |     | t  | i  | Ť     | t    | t          | t   | t   | ÷     | -   | t   |   |
| _  | AY185506.1 Sepia                     |   | Ì  |     | -   |     | t    | A       |     |       | +-   | t  | Ľ.    | Ì  | t                                       | t  | t     | e     | Ì |       | ,     | ť.   | Ċ   |      | t   | t   | Ċ                 | c          | Ì           |     | Ť.           | Ê  | Ĥ   | t  | t  | Ť     | Ť    | g          | Ē   | t   | à     | ÷   | t   |   |
|    | KF009663.1 Sepia                     |   | Ì  | Ť   | +   |     | t    |         | t   |       |      | Ľ. | Û     | Ì  | t                                       | t  | ť.    |       | İ | Ť     | Ť.    | Ť.   | Ċ   |      | t   | t   | Ľ.                | Ĩ          | Ì           | t   | Ť.           | t  | Û   | t  | Ì  | Ť     | t    | t          | t   | t   |       | -   | t   |   |
|    |                                      | - | -  |     |     |     | -    | 1.001   |     | - 1.2 |      | -  |       | -  | - 1.                                    | -  | 1.1.1 | -     |   | -     |       |      |     |      |     | 1.0 | -                 | -          |             | -   | -            |    |     | -  | -  | -     | -    | -          | ÷   | -   | -     | -   | -   |   |
|    | _                                    | _ | _  | _   |     | _   | _    |         |     |       | _    |    |       |    |   |    | _     |       |   |       | Wit   | ndov | C 2 | 01-2 | 50  |     |                   |            |             |     | _            |    |     | _  |    |       |      |            |     |     |       |     |     |   |
|    |                                      |   |    |     |     | 1   |      |         |     | 1     |      |    |       |    |   |    |       |       |   | 1     | 1     |      |     |      |     |     |                   |            |             |     |              |    |     | 1  |    | 1     | 1    |            |     |     |       |     |     | i |
|    |                                      |   |    | -   |     | 1   |      |         | -   |       |      |    |       |    |   |    |       |       |   |       |       |      |     |      |     |     |                   |            | 4           |     |              |    |     |    | -  | 1     | _    | -          |     | _   |       |     |     | 1 |
|    | 1542916 Sepia la                     | G | A  | t 3 | A 1 | À   | G    | C       | t   | 7 7   | t    | C  | C     | al | CC                                      | t  | A     | 7     | A | A J   | A c   | A    | A   | 7 2  | 1 7 | A   | A                 | G          | a           |     | 1            | T  | G   | a  | 7  | 1 2   | i c  | 7          | λ   | C   | C     | C   | C   |   |
|    | 1626051 Sepia la                     | • | +  | -   | -   |     | ŀ    | -       | •   | •     | -    | -  | -     | -  |   | +  | +     | -     | + | -     |       | +    | •   | -    | +   | -   | ÷                 | ÷          | •           | +   | ŀ            | -  | •   | -  | -  | -     | -    | -          | Ŀ   | -   | -     | Ŧ   | -   |   |
|    | 1626052 Sepia la                     |   | +  |     |     |     |      | +       |     |       |      | -  | -     | -  |   |    | +     | -     | + |       |       | +    |     |      |     | -   | -                 | +          |             |     |              | -  |     | -  |    |       |      |            | Ŀ   |     | -     | -   | -   |   |
|    | 1626057 Sepia la                     |   | +  |     |     |     |      | +       | a   |       |      | -  | -     | -  |   | a  |       |       |   |       | t     | +    |     |      |     |     |                   |            | ŧ.          |     |              |    |     | 1  |    |       | t    |            |     |     | -     | a   |     |   |
| 2  | 1733049 Sepia la                     |   |    |     |     |     | 1.   |         | 4   |       |      | -  |       |    |   |    |       |       | 1 |       | .].   |      |     |      |     | -   |                   |            |             |     |              |    |     | -  | -1 |       |      |            |     |     |       |     |     |   |
|    | 3369262 Sepia la                     |   |    |     |     |     | 1.   |         | 4   |       |      |    | -     |    |   |    |       |       |   |       | 1.    |      | 4   |      |     |     |                   |            |             |     |              |    | i.  |    | -  |       | . 4  |            |     |     | +     |     |     |   |
|    | 3369264 Sepia la                     |   |    |     |     |     | 1.   |         | 4   |       |      |    |       |    |   |    |       |       |   |       | 1.    |      |     |      |     |     | 1                 |            |             |     |              |    |     |    | -  |       |      |            |     |     |       |     |     |   |
|    | AB192338.1 Sepia                     |   |    |     |     |     |      |         | a   |       |      | -  |       |    |   |    |       |       | , |       | . ] . |      |     |      |     | -   |                   |            |             |     |              |    | ,   |    |    |       | . t  |            |     |     | +     |     |     |   |
| 1  | AB430406.1 Sepia                     |   |    |     |     |     |      |         | a   |       |      | -  | -     |    |   |    |       |       |   |       |       |      |     |      |     | -   | 1                 |            |             |     |              |    |     |    |    |       | t    |            |     |     | +     | -   |     |   |
| 5  | AY185506.1 Sepia                     |   |    | c . |     |     | 11   |         | c   |       | c    | -  |       | t  |   | a  |       |       |   |       |       |      |     |      | 1.  | -   | 1                 |            | t           |     |              |    |     | g  |    |       | . t  |            |     |     |       | t   |     |   |
|    | KF009663.1 Sepia                     |   |    |     |     |     | 1.   |         | a   |       | 1.   |    |       |    |   |    | 1.    |       |   |       |       |      |     |      | 1.  |     | 0                 |            |             |     | 1.           |    |     | 1  | .1 |       |      |            |     |     |       | 1   |     |   |
| -  |                                      | - | -  | -   | -   | -   | -    | inter i | -   | -     | -    | -  | -     | -  | inini i                                 | -  | -     | -     | - | in in | 140   |      |     |      | 100 | -   | int in the second | tente<br>I | <u>inin</u> | -   | -            | -  |     | -  | -  | -     | -    | -          | -   | -   | -     |     | -   |   |
|    | -                                    | _ |    |     |     |     |      |         |     |       | -    |    |       |    |   |    |       |       |   |       | 10    | noo  | W   | 51-3 | 300 |     | _                 |            |             |     |              | _  |     |    |    | _     | _    |            | _   | _   |       |     |     |   |
|    |                                      |   |    |     | 4   | +   | -    | -       |     | 4     | +    | -  |       |    | 4                                       | +  | 1     | -     |   | 4     | +     | +    | 1   |      | +   | +   | Ļ                 |            |             | +   | 4            | 1  | -   |    |    |       | 4    | -          | 4   | 4   | L     | Ļ   | 1   |   |
|    | Internation Provider No.             |   |    |     |     |     |      |         |     |       |      |    |       |    |   |    |       |       |   | -     |       |      |     |      |     |     |                   |            |             |     |              |    | 1   |    |    |       |      |            |     |     |       |     |     |   |
|    | 1542916 Sepia la                     | a | T. | -   | a   | -   | - 14 | 4       | -   | a     | 1    | a  | C     | 1  | -                                       | 7  | a     | 1     | C | C     | 14    | a    | 1   | C    | t a | ı t | T                 | a          | T           | -   | <i>a</i> . n | 1  | •   | 4  | a  | 4     | Gł   | 2 0        | 1   | - 4 | G     | 6   | +   |   |
|    | 1626051 Sepia la                     | 4 |    |     | 4   |     | 4    |         | 4   | •     | -    | +  |       | 4  | •                                       | +  | -     |       | + | *     | -     |      | 1   | +    | -   | •   | -                 | •          | +           | •   | • •          | 1  |     | +  | •  | *     | -    |            | +   |     | +     | +   | ł   |   |
| Į. | 1626052 Sepia la                     | ÷ | 4  | •   | 1   | • • | 1.   |         | •   | •     | •    |    | •     | *  | •                                       | 4  | •     |       | • | •     |       | •    | ŀ   | +    | -   | •   | •                 | -          | ÷           | •   | -            | 1. | ÷   | ÷  | +  | ÷     | -    | +          | 1   | -   | 1     | ŀ   | 4.  |   |
| 5  | 1626057 Sepia la                     | t | •  |     |     |     | 4    |         | -   |       | -    | -  |       |    |   | -  |       | -     | - | a     | -     |      | -   |      | -   |     |                   | -          | +           | •   |              | 1  | -   | +  | •  | -     | - 1  | <b>x</b> : | 1   | t   | 1     | ŀ   | 1   |   |
| 1  | 1733049 Sepia la                     | 4 |    |     | -   |     | 1    |         |     |       |      | +  |       | +  |   |    |       |       | - |       | -     |      |     | ÷    |     |     |                   | -          | ÷           |     |              |    |     | ÷  | -  | +     |      |            | -   |     | +     |     |     |   |
|    | 3369262 Sepia la                     | 1 |    |     |     |     | 1    |         | -   |       |      | 4  |       | -  |   |    |       |       |   |       |       |      | -   | 1    |     |     | -                 |            | -           |     |              |    |     | ÷. | -  |       | +    |            |     |     | 1     |     |     |   |
|    | 3369264 Sepia la                     |   |    |     | -   | _   | -    | 1       |     | -     |      | Т  |       |    |   |    |       |       |   |       |       |      |     |      |     |     |                   |            |             |     |              |    | 1.  | -  | Ţ  | -     |      |            |     |     |       | 1   | Ţ.  |   |
| 8  | AB192338.1 Sepia                     |   |    | -   | +   |     | 17   | 17      | m   | -     | -    | -  | t     |    | a                                       |    | t     |       |   | a     | Ť.    | T.   | İ.  | 1.1  | 1   | 1.  | İ.                |            |             | Ţ   | Τ.           | t. | İ.  |    | t  |       | T    | t.         | 7   | T   | Ť.    | Ť.  | Ť   |   |
|    | AB430406.1 Sepia                     | 1 |    |     | -   |     | 177  | 10      |     | -     |      |    | t     | -  | a                                       | 12 |       |       |   | a     | t     | t    | t   | 11   | Ť   | ť   | t                 | f          | Ì           | Ť   | ť            | t  | t   |    |    |       | f    | Ť          | t   | ť   |       | t   | f   |   |
| -  | AY185506.1 Sepia                     | , |    |     | -   |     | 1.   | 1.0     |     | ŧ     |      | -  |       | -  | a                                       |    | +     | f     |   | a     |       | +    | t.  |      |     | 7 c | f                 |            | Ť           | Ť   | Ť            | f  | f   |    | Ť  | Ť     | Ť    | Ť          | ť   | 1.  |       | +   | İ,  |   |
| -  | KF009663.1 Sepia                     | t | -  |     | *   |     | t    | ++      | -   |       |      | -  | t     |    | a                                       |    | +-    |       |   | -     | +     | -    | +   | -    | 1   | -   | ŀ                 | 4          | *           | +   | ÷            | f  | f   | -  | -  | -     | ÷    | ÷          | ť   | +   | ÷     | ť   | f   |   |
|    |                                      |   |    |     |     |     |      | 100     |     |       | 10.0 | 14 | 1.851 |    | - C - C - C - C - C - C - C - C - C - C |    |       | 10.00 |   | ā     |       |      |     |      |     |     |                   | e - 1      |             | 100 | 182          | 1  | 121 |    | t  | 10.00 | - P. | 1.10       | 100 | 110 | 11.27 | 1.2 | 114 |   |

| _  |                  | _  |    |    |    |    |    | _  | _  |     |     |     |     |    |    |    |    |    |     |   |     |     | Wi       | ndo   | sw. | 30            | 1-25 | 0  |    |     |     |     |     |    |    |    |   |     |     |      |     |       |     |       |     |     |     |
|----|------------------|----|----|----|----|----|----|----|----|-----|-----|-----|-----|----|----|----|----|----|-----|---|-----|-----|----------|-------|-----|---------------|------|----|----|-----|-----|-----|-----|----|----|----|---|-----|-----|------|-----|-------|-----|-------|-----|-----|-----|
|    |                  | Ļ  | -  | ŀ  | -  | -  |    | -  | +  | +   | +   | -   | -   | -  | -  |    |    |    |     |   | +   | +   | +        | +     | +   | $\frac{1}{1}$ | -    |    | -  | +   | +   | ł   |     |    |    | +  | + | +   | +   | +    | +   | +     | ł   | ł     | +   | +   | -   |
| 1  | 1542916 Sepia la | с  | t  | G  | G  | λ  | Ŧ  | G  | λ. | A ( | 2 2 | 10  | Ŧ   | ŧ  | 7  | λ  | t  | с  | с   | t | c   | c 1 | . 1      | 1     | à   | 1             | c    | a  | A  | G 1 | t A | A   | Ŧ   | t  | Ŧ  | a  | T | c   | a ( | c 2  | A ( |       | ; c | c t   | G   | ; ; | a   |
| 2  | 1626051 Sepia la |    | -  |    |    |    |    |    |    |     |     |     | ŀ   |    |    |    | 1  |    |     |   |     | , . |          |       |     |               | ,    |    | -  |     |     |     |     | 4  | -  | -  |   |     |     |      |     |       |     | . ] . |     |     |     |
| 3  | 1626052 Sepia la |    | -  | 1  |    | i. | 4  | -  |    |     |     |     |     |    |    |    |    |    | 1   |   |     |     |          |       |     |               |      |    | -  |     |     |     |     |    | -  | 1  | - | -   |     |      |     |       |     |       |     |     | -   |
| 4  | 1626057 Sepia la | 4  | a  | 1  |    | 1  | 4  | -  | 1  | 1   |     |     |     | a  | -  |    | ŝ. |    | 1   | a |     | . 1 | κ.       |       |     |               |      | -  | 1  | . 1 | a . |     |     | c  |    | c  |   | 1   |     | -    | . 1 | t.    |     | а     |     |     | 4   |
| 5  | 1733049 Sepia la | 4  |    |    |    | ŝ  |    | -  |    |     |     |     |     |    | -  |    | 4  |    | 1   |   |     |     |          |       |     | 1             |      |    | -  |     |     |     |     |    |    |    |   | 1   |     |      |     |       |     |       |     |     | 4   |
| б  | 3369262 Sepia la |    | -  |    |    | ÷  | ÷  | -  | 1  |     |     |     |     |    |    |    | Ş. |    | +   |   |     |     |          |       |     |               |      |    | -  |     |     |     |     | -  | -  | ÷  | - | -   |     |      |     |       |     | -     |     |     |     |
| 7  | 3369264 Sepia la | -  | -  |    | -  |    |    | -  |    | -   |     |     |     |    |    |    | +  |    | ÷   |   | -   |     |          |       |     |               |      | -  | +  |     |     |     |     |    | -  | +  | - | -   | -   |      |     |       |     | -     |     |     |     |
| 8  | AB192338.1 Sepia | ×  | -  |    |    | -  |    |    | -  | -   |     |     |     | ¢  |    |    | c  |    | į.  | a |     |     |          |       |     | 1             |      | t  | 4  | 4   |     |     |     | ×  |    |    | - | -   | -   | -    | 1   | t i   |     | -     |     |     |     |
| 9  | AB430406.1 Sepia | ÷  |    | 1. |    |    |    | -  |    | -   |     |     | k   |    |    |    | c  |    |     | a |     | . 4 | ١.       |       |     | 1             |      | t  |    |     |     |     |     | ×  |    |    |   |     |     | -    |     | ŧ.    |     |       |     |     |     |
| 10 | AY185506.1 Sepia |    |    | ÷  |    | ÷  | +  |    | +  |     |     |     |     | a  |    |    | ÷  |    | +   |   |     | . 0 |          |       |     |               |      | ŧ  |    |     |     |     |     | ¢  |    | +  | - | ÷   | t.  |      | . 1 | ε,    | . 1 |       |     |     | c   |
| 11 | KF009663.1 Sepia | ŀ  | ŀ  | ŀ  |    | ŀ  |    |    | •  |     | .1. | 1.  | ŀ   | c  |    |    | c  |    | • 1 | a |     | . 1 | <b>1</b> |       | 1.  |               |      | t  | •  |     | 4.  | ŀ   | ŀ   | -  |    |    | • |     | •   |      | 1   | t   . |     | 4.    | ŀ   | ŀ   | Ŀ   |
| _  |                  |    |    |    |    |    |    |    | _  |     |     |     |     |    |    | _  |    |    |     |   | _   |     | W        | indo  | W.  | 35            | 1-40 | 0  |    |     |     |     | _   | _  |    |    |   | _   | _   | _    |     |       |     |       | _   |     |     |
| -  |                  |    | +  | +  |    | +  |    | -  | +  | +   |     | +   | +   | +  | +  | ŀ  | 1  |    | _   |   | +   | +   | +        | ł     | ł   | +             | ł    |    | -  | +   | +   | ł   | +   | -  |    | -  |   |     | +   | +    | +   | +     | ł   | +     | +   | +   | +   |
| 1  | 1542916 Sepia la | c  | c  |    | T  | c  | a  | G  | 7  | t ( | 5 7 | 1 1 | c   | 7  | A  | G  | с  | 8  | λ   | T | t : | 7 1 |          | . 1   | . 0 |               | c    | 7  | t  | c   | A 1 | 1   | Ŧ   | A  | G  | с  | g | G   | G   | a: / |     | 7 (   | . 1 | T C   | 2 0 | 1   | c   |
| -  | 1626051 Sepia la |    |    | ١. | 1. |    | t  |    |    |     |     | 1.  | 1.  |    |    |    |    |    |     |   |     |     |          | Ĩ.    |     | 1.            | 1.   | 1  |    |     |     |     |     |    |    |    |   |     | T   |      |     |       |     |       |     |     | T.  |
| 3  | 1626052 Sepia la |    |    |    | Į. |    | t  |    |    |     | Ţ   | 1   |     | -  |    |    |    | -  | +   |   |     | -   | Ţ        |       |     | 1.            | 1.   |    |    | -   |     | Ţ.  |     |    | +  |    |   | -   | -   | -1   |     |       | T   |       |     | Ţ   | Τ.  |
| ŧ. | 1626057 Sepia la |    |    | t  |    |    |    |    |    | a   | Ţ   |     | t   |    |    |    |    | t  | +   |   | c   |     | . 1      | e .   |     |               |      |    | a  |     |     | c   |     |    |    | +  | a |     |     | t    | g   | . 1   | t.  |       |     |     | Γ.  |
| 5  | 1733049 Sepia la | ,  |    | Ţ. | Į. |    |    |    |    |     |     |     |     |    |    |    |    |    |     |   |     |     |          |       |     |               |      |    |    |     |     | Ι.  |     |    | +  |    |   |     | -   |      |     |       |     |       |     | Π.  | Π.  |
| 6  | 3369262 Sepia la | ,  |    |    | ,  |    | t  | -  | ,  |     |     | Ι.  | ,   |    |    | ,  | ,  | ,  |     |   |     | -   |          | , ] , |     |               |      | 4  |    | ,   |     | ,   |     | ,  | ,  |    | , |     |     | ,    | ,   |       |     | , ,   |     | Π.  | Ι,  |
| ,  | 3369264 Sepia la |    |    |    |    |    | ţ  |    |    |     |     |     |     |    |    |    |    |    |     | - | +   |     |          |       |     |               | -    |    |    |     |     |     |     |    |    | 4  |   |     | -   | -    |     |       |     |       | . t |     |     |
| 8  | AB192338.1 Sepia |    |    |    |    | 1  | t  | -  |    | 2   |     |     | t   |    |    | 1  |    | t  | 4   | 1 |     | -   | . 9      | ε.    |     |               |      | 4  |    | -   |     |     |     |    |    |    | c |     | 1   | t    | g   | . 1   | a . |       | . a |     |     |
| 9. | AB430406.1 Sepia |    | -  |    |    |    | t  | -  | -  | -   | 4   |     | t   |    |    |    | -  | t  | ÷   | - | -   | -   |          | ŧ .   |     |               |      | 4  | -  | -   | -   | -   | -   | -  |    |    | c | 2   | . 1 | t    | g   | - 1   | a . |       | . 2 |     |     |
| -  | AY185506.1 Sepia | •  | ŀ  | ŀ  | ÷  | -  | t  | ÷  |    | a . | 4   | 4   | +-  |    | -  | ŀ  |    | t  |     | - |     | -   | n-       | ŧ .   | ŀ   | . t           | t    | •  | a  | -   | +   |     |     |    | 4  | •  | t | -   | - 1 | t i  | g   | - 1   | £ . | -     | -   |     | -   |
| 11 | KF009663.1 Sepia | ÷  | ŀ  | 1. | ł÷ |    | t  | 4  | •  | - 1 | •   | 4   | t   |    | ŀ  |    | ŀ  | t  | +   | • | •   | - 1 | 1        | t   . | ŀ   | -             | ŀ    | 4  | •  | ÷   | ÷   | ŀ   | -   | ÷  |    | ÷  | c | - 1 | - 1 | t    | g   | 4     | a j | d le  | . 1 | 1   | ł   |
|    |                  |    |    |    |    |    |    |    |    |     |     |     |     |    |    |    |    |    |     |   |     |     | W        | ind   | ow  | 40            | 14   | 50 |    |     |     |     |     |    |    |    |   |     |     |      |     |       |     |       |     |     |     |
| _  |                  |    | -  | +  | ŀ  | -  | F  |    |    |     | -   | +   | +   | ŀ  | ŀ  |    |    | -  | -   |   |     |     |          |       | +   | +             | -    | ŀ  |    |     | -   | +   | ŀ   | -  | -  | -  | - |     |     |      |     |       |     | -     | -   | -   | -   |
| 1  | 1542916 Sepia la | λ  |    | 1  | t  | Ŧ  | Ŧ  | a  | G  | G   | ti  | G ( | : a |    | 1  | t  | A  | λ  | Ŧ   | Ŧ | 7   | t   | Å        | Ŧ .   | 7.1 | A (           | c .  | A  | с  | a   | λ   | . 1 | 1 0 | 1  | a  | λ  | λ | 7   | λ   | Ŧ    | g   | с     | G   | t     | 7   | G   | A C |
| 2  | 1626051 Sepia la |    | Ι. |    | Ι. | -  |    |    | +  |     |     |     |     | Ι. | Ι. |    |    |    | 1   |   |     |     |          |       |     |               |      | +  |    |     | +   |     |     |    |    |    |   |     |     |      | a   | +     |     | +     |     |     |     |
| 3  | 1626052 Sepia la |    | Τ, | 1. | 1. | 1  | 1. |    |    |     |     |     | T.  | T. | Ţ. | Τ. |    |    |     |   |     |     | .1       |       |     |               |      | Τ. |    |     | ,   |     | Π.  | 1. |    |    |   |     |     |      |     |       | Ţ   | Ţ     | Ţ   | Ţ   | T.  |
| 4  | 1626057 Sepia 1a | -  | Ť. | t. | t. | t. | Í, | g  |    |     | 1   |     |     |    | T. | İ. | Í. | Í. | 1   |   |     | c   | Ì        |       |     |               | 1.   | t. | 1  |     | 1   |     | t   | t. | 1  | I. |   |     |     |      | a   |       | 1   | a     | t   | t   | t   |
| 5  | 1733049 Sepia 1a | Í. | t  | t  | t  | ť. | ť. | Í. | Ì  | Ì   |     |     | Ť.  | Ť. | t  | ť. | Í. | t  | Í.  |   | Ē.  |     | 1        | 1     | 1   | Ì             | Ť    | Ĺ  | Í. |     | Ì   | t   | t   | t  | ť. | Í. | Û |     | Î   | Î    |     |       | đ   | Î     | t   | t   | t   |
| 6  | 3369262 Sepia la | ľ  | ť  | ľ  | ľ  | ľ  | İ  | Í  |    |     |     |     | t   | ť  | t  | ľ  | Ê  | ľ  | ľ   |   |     |     | 1        | 1     |     |               | ľ    | 1  | ĺ. |     | 1   |     | ť   | t  | É  |    |   |     |     | Í    |     |       | Ť   | 1     | 1   | 1   | ť   |
| 7  | 3369264 Sepia la |    | -  |    | 1  |    | -  |    |    |     |     |     |     | Ť. | 1  | 1. | -  |    |     |   |     |     |          |       |     |               |      |    | f  |     |     |     | ť   | ľ  |    |    |   |     | _   |      | _   |       |     | +     | _   | _   | _   |
| -  | AB192338.1 Sepia | -  | +  | +  |    | 1  | 1  |    |    |     | -   | -+- |     |    | -  | -  | -  | -  | 1   |   | H   |     | 1        | *     | 1   | +             | +    |    | 1  | -   | 1   | -   | ť   | f  | -  | -  | - |     |     |      |     |       |     |       |     | +   | +   |
| -  |                  | -  | T  |    | +  | 1  |    |    |    |     | c   | T   | Τ.  | Т  | Т  | c  | -  | -  | 1   | - | ÷   | *   | 1        | *     | -   | -             | ł    | +  | -  | *   | +   | -   | +   | -  | -  | -  |   | •   |     |      | a   |       | +   |       |     | +   | Ŧ   |
| -  | AB430406.1 Sepia | -  | +- | ŀ  | +- | +  | ŀ  |    |    |     |     | +   | ÷   | ÷  | ÷  | C  | -  |    | -   | * | -   | 4   | 1        | *     | •   | +             | +    | 4  | -  | *   | •   | -   |     | f  | 1  | -  |   | *   | -   |      | a   |       | 4   |       |     | -   | +   |
| -  | AY185506.1 Sepia | -  | T  | T  | -  | -  | 1  | +  | +  | *   | -   | -   |     | +  | +  | +  | ŀ  | -  | ŀ   |   | *   | *   | +        | -     | -   | +             | +    | -  | *  | t   | +   | +   | . t | -  |    | -  | - | *   | -   |      | Δ.  |       |     | -     |     | +   |     |
| 11 | KF009663.1 Sepia |    |    | 1  | 1  | 1. | 4  | 4  | ÷  |     | •   | -   | 1   | 1  | 1  | 1  | i. | +  | 4   | 4 | 1   | +   | 8        | - 1   |     | -             | 1    | 1. | 1  | +   | +   |     | 1   |    | C  | 1+ |   | +   | +   | 4    | a   |       | 4   | +     | +   | +   | 0.0 |



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 reefsurrounded 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.

**Acknowledgements**. We would like to appreciate the Ministry of Research and Technology, Directorate General of Higher Education for financial support of this study through University Superior Basic Research Grant 2018. Great gratitude is also given to Mr. Andre Kambey and Ms. Inggrid Akerina for field sampling activities.

### References

- Alberto F., Raimondi P. T., Reed D. C., Coelho N. C., Lebois R., Whitmer A., Serräo E. A. 2010 Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. Ecology, 91(1), 2010, pp. 49–56.
- Allen J. J., Mäthger L. M., Barbosa A., Hanlon R. T. 2009 Cuttlefish use visual cues to control three-dimensional skin papillae for camouflage. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural and Behavioral Physiology 195:547–555.
- Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W., Lipman D. J. 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acids Research 25:3389-3402.
- Arkley K., Jacklin M. S., Boulter M., Tower J. 1996 Cuttlefish: a guide to its exploitation in UK waters. The sea fish Industry Authority. Seafish Report no. SR467. 81 pp.
- Barbosa A., Mäthger L. M., Buresch K. C., Kelly J. C., Chubb C., Chiao C., Hanlon R. T. 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive patterns. Vision Research 48:1242–1253.
- Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T. 2012 Cuttlefish use visual cues to determine arm postures for camouflage. Proceedings of the Royal Society B Biological Sciences. Vol. 279: 84-90.
- Chiao C.-C., Chubb C., Buresch K. C., Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T. 2010 Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. Journal of Experimental Biology 213:187–199.
- Drummond A. J., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowitz S., Moir M., Stones-Havas S., Sturrock S., Thierer T., Wilson A. 2012 Geneious v5.5.6, http://www.geneious.com.
- Fernandez L. D., Hernandez C. E., Schiaffino R. M., Izaguirre I., Lara E. 2017 Geographical distance and local environmental conditions drive the genetic population structure of a freshwater microalga (Bathycoccaceae; Chlorophyta) in Patagonian lakes. FEMS Microbiology Ecology, 2017, Vol. 93, No. 10. 10 pp.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5):294-299.
- Hanlon R. T., Chiao C.-C., Ma<sup>°</sup>thger L. M., Barbosa A., Buresch K. C., Chubb C. 2009. Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Philosophical Transactions of the Royal Society B364, 429 – 437.

- Hays G. C. 2017 Ocean currents and marine life. Magazine. Current Biology 27, issue 11, R431-R450. 4 pp.
- IUCN, 2014 The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 25 September 2014.
- Kelman E. J., Osorio D., Baddeley R. J. 2008 A review of cuttlefish camouflage and object recognition and evidence for depth perception. Journal of Experimental Biology 211:1757–1763.
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018 MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
- Leopold A. 1933 Game Management. Charles Scribner's Sons. New York. 481 p.
- McManus M. A., Woodson C. B. 2011 REVIEW Plankton distribution and ocean dispersal. The Journal of Experimental Biology 215, 1008-1016.
- Murphy J. M., Balgueri ´as E., Key L. N., Boyle P. R. 2002 Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the Northwest African Coast. Bulletin of Marine Science, 71: 545–553.
- Neves A., Cabral H. N., Sequeira V., Figueiredo I., Moura T., Gordo L. S. 2009 Distribution patterns and reproduction of the cuttlefish, Sepia officinalis in the Sado estuary. (Portugal) Journal of the Marine Biological Association of the United Kingdom 89(3):579–584.
- Pascual M., Palero F., García-Merchán V. H., Macpherson E., Robainas-Barcia A., Mestres F., Roda T., Abelló P. 2016. Temporal and spatial genetic differentiation in the crab Liocarcinus depurator across the Atlantic-Mediterranean transition. Scientific RepoRts 6:29892
- Pawson, M.G. 1995 Bio-geographical Identification of English Channel fish and shellfish stocks. CEFAS Fisheries Research Technical Report No 99. 72 pp.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D. 2015 Size at First maturity of cuttlefish, *Sepia latimanus*, from North Sulawesi Waters, Indonesia. Marine Science 5(1):6-10.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D. 2016 Mitochondrial CO1 genetic markerbased species diversity of cuttlefish (Cephalopod; Mollusk) in Manado Bay and Lembeh Strait, North Sulawesi, Indonesia. AACL Bioflux 9(6):1345-1354.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D. 2017 Egg placement habitat selection of cuttlefish, Sepia latimanus (Sepiidae, Cephalopoda, Mollusca) in North Sulawesi waters, Indonesia. AACL Bioflux 10(6):15141523.
- Ramachandran S., Deshpande O., Roseman C. C., Rosenberg N. A., Feldman M. W., Cavalli-Sforza L. L. 2005 Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proceedings of the National Academy of Sciences USA vol. 102 (44): 15942-15947.
- Ratnasingham S., Hebert P. D. N. 2007 BOLD: the barcode of life data system. Molecular Ecology Notes 7:355–364.
- Reid A., Jerep P., Roper C. F. E. 2005 Family Sepiidae. In: Cephalopods of the world. An annotated and illustrated catalogue of Cephalopod species known to date. Volume 1. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). Jerep P. & Roper C.F.E. pp 54-152. FAO, Rome.
- Rozas J., Ferrer-Matta A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., Sánchez-Gracia A. 2017 DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12): 3299-3302.
- Shohet A. J., Baddeley R. J., Anderson J. C., Kelman E. J., Osorio D. 2006 Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage. Journal of Experimental Biology 209:4717–4723.
- Tricarico E., Amodio P., Ponte G., Fiorito G. 2014 Cognition and recognition in the cephalopod mollusc *Octopus vulgaris*: coordinating interaction with environment and

conspecifics. In Witzany, G. (ed.). *Biocommunication of Animals*. Springer. pp. 337–349. ISBN 978-94-007-7413-1.

Received: 25 April 2019. Accepted: 31 May 2019. Published online: xx June 2019. Authors:

Silvester B. Pratasik, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: spjong07@yahoo.com

Harjany Sambali, Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: hariyanisambali@unsrat.ac.id Lefrand Manoppo, Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam

Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: lefrandmanoppo@vahoo.com

Frans F. Tilaar, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: fftilaar@unsrat.co.id Meiske S. Salaki, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: mssalaki@unsrat.co.id This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

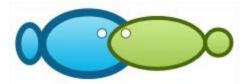
How to cite this article:

Pratasik S. B., Sambali H., Manoppo L., Tilaar F. F., Salaki M. S., 2019 Genetic variations of cuttlefish *Sepia latimanus*, cephalopoda, Sepiidae, in North Sulawesi waters, Indonesia. AACL Bioflux 12(3):xxx-xxx.

AACL Bioflux, 2019, Volume 12, Issue 3. http://www.bioflux.com.ro/aacl

AACL Bioflux, 2019, Volume 12, Issue 3. http://www.bioflux.com.ro/aacl

| 5  | FINANC   | æ  | SPORTS   | ENTERTAIN  | MENT      | LIFE       | SHOPPING        | YAHOO P      | LUS      | MORE            |              |        |
|--|--|--|--|--|-----------|------------|-----------------|--------------|----------|-----------------|--------------|--------|
| nd me                                      | essages,   | , doc  | uments, pho  | tos or people  | 2         |            | ~               | Q            |          |                 |              |        |
| - Back                                     | •  | *  | +  | iii  | Archive   | Move       | 💼 Delete        | 😵 Spam       |          |                 |              | • >    |
| the  | final fo   | orm  | of your ow   | n paper: Ge  | netic va  | riations o | of cuttlefish : | Sepia latir  | nanus    | Ya              | ahoo/Inbox   |        |
| 1  | 1.177  |  |  | <ionelclaudiu@< td=""><td>@yahoo.co</td><td>&gt;m&gt;</td><td></td><td>-</td><td>⊗ Tu</td><td>ie, Jul 2, 2019</td><td>9 at 2:54 PM</td><td>*</td></ionelclaudiu@<> | @yahoo.co | >m>        |                 | -            | ⊗ Tu     | ie, Jul 2, 2019 | 9 at 2:54 PM | *      |
| J  | To: Be   | enny F   | Pratasik   |  |           |            |                 |              |          |                 |              |        |
|  |  |  |  |  |           |            |                 |              |          |                 |              |        |
| Dea  | r Profe  | SSOL   | Pratasik   |  |           |            |                 |              |          |                 |              |        |
|  |  |  | Pratasik,  |  |           |            |                 |              |          |                 |              |        |
|  |  |  |  | er is ready. I l   | have ma   | ade severa | al corrections  | s within the | e refere | nces sect       | tion.        |        |
| The  | final fo   | orm c  | f your pape  |  |           |            |                 |              |          |                 |              | 5      |
| The<br>Kinc                                | final fo<br>dly cheo   | orm o<br>ck it<br>biofl                            | f your pape<br>carefully an<br>ux.com.ro/h                 | er is ready. I h<br>d then let me<br>lome/volume   | e know i  | f you agre |                 |              |          |                 |              | ç.     |
| The<br>Kinc                                | final fo<br>dly cheo   | orm o<br>ck it<br>biofl                            | f your pape<br>carefully an<br>ux.com.ro/h                 | d then let me  | e know i  | f you agre |                 |              |          |                 |              | Ē.     |
| The<br>Kinc<br><u>http</u><br>Tha          | final fo<br>dly cheo   | orm o<br>ck it<br>biofl<br>very                    | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | ,<br>, |
| The<br>Kind<br><u>http</u><br>Tha          | final fo<br>dly cheo<br>://www.<br>nk you                    | orm c<br>ck it<br>biofl<br>very<br>ours            | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | C)     |
| The<br>Kinc<br><u>http</u><br>Tha          | final fo<br>dly cheo<br><u>//www.</u><br>nk you<br>dially yo | orm c<br>ck it<br>biofl<br>very<br>ours            | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | Ċ      |
| The<br>Kinc<br><u>http</u><br>Tha          | final fo<br>dly cheo<br><u>//www.</u><br>nk you<br>dially yo | orm c<br>ck it<br>biofl<br>very<br>ours            | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | 5)     |
| The<br>Kinc<br><u>http</u><br>Tha          | final fo<br>dly cheo<br><u>//www.</u><br>nk you<br>dially yo | orm c<br>ck it<br>biofl<br>very<br>ours            | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | 5      |
| The<br>Kinc<br><u>http</u><br>Tha          | final fo<br>dly cheo<br><u>//www.</u><br>nk you<br>dially yo | orm c<br>ck it<br>biofl<br>very<br>ours            | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | C      |
| The<br>Kinc<br><u>http</u><br>Tha          | final fo<br>dly cheo<br><u>//www.</u><br>nk you<br>dially yo | orm c<br>ck it<br>biofl<br>very<br>ours            | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | 2      |
| The<br>Kinc<br>http<br>Tha<br>Cord<br>Clau | final fo<br>dly cheo<br><u>//www.</u><br>nk you<br>dially yo | orm c<br>ck it i<br>bio <u>fil</u><br>very<br>ours | f your pape<br>carefully an<br><u>ux.com.ro/h</u><br>much! | d then let me  | e know i  | f you agre |                 |              |          |                 |              | ŝ      |



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

<sup>1</sup>Silvester B. Pratasik, <sup>2</sup>Hariyani Sambali, <sup>3</sup>Lefrand. Manoppo, <sup>1</sup>Frans F. Tilaar, <sup>1</sup>Meiske S. Salaki

<sup>1</sup> Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>2</sup> Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Corresponding author: S. B. Pratasik, spjong07@yahoo.com

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).

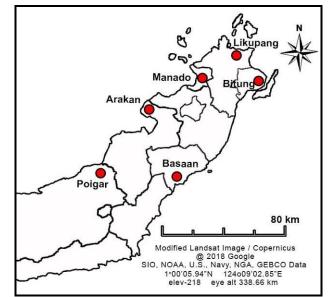


Figure 1. Map of North Sulawesi with sampling points (marked red).

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°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).

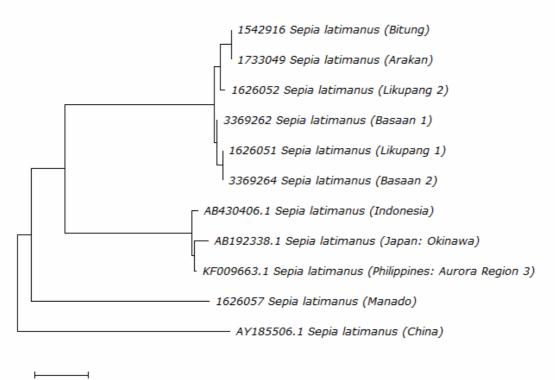
**Results and Discussion**. During the study, 7 individuals of *S. latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not 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 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 of Basaan waters. On the other hand, both *S. latimanus* samples from Likupang waters (Figure 2).

Table 1

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

Genetic distance of cuttlefish Sepia latimanus in North Sulawesi waters



0.010

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.

| -  |  |   |         |                 |    |          | _  | _   | _     | _     | _   | _           | _ | _                |       |                           | _   | _   | _   | W   | ndo  | w:                                    | 1-50 |       |                 | _   | _ | _   | _   | _   | _  | _  | _  | _  | _ |   | _        | _                |   |   | _ |     |                  |   |
|----|--|---|---------|-----------------|----|----------|----|---|-------|-------|-----|-------------|---|------------------|-------|---------------------------|---|-----|-----|-----|------|---------------------------------------|------|-------|-----------------|-----|---|-----|-----|-----|----|----|--|----|---|---|----------|------------------|---|---|---|-----|------------------|---|
|    |  |   |         | 4               | 4  | -        | 1  | Ц   |       | 1     | +   | 1           |   |                  |       | +                         | 1   |     |     | 1   | +    | Ļ                                     |      |       | 4               | +   | Ļ | +   | Ļ   | Ļ   | Ļ  | ļ  |  |    |   |   |          |                  |   |   |   |     | -                |   |
| _  |  | _ |         |                 |    |          | +  |   |       | +     | +   | -           | _ |                  | +     | +                         | -   |     |     | +   | +    |                                       |      |       | +               | +   | + | +   | +   | -   | -  |    | -  |    |   | _   |          | _                |   |   |   | _   |                  |   |
|    | 1542916 Sepia la   | 7 | t       | T C             | a  | T C      | a  | G   | G     | T 1   | 1   | Å           | T | T                | 9 0   | 0                         | g   | λ   | С   | a 1 | r (C | λ                                     | t    | Ŧ.    | λJ              | 10  | 1 | t   | 1   | À   | A  | T  | t  | λ  | 7 | t   | С        | G                | t | λ   | G | t   | G                | A |
|    | 1626051 Sepia la   | * |         |                 | ×  |          |    | •   | •     |       |     |             |   | •                |       |                           |   |     | -   | •   |      |                                       |      |       | •               |     |   |     |     |     |    |    |  |    |   |   |          |                  |   | á   |   |     |                  |   |
| -  | 1626052 Sepia la   |   | •       |                 |    | • •      |    |   | -     | •     |     |             |   | •                | •     | • •                       |   |     | -   | •   | • •  |                                       |      | •     | •               | •   |   |     | •   |     |    |    | •  |    |   | •   | •        |                  |   |   | • |     | •                |   |
|    | 1626057 Sepia la   |   | a       |                 | g  |          |    |   |       | • •   |     |             |   |                  | a.    |                           | t   |     | -   |     |      |                                       | c    | •     |                 |     | a | ( C | : . |     |    |    |  |    |   | c   |          |                  | a |   |   |     |                  |   |
| 5  | 1733049 Sepia la   |   |         |                 |    |          |    |   |       |       |     |             |   |                  |       |                           |   |     |     |     |      |                                       |      |       |                 |     |   |     |     |     |    |    |  | i  |   |   |          |                  |   |   |   |     |                  |   |
|    | 3369262 Sepia la   |   |         |                 |    |          |    |   | •     | • •   |     |             |   |                  | •     |                           |   |     | -   |     | •    |                                       |      |       |                 | 4   |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   |     |                  |   |
|    | 3369264 Sepia la   |   |         |                 |    |          |    |   |       |       |     |             |   |                  | •     |                           |   |     | -   |     |      |                                       |      |       | •               |     |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   |     | •                |   |
| 1  | AB192338.1 Sepia   |   |         |                 | g  |          | g  |   |       |       |     |             |   |                  | a.    |                           | a   |     | -   |     |      |                                       | c    |       |                 |     |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   | a   |                  |   |
|    | AB430406.1 Sepia   |   |         |                 |    |          | g  | 0   |       |       |     |             |   |                  | a .   |                           | a   |     |     |     |      |                                       | c    |       |                 |     |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   | a   |                  |   |
| 10 | AY185506.1 Sepia   |   |         |                 |    |          |    |   |       | • •   |     |             |   |                  | a .   |                           | t   |     |     | t   |      |                                       |      |       |                 |     |   |     |     |     |    |    | a  |    |   |   |          |                  | a |   |   | a   |                  | ł |
| 11 | XT009663.1 Sepia   |   |         |                 | g  |          | 9  |   |       |       |     |             |   |                  | a .   |                           | a   |     | -   |     |      |                                       | c    |       |                 |     |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   | a   |                  |   |
|    |  |   |         |                 |    |          |    |   |       |       |     |             |   |                  |       |                           |   | _   |     | W   | indo | NC.                                   | 51-1 | 00    | 1               | _   |   | _   | _   | _   |    | _  |  |    |   | _   | _        | _                | _ | _   |   |     | _                |   |
|    |  |   |         |                 |    |          |    |   |       |       |     |             |   |                  |       |                           |   |     |     |     |      |                                       |      |       |                 |     |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   |     |                  | 0 |
|    |  |   |         |                 |    |          |    |   |       |       |     |             |   |                  |       |                           |   |     |     |     |      |                                       |      |       |                 |     |   |     |     | Ļ   |    | L  |  |    |   |   |          |                  |   |   |   |     |                  | Ĺ |
| -  | 1542916 Sepia la   |   | T       | g G             | G  | t A      | A  | λ   | C     | C     | G   | G           | T | λ                | C a   | t                         | T   | a   | t   | T 1 | N A  | A                                     | t    | G.    | A 1             | 6   | 3 | . 0 | C   | 3   | λ  | t  | T  | λ  | Ŧ | λ   | t.       | A                | A | T   | G | T   | c                | a |
| _  | 1626051 Sepia la   |   | -       | • •             | ŀ  |          |    | •   |       |       |     | •           |   |                  |       |                           |   | •   |     |     |      |                                       |      |       |                 | 4.  |   |     |     |     |    |    |  |    |   | •   |          |                  |   | -   |   |     |                  |   |
|    | 1626052 Sepia la   |   |         |                 | ·  |          |    | •   |       |       |     |             |   |                  | .  .  |                           | -   |     |     | •   |      |                                       |      |       | •               | +   |   |     |     | •   |    | ŀ  |  |    |   | •   |          | •                |   | •   |   |     |                  | • |
|    | 1626057 Sepia la   | ŀ | . 4     | а.              | +  | • •      | •  | •   |       | . 4   | ۱.  | •           | • | •                |       | c                         |   | t   | c   | •   | +    |                                       | c    |       | . 4             | :   |   | 1   |     |     |    | c  | -  | •  |   | •   | •        | -                |   | -   | • |     | t                |   |
|    | 1733049 Sepia la   | · | -       | • •             | •  | • •      | •  | •   |       | •     |     | •           |   | •                | •     | +                         |   | •   |     | •   | ÷    |                                       | •    | •     | •               | ÷   |   | •   | +   | •   |    | ŀ  | ŀ  | •  | • | -   | •        | -                | • | •   | • | +   | +                | • |
| -  | 3369262 Sepia la   |   | -       | •               | H  | • •      | +  | H   | •     | +     | -   | •           | • | •                | +     | •                         |   | •   | +   | •   | •    |                                       | •    | •     | +               | +   | + | +   | -   | +   |    | ŀ  |  | •  | - | +   |          | +                | • | -   | • | •   | t                | • |
|    | 3369264 Sepia la<br>AB192338.1 Sepia   |   |         | <br>а.          | ÷  |          | •  | H   | •     | •     |     | •           | • | •                | •     | c                         | -   | •   | · · | •   | +    |                                       | -    | •     |                 | +   | + |     |     | +   |    | ŀ  | +  | •  | • | -   | ·<br>c   | 1                | • |   | • |     | •<br>t           | 1 |
| -  | AB192338.1 Sepia   |   | -       | а.<br>а.        | Ħ  |          |    | H   | Ť     | ł     |     |             | ÷ | Ť                | Ŧ     | c                         | -   |     | e   | +   | Ť    |                                       |      | +     | f               | ť   | 1 | t   | +   | ť   |    |    |  |    | ÷ | -   | c        | ÷                | İ | i   | ÷ | -+  |                  | • |
|    | AY185506.1 Sepia   |   |         | a.              |    | а.       | ť. | į.  |       |       |     | i.          | ÷ |                  |       | : c                       | -   |     | -   | Ì   |      |                                       | i.   | ;     |                 | t   |   | t   | +   | t,  |    | İ. | ť.   | i  |   | Ť   | -        | j                |   | j   |   | -   | t i              | - |
| -  | KF009663.1 Sepia   |   | -       | а.              | ÷  | -        | ÷  | ÷   | ÷     | -     | +   | ÷           | ÷ | ÷                | -     | c                         | -   | ÷   | c   | -   | +    | ÷                                     | ÷.   | ÷     | -               | Ŧ   | - | ł   | -   | t   | -  | ŕ  | ÷  | ÷  | ÷ | ÷   | c        | Ì                | ÷ |   | ÷ |     | t                | * |
|    | 1542916 Sepia la   | 2 | C I     | <del>7</del> +  | 2  | C .      | c  | c   | c     | с э   | l t | G           | G | t                | T 1   |                           | A   | -   | +   | 2 * |      | 2                                     | -    | +     |                 |     |   |     |     |     |    | 1  | c  | 7  | a | 2   | <b>9</b> | 2                | c | c   | - | A   | -                |   |
| -  | 1626051 Sepia la   |   |         |                 | -  |          |    | -   | ~ ·   |       |     | -           | • | *                |       |                           | -   | •   |     |     |      |                                       |      |       |                 |     |   | +   |     | -   |    | -  |  | •  | - | **  |          |                  | ~ | •   | - | -   |                  | T |
| -  |  |   | -       |                 | -  |          | •  | -   | •     |       | •   | ÷           |   | •                |       |                           | •   |     | -   | •   | -    | •                                     |      | C     | •               |     |   | +   |     | -   | ÷  | ŀ  | -  | •• | - | •   | •        | -                | • |   | • |     | -                | • |
|    | 1626052 Sepia la   |   | -       |                 | •  |          | •  | -   | •     |       | •   | -           | - | •                |       |                           | -   | ••• | -   | •   | -    | •                                     |      | С     | •               |     |   | +   |     | -   | •  | ŀ  | -  | •  | • | -   | •        | ••••             | • | -   | • | •   | - 1              | C |
|    | 1626057 Sepia la   |   | -       | • •             | •  |          |    | -   | •     |       |     | -           | • | a                | -     | t                         | •   |     | -   | •   | -    | •                                     |      |       | •               |     |   | •   | C   | : 0 |    | •  |  | •  |   | •   |          | •                | • |   | • |     | -                | • |
| 5  | 1733049 Sepia la   |   | •       |                 | •  |          | •  | -   | •     |       |     |             |   |                  |       | •                         |   | ••  |     | •   |      | •                                     | •    |       | •               | •   |   | •   | •   |     | •  | ŀ  | ••   | •  | • | •   |          | •                | • | •   | • | ••• | •                |   |
|    | 3369262 Sepia la   |   | •       |                 | •  |          |    | •   | •     |       |     |             |   |                  |       |                           |   |     |     |     |      |                                       |      | C     |                 |     |   | +   | •   |     |    |    | -  |    |   |   |          | •                |   |   | • |     |                  |   |
| -  |  |   |         |                 |    |          |    |   |       |       |     |             |   |                  | -     | +                         |   |     |     |     | 111  | Ι.                                    |      | c     |                 |     |   |     |     |     |    |    |  |    |   |   |          |                  |   |   |   |     |                  |   |
| -  | 3369264 Sepia la   |   | -       | +               | 11 | • •      |    | •   | •     |       | •   |             |   |                  |       |                           |   |     |     |     |      | -                                     |      |       |                 | . 1 |   | 1   | 11. |     | 11 | 11 |  |    |   | -   |          |                  |   |   |   |     | -                |   |
|    | 3369264 Sepia la<br>AB192338.1 Sepia   |   |         |                 | ++ | • •      | +  | •   |       |       |     |             | • | -                |       | +                         | +-  |     |     | +   |      |                                       |      | -     | •               | -   |   |     | C   |     |    |    |  |    |   |   |          |                  |   |   |   |     | 111              |   |
|    |  |   |         |                 | ++ | -        | +  | •   | •     |       |     | •           |   | g                |       | +                         |   |     |     | +   | +    |                                       |      |       |                 | . t |   | +   | . c | +   | •  |    |  |    |   | •   |          |                  |   |   |   |     | -                |   |
|    | AB192338.1 Sepia   |   |         |                 |    | -        |    | -   | •     | · ·   |     | •           |   | g                |       | t                         |   |     |     | +   | +    |                                       |      | •     |                 |     |   | +   | +   | +   | •  |    |  |    | t | •   |          |                  | • |   | • | •   | •                |   |
| 0  | AB192338.1 Sepia<br>AB430406.1 Sepia   | • |         |                 |    |          |    | •<br>•<br>•   | •     | · ·   |     | ·<br>·<br>· |   | g<br>g           |       | . t                       |   |     |     | +   | +    |                                       |      | •     | •               |     |   |     | +   |     |    |    |  |    | t | •   | •        | -                | • |   | • |     | •                |   |
| 0  | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia   | • |         | <br>            |    |          |    | -   | •     | · ·   | -   |             |   | g<br>g           |       | . t                       |   | •   | c   |     | +    |                                       |      | 200   | •               |     |   |     |     |     |    |    |  |    | t | •   | •        | -                | • |   | • |     | -                |   |
| .0 | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia   | • |         | <br>            |    |          |    | · · · · · · · · · · · · · · · · · · ·   | •     | · ·   | -   |             |   | g<br>g           |       | . t                       |   | •   | c   |     |      |                                       |      | 200   | •               |     |   |     |     |     |    |    |  |    | t | •   |          |                  | • |   | • | •   | -                |   |
| .0 | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia   |   |         | <br>. a         |    |          |    |   |       |       |     |             | • | g<br>g<br>g      |       | . t                       |   |     | c   |     | ndo  |                                       |      |       |                 |     |   |     | . c |     |    |    |  |    | - | •   |          |                  | • |   | • | •   |                  |   |
| .0 | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia   |   |         | <br>. a         |    |          |    |   |       | · · · |     | T           | • | g<br>g<br>g<br>g | <br>  | . t                       |   |     | c   |     | ndo  |                                       |      |       |                 |     |   |     | . c |     |    |    |  |    | - |   | G        | c                |   |   |   |     |                  |   |
| .0 | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la   |   |         | <br>. a         |    |          |    |   |       |       |     |             | • | g<br>g<br>g<br>g |       | . t                       |   |     | c   |     | ndo  |                                       |      |       |                 |     |   |     | . c |     |    |    |  |    | - |   | G        |                  | a |   |   |     | -<br>-<br>-      |   |
| .0 | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la   |   |         | <br>. a<br><br> |    | <br><br> |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-   |       |       |     |             | • | g<br>g<br>g<br>g |       | . t                       |   |     |     |     | ndo  |                                       |      | T     | t (             |     |   |     | . c |     |    |    | ·<br>·<br>·<br>·<br>·  |    | - |   |          | G                |   |   |   |     | - (              |   |
| 0  | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la   |   |         | <br>. a         |    |          |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-                                    |       |       |     | T           | • | g<br>g<br>g      | · · · | . t                       |   |     | c   |     | ndo  |                                       |      | T     |                 |     |   |     | . c |     |    |    | -<br>-<br>-<br>-<br>-  |    | - |   |          | G                |   |   |   |     | -<br>-<br>-<br>- |   |
|    | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>I542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la   |   |         | <br>. a<br><br> |    | <br><br> |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-                               |       |       |     |             | • | g<br>g<br>g      |       | . t                       |   |     |     |     | ndo  |                                       |      | T     | t (             |     |   |     | . c |     |    |    | ·<br>·<br>·<br>·   |    | - |   | G        |                  |   | -<br>-                                    |   |     |                  |   |
|    | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>IS42916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la   |   |         | <br>. a<br><br> |    | <br><br> |    |   |       |       |     | T           | • | g<br>g<br>g      | · · · | . t                       |   |     |     |     | ndo  |                                       |      | T     | t (             |     |   |     | . c |     |    |    | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·                               |    | - | ·<br>·<br>·<br>·<br>·   |          | G                |   | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |   |     |                  |   |
|    | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>I542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la   |   | A )<br> | <br>. a<br><br> |    | <br><br> |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |       |       |     | T           | • | g<br>g<br>g      | · · · | . t                       |   |     |     |     | ndo  |                                       |      | T     | t (             |     |   |     | . c |     |    |    | ·<br>·<br>·<br>·<br>·<br>·   |    | - |   |          | -<br>G<br>-<br>- |   |   | • |     |                  |   |
|    | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>I542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369264 Sepia la                     |   | A )<br> | <br>. a<br><br> |    | <br><br> |    |   | . 1   | · ·   |     | T           | • | g<br>g<br>g      | · · · | . t                       |   |     |     |     | ndo  | · · · · · · · · · · · · · · · · · · · | g    | T     | t (             |     |   |     | . c |     |    |    | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>· | t  | - | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |          | C                |   |   | • | t   |                  |   |
|    | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>3369262 Sepia la<br>3369263 Sepia la<br>3369263 Sepia la |   | A 2<br> |                 |    | <br><br> |    |   | · · · |       |     | T           |   | g<br>g<br>g      |       | . t<br>t<br>t<br>. t<br>t | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |     |     |     |      |                                       | g    | T<br> | t (<br><br><br> |     |   |     |     |     |    |    | •  |    | - | •   |          | •                | t | · · · · · · · · · · · · · · · · · · ·     |   |     |                  |   |

|    |                  | _ |   |   |        |     | _ |     |     |    |    |   |   |   |     |     |     |     |     |    |    | W | lind | ow:  | 20  | 1-2  | 50 |   |     |        |     | 1001 |   |          |     |     |     |     |     |     | _   | _   |     |    |    |   |
|----|------------------|---|---|---|--------|-----|---|-----|-----|----|----|---|---|---|-----|-----|-----|-----|-----|----|----|---|------|------|-----|------|----|---|-----|--------|-----|------|---|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|---|
|    |                  | _ |   | _ | -      | -   | + | +   | ł   | -  |    |   | _ | + | +   | +   | +   | t   |     | -  |    | _ | +    | +    | +   | ╞    | -  | _ | -   | +      | +   |      |   | +        | +   | +   | +   | +   | ł   | +   | ┝   | +   | +   | -  | -  | - |
| 1  | 1542916 Sepia la | G | A | t | A      | T i | A | G   | t   | T  | T  | t | С | С | a ( | c ( | G 1 | : A | 7   | λ  | A  | A | c.   | A 2  | 1 1 | A    | T  | λ | A   | 3 8    | 1   | 7    | Ŧ | 7        | G   | a 1 |     | T A | c   | 7   | à   | c   | С   | c  | С  | ¢ |
| 2  | 1626051 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     | Τ.  |     |     |     |    |    |   |
| ¢, | 1626052 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    |   |
|    | 1626057 Sepia la |   |   |   |        |     |   |     | a   |    |    |   |   |   |     |     |     | ۱.  |     |    |    | ÷ | t    |      |     |      |    |   |     | . t    |     |      |   |          |     |     |     |     | t   | : . |     |     |     | a  |    |   |
|    | 1733049 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    |   |
|    | 3369262 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    |   |
|    | 3369264 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    |   |
|    | AB192338.1 Sepia |   |   |   |        |     |   |     | a   |    |    | , |   |   |     |     |     |     |     |    | ,  |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     | t   |     |     |     |     | ,  |    | Ī |
|    | AB430406.1 Sepia |   |   |   |        |     |   |     | a   |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     | t   |     | Ι.  |     |     |    |    | T |
| 0  | AY185506.1 Sepia |   |   | c |        |     |   |     | c   |    |    | a |   |   | t   |     |     | ι.  |     |    |    |   | t    |      |     |      |    |   |     | . t    |     |      |   |          | . 1 | g . |     |     | t   |     |     |     |     | t  |    | Ī |
| 1  | KF009663.1 Sepia |   |   |   |        |     |   |     | a   |    |    |   | - |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     | t   |     |     |     |     |    |    | Ī |
|    |                  |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    | N | Vinc | low  | 25  | i1-3 | 00 |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    | Ī |
|    |                  |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     | T   | Ι   | Ι   | Τ   | Τ   |    | Γ  | Ī |
|    |                  |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    |   |
|    | 1542916 Sepia la | a | T | C | a      | T   | T | A a | a C | a  | с  | T | a | с | T   | t   | t   | 7 a | 1   | C  | с  | T | C    | a    | 7 ( | C t  | a  | t | T   | a      | t A | G    | A | A        | A   | G   | a   | G   | G t | t G | C C | 2 a | i G | G  | t  |   |
|    | 1626051 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    | _ |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     | Π.  |     | Π.  |     | Γ. |    | I |
|    | 1626052 Sepia la |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    | 1 |
|    | 1626057 Sepia la | t |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    | a  |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     |     | λ.  | Ţ   | . t |     | T. | Ϊ. | 1 |
|    | 1733049 Sepia la |   |   |   |        |     |   |     | 1.  | Ť. | Ϊ. |   |   |   |     |     |     |     |     | ١. |    | _ |      |      |     |      | 1. |   |     |        |     | Τ.   |   |          |     |     |     |     | T.  |     | t.  | T.  | Τ.  | t. | Γ. | Î |
|    | 3369262 Sepia la |   |   |   |        |     |   |     | T.  | t. |    |   |   | _ |     |     |     |     | 1   | Ϊ. |    | _ |      |      |     |      | T. |   |     |        | Ť   | 1.   |   |          |     |     |     |     | T.  |     | t   | T.  | t.  | t. | Τ. | Ì |
| Ī  | 3369264 Sepia la |   |   |   |        |     |   |     | t   | Ť. |    |   |   |   |     |     | Ť   |     |     | Ť. | 1  |   |      | Ť    |     |      | Ť. |   |     |        | T   | Ť.   |   |          |     | Ť   | Ť   | T   | t   | T   | t   | T   | Ť   | t  | t. | 1 |
|    | AB192338.1 Sepia | - | ÷ | - |        |     |   |     |     | Ť. | t  |   |   | t |     | a   | c   |     |     |    | a  |   |      |      |     |      | t  |   |     |        | T   | Ť.   |   |          |     |     | t   | T   | T   | T   | Ť   | T   | Ť   | t  | Ē  | 1 |
| -  | AB430406.1 Sepia | - | - | - |        |     | - |     |     | t  | t  |   |   | t | -   | a   | -   |     |     |    | a  | - | •    | -    |     |      | t  | - | -   | -      | +   | ÷    | ÷ | -        |     | -   | t   | -   | +   |     | -   | +   | -   | ÷  | ÷  | 1 |
| -  | AY185506.1 Sepia | * |   |   | ·<br>t | •   |   | • • |     | t  | -  | • | t | - | -   | a   | •   |     | r . |    | a  | - | -    | •    |     |      | g  | - | •   | -<br>a | -   | +    | • | -        | •   |     | ÷   |     |     |     |     |     | -   | ÷  | ÷  |   |
| -  | KF009663.1 Sepia | - |   | - | £      | •   |   | •   | -   | -  | t  |   | • | - | -   | -   | -   |     |     | ŀ  | a  | - | •    | ç    |     | . a  | 9  | ¢ | •   | 9      | +   | ·    |   | -        |     | •   | t   |     | -   |     | -   | - 5 | •   | ÷  | a  | 1 |
| 1  | Mr009665.1 Sepia | ¢ | • |   | •      | •   | • | • • | •   | •  | ¢  |   | • | ç | •   | a   | ¢   | . 3 |     |    | a  | - | •    | •    | • • | •    |    |   | •   | •      |     |      | • |          | •   | •   | ¢.  | •   |     |     | -   |     | •   |    | ÷  | ł |
|    |                  |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    | W | lind | low: | 30  | 1-3  | 50 |   |     |        |     |      |   |          |     |     |     |     |     |     |     |     |     |    |    |   |
|    |                  |   |   |   |        |     |   |     |     |    |    |   |   |   |     |     |     |     |     |    |    |   |      |      |     |      |    |   |     |        |     |      |   |          |     |     |     |     | T   |     |     | I   |     |    |    | 1 |
|    |                  | - |   |   |        |     | _ |     | _   | -  |    |   | _ |   | _   |     | _   | 2 0 |     | c  |    |   |      | _    |     | _    | -  | λ | c · | _      | -   | -    |   | _        | _   | _   | _   | _   |     | +   | +   | e c |     | -  | c  |   |
|    | 1542916 Sepia la | C | t | C | C      | x   | T | C 2 | / A | C  | λ  | C | 7 | t | 7.  | λ   | t ( | C ( |     | C  | C  | t | T    | 7 2  | 1 5 | r c  | a  | λ | C   | t 2    |     | T    |   | 7        | a   | 7 ( | 6 1 | a C | C 2 | ه ۱ | ) C | ; c | 1 1 | C  | C  | 1 |
|    | 1626051 Sepia la |   | • | • | •      | •   | • | • • |     | •  | •  | • | • | • | •   | •   | •   | +   | •   |    | •  | • | •    | •    | +   |      | ŀ  | • | ÷   |        |     | •    | • | •        | •   | •   | •   | •   |     |     | +   | -   | -   | ŀ  | ŀ  |   |
|    | 1626052 Sepia la |   |   | • | •      | •   | • |     |     | •  |    | - | • | • | -   | •   | •   | •   |     | •  | •  | - | •    | •    | • • | •    | •  |   | -   |        |     | •    | • | -        | •   |     | •   |     | •   |     | -   | -   | -   | ·  | ŀ  |   |
|    | 1626057 Sepia la |   | a | - | •      | •   |   | •   |     | +  | •  |   |   |   | -   | -   | +   | _   | a   | -  | •  |   | •    | +    | +   |      | •  | • | - 1 | R .    | ŀ   | •    | C |          | C   | •   | •   | • • | •   | t   | 1.  | ÷   | a   | •  | -  | 1 |
| -  | 1733049 Sepia la |   |   | • | •      |     | • | +   |     |    | •  |   |   |   | •   | •   | •   |     |     |    |    | • |      |      | ÷   |      | •  | • | •   | +      | +   |      | • | ·        |     |     |     |     | +   |     | +   | +   |     | +  | -  | 1 |
|    | 3369262 Sepia la |   | - | • | -      | •   | • |     | •   |    | •  | - | • |   | -   | •   | •   | ÷   | •   | -  | •• | - | •    |      | -   |      |    | • | •   | +      | +   | •    | • | -        | •   | •   | •   |     | +-  |     | +   | •   | -   | -  | -  | 1 |
|    | 3369264 Sepia la |   | - | • |        |     | + | • • | +   | +  | •  |   | - | • | -   | •   | -   | -   | •   | -  | •  | • |      | +    | •   |      | ÷  |   | +   | •      | +   | 1    | • | $\vdash$ | -   | +   | •   | +   | ÷   | -   | ÷   | ÷   | -   | ŀ  | •  | 1 |
| -  | AB192338.1 Sepia | · |   | • |        | •   | + | -   | -   | -  | -  |   |   |   | -   | -   | -   | _   | -   | -  |    |   | -    | -    | +   | -    | -  |   | •   | -      | -   | -    | · |          | -   | +   | +   | +   | +   |     | -   | •   | +   | -  |    | 1 |
| -  | AB430406.1 Sepia |   | - | • | •      |     | + | -   | •   | -  | •  |   | - |   | -   | -   | -   |     | a   |    | -  | a | _    | -    | •   |      | t  |   | -   |        | +   | -    |   |          | +   | +   | -   | -   | ÷   | -   | -   | •   | -   | +  | •  | t |
| -  | AY185506.1 Sepia |   | - | • |        |     | + |     | +   | -  | •  |   | - | - | -   | •   | -   |     | •   | -  | -  | с | -    | -    |     | -    | t  | - | •   |        | -   |      | - | -        | +   | •   | -   | t.  | -   | _   | _   |     | _   | -  |    | ÷ |
| 1  | KF009663.1 Sepia |   |   |   |        |     |   |     |     |    |    |   |   | c |     |     | c   |     | 9   |    |    | a |      |      |     |      | t  |   |     |        |     |      |   |          |     |     |     |     |     | 1   | 2   |     |     |    |    |   |

|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     | W   | ind  | ów: | 35   | 1-40 | 0  |   |    |     |     |     |    |   |   |          |     |     |      |     |     |     |          |    |   |    |
|----|--------------------------------------|---|----|---|---|---|--------|---|-----|-----|-----|-----|----|----|----|---|---|---|---|---|---|-----|-----|------|-----|------|------|----|---|----|-----|-----|-----|----|---|---|----------|-----|-----|------|-----|-----|-----|----------|----|---|----|
|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     | _   |      |     |     |     |          |    |   |    |
| 1  | 1210010 00010 10                     |   |    | _ | - |   |        | G | _   |     |     |     |    | _  |    |   | С |   |   |   | _ | T   | _   |      |     |      | -    | -  | t | _  |     |     |     |    |   | С | _        | _   |     | _    |     | +   | : 1 |          | c  | _ | -  |
| 2  | 1542916 Sepia la<br>1626051 Sepia la | L | 6  | a | Т | 6 | a<br>t | 6 | T   | C 1 |     | 1 1 | C  | T  | A  | 0 | 6 | a | • | T | 5 | T   | T   | G    | T L | 8    | C    | T  | 5 | 63 | -   | 1 1 | 1   | •  | U | 6 | <b>a</b> | 6   | 2 2 | 1 -6 | 1 1 | C   | T   | 6        | •  | T | -  |
| -  | 1626052 Sepia la                     |   | -  |   |   |   | t      | - |     |     |     |     |    |    | -  | - |   |   | - | • |   |     | -   | -    | 1   |      |      | İ  |   | -  | -   |     |     | -  | • |   |          | -   | +   | +    | -   | -   | ÷   | ÷        |    | - | Ì  |
| -  | 1626057 Sepia la                     |   |    | t |   |   |        |   |     | a   |     |     | t  |    | 1. |   |   | t |   |   | c |     |     | t    |     |      | Ť.   | t. | a |    |     |     |     | İ. |   |   | a        |     |     | t .  | 1   | t   | Ť   | İ.       |    | Ē | Ċ  |
| -  | 1733049 Sepia la                     |   |    |   |   |   |        |   |     |     | T.  |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      | Ϊ.   | Ī. |   |    |     |     |     |    |   |   |          |     |     | T    |     | T.  | T.  |          |    |   | Ì. |
| 6  | 3369262 Sepia la                     |   |    |   |   |   | t      |   |     |     |     |     | ١. |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     |     |      |     | T.  |     | 1.       |    |   |    |
| 7  | 3369264 Sepia la                     |   |    |   |   |   | t      |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     |     |      |     |     |     |          | t. |   |    |
| _  | AB192338.1 Sepia                     |   |    |   |   |   | t      |   |     |     |     |     | t  |    |    |   |   | t |   |   |   |     | . 1 | t    |     |      |      |    |   |    |     |     |     |    |   |   | с        |     | . t | t q  | , . | a   | ι.  |          | a  |   |    |
| 9  | AB430406.1 Sepia                     |   |    |   |   |   | t      | 1 |     |     |     |     | t  |    |    |   |   | t |   |   |   |     |     | t    |     |      |      |    |   |    | -   |     |     |    |   |   | c        |     | . t | t 9  | ,   | a   | ι.  |          | a  |   |    |
| 10 | AY185506.1 Sepia                     |   |    |   |   |   | t      |   |     | a   |     |     | t  |    |    |   |   | t |   |   |   |     | h   | t    |     | . 1  | t    |    | a |    | -   |     |     |    |   |   | t        |     | . 1 | t 9  |     | t   | : . |          |    |   |    |
| 11 | KF009663.1 Sepia                     |   |    |   |   |   | t      |   |     |     |     |     | t  |    |    |   |   | t | - |   |   |     | Ŀ   | ŧ.   |     |      |      |    |   |    |     |     |     |    |   |   | c        | -   | . t | t g  | . 1 | . a | 1 - |          | ð. |   |    |
|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     | W   | lind | ow. | 40   | 14   | 50 |   |    |     |     |     |    |   |   |          |     |     |      |     |     |     |          |    |   |    |
|    |                                      |   |    |   |   |   |        |   |     | _   |     |     | _  |    | _  |   |   | _ |   |   |   |     | _   |      | _   | _    |      | _  |   |    | _   | _   | +   |    |   |   | _        |     |     | _    | +   | _   | _   | _        | _  |   |    |
| 1  | 1542916 Sepia la                     | λ | λ  | T | t | T | Ţ      | a | G   | G   | t ( | . 0 |    | λ  | T  | t | λ | λ | Ŧ | T | T | t   | λ   | T    | 7   | A. 1 | C a  | λ  | С | a  | λ   | T   | r c | T  | a | λ | λ        | Ŧ   | λ,  | T ç  | 3 ( | C C | 3 t | T        | G  | λ | G  |
| 2  | 1626051 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   | 4   | _   |      |     |      |      |    |   |    |     | -   |     |    |   |   |          |     | -   | . 8  | a . |     |     |          |    |   |    |
| 3  | 1626052 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     |     |      | Ţ   | . , |     |          |    |   |    |
| 4  | 1626057 Sepia la                     |   |    |   |   |   |        | g |     |     |     |     | t  |    |    |   |   |   |   |   |   | с   | _   |      |     |      | . c  |    |   |    |     | -   | . t |    |   |   |          |     | -   | . 1  | a   |     | . a | Π.       |    |   |    |
| 5  | 1733049 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     |     |      |     |     |     |          | Γ. |   |    |
| 6  | 3369262 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     | _   |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     |     | . 2  | a   |     |     |          |    |   |    |
| 7  | 3369264 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    |   |   |          |     |     | . ?  | 8   |     |     |          | Γ. |   |    |
| 8  | AB192338.1 Sepia                     |   |    |   |   |   |        |   |     |     | c . |     |    |    |    | c |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     | -   |     |    | c |   |          |     |     | . 2  | a . |     |     |          |    |   |    |
| 9  | AB430406.1 Sepia                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    | c |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    | c |   |          |     |     |      | 8   |     |     |          |    |   |    |
| 10 | AY185506.1 Sepia                     |   |    |   | c |   |        |   |     |     |     |     | t  |    |    |   |   |   |   |   |   |     | _   |      |     |      |      |    |   | t  |     |     | . t |    |   |   |          |     |     |      | a . |     | . g | Π.       | Γ. |   |    |
| 11 | KF009663.1 Sepia                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     |     |      |     |      |      |    |   |    |     |     |     |    | c |   |          |     | T   |      | 8   |     |     |          | Γ. |   |    |
|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |   |   |     | W   | inde | w.  | 45   | 1-50 | 0  |   |    |     |     |     |    |   |   | Ī        |     |     |      | -   |     |     |          |    |   |    |
|    |                                      |   |    |   |   |   |        |   |     |     |     | [   |    |    |    |   |   |   |   |   |   |     | I   | I    | I   | T    |      |    |   |    |     | I   |     |    |   |   |          | I   | I   | T    | Γ   | Γ   | Γ   |          |    |   |    |
| 1  | 1542916 Sepia la                     | λ | a  | C | C | 7 | c      | 7 | λ   | c , | A g | λ   | 7  | λ  | C  | λ | λ | С | C | a | t | 7 . | a ( | C (  |     | : t  | 7    | λ  | Ŧ |    | 7 0 | 7   | t   | T  | C | λ | 7 0      | C 1 | t C | 1    | a   | 1   | 7   | t        | λ  | 7 | 7  |
| 2  | 1626051 Sepia la                     |   | Ī. |   |   |   | t      |   |     |     | T.  | T.  |    |    |    |   |   |   |   | a |   | Ţ   |     |      | Ť.  |      |      |    |   |    |     |     |     |    |   |   |          |     | T.  | T.   | g   | Γ.  | Γ.  |          |    |   |    |
| 3  | 1626052 Sepia la                     |   |    |   |   |   | Ĩ      | Ì | Ť   |     | t   | t.  | Ē  | Ť. | Ē  |   |   |   | Ĩ | a | Ì | Ť   |     |      | t   |      | ť.   |    |   |    |     | t   | ť.  |    | - | Ť | 1        | t   | Ť   | t    | Ť.  | t   | Ť.  | Í        | Ť, |   | Ĩ  |
| 4  | 1626057 Sepia la                     | - | a  |   | - |   | t      |   | Ť   |     |     | t   |    |    |    |   | * |   | - | a | - | Ť   |     | Ť    | ť   | t    |      |    |   | Í  |     | t   | f   |    |   | Ť | 1        | t   | ť   | ť    | ,   |     | Ť   | c        | -  |   |    |
| 5  |                                      | * | -  |   | - | - | -      |   | +   |     | +   | ÷   | *  | ŕ  | ŕ  |   | - | * | - | - | - | +   | 1   |      | ť   |      | ť    |    |   | -  |     | +   |     |    |   | • |          |     |     | ť    | -   | +-  | +-  | -        | -  |   | *  |
|    | 1733049 Sepia la                     | _ | _  |   | _ |   |        |   |     |     | _   |     | -  |    | _  | - |   |   | _ |   |   | _   | _   | _    | _   | _    | -    | -  |   | _  |     | _   | -   |    |   | _ | _        | _   | _   |      | _   | -   | -   |          | _  |   | _  |
|    | 3369262 Sepia la                     |   |    |   |   |   |        |   | - 1 | _   | -   |     | -  |    |    |   |   |   |   |   |   |     | -   |      | Т   | 1    | 1    |    |   |    | 1   | -   | T   |    |   |   |          |     |     |      |     |     |     |          |    |   |    |
|    | 3369264 Sepia la                     |   |    |   |   | - |        | - | -   |     |     | Т   | -  | 1  | •  |   |   |   |   |   |   | •   | T   | -    | +   | -    | +    |    |   | •  | +   | +   | •   |    |   |   | •        | +   |     | •    | T   |     |     |          | •  |   |    |
| -  | AB192338.1 Sepia                     |   |    |   |   |   |        |   | _   | _   | -   |     | -  | •  | t  |   |   |   |   | a | - | •   | +   | +    | +   | +    | -    | -  |   | +  | +   | •   | ۴   |    | * | - | +        | +   | • • | +    | +   |     |     |          | •  |   |    |
| -  | AB430406.1 Sepia                     | - | a  |   | - | - | _      |   | •   | •   | . a | •   |    | •  | •  |   | • | - |   | a | - | •   | +   | +    | . 3 | L C  |      | •  | + | •  |     | •   | 9   |    |   | • | •        | +   |     | •    |     |     |     |          | •  |   |    |
| -  | AY185506.1 Sepia                     | - |    |   | - | - | t      | - | 4   | -   | . a | +-  |    | •  | •  |   |   |   |   | a | - | •   | +   | +    | -   |      |      |    |   |    |     |     |     | •  |   |   | •        | +   | с.  | ÷    |     | ŀ   |     | $\vdash$ |    |   |    |
| 11 | KT009663.1 Sepia                     |   | a  |   |   |   | t      |   |     |     | . a |     |    |    |    |   |   |   |   | a |   |     |     |      |     | c    |      |    |   |    |     |     | 9   |    |   |   |          |     |     |      |     |     |     |          |    |   |    |

|   |  |   |   |     |     |   |                                      |             |     |       |             |      |   |     |                     |       |     |   |          | 1      | Vind             | W.        | 551-6                            | 00             |          |   |                                       |                                       |   |   |   |                   |       |                                       |   |             |    |     |     |   |   |
|---|--|---|---|-----|-----|---|--------------------------------------|-------------|-----|-------|-------------|------|---|-----|---------------------|-------|-----|---|----------|--------|------------------|-----------|----------------------------------|----------------|----------|---|---------------------------------------|---------------------------------------|---|---|---|-------------------|-------|---------------------------------------|---|-------------|----|-----|-----|---|---|
|   |  |   |   |     |     |   |                                      |             |     | _     |             |      |   |     |                     |       |     |   |          |        |                  |           |                                  |                |          |   |                                       |                                       |   |   |   | _                 |       |                                       |   |             |    |     |     |   | I |
|   | 1542916 Sepia la   | a   | 1 | 7.3 |     |   |                                      | 7           | 7.3 |       | A (         | : e  | G | 2   | t C                 | : G   | a   |   | A t      |        | Ŧ. 1             |           | A                                |                | e 1      |   |                                       |                                       |   |   | 7 |                   | r G   |                                       |   | с           | с  | a 2 |     | 1 |   |
| _ | 1626051 Sepia la   | •   | n |     |     |   | ~                                    | -           |     |       | n .         |      | 4 | ~   |                     |       | •   | - | <b>^</b> |        |                  | -         | -                                | -              | -        | - | -                                     |                                       |   | F | - | +                 | +     |                                       | - | -           |    | a / |     | - | f |
| _ | 1626052 Sepia la   | •   | • |     | ÷   | - | •                                    |             |     | -     | +           | ÷    | ÷ |     |                     | -     |     | - | •        | +      | •                | •         |                                  | •              | •        | + | •                                     | -                                     |   | ŀ | • | •                 | • •   |                                       | • | •           | •  |     |     | • | - |
| - | 1626057 Sepia la   | ·<br>t  | • |     | +   | - | •                                    | ŀ           |     | -     |             | a    | ÷ |     | <br>c .             |       | t.  | - | • •      |        |                  |           |                                  |                | •        | + |                                       | a                                     |   | ÷ | ÷ | +                 | +     | •                                     | - | •           | +  |     |     | • | ł |
| - | 1733049 Sepia la   | £.  | • | • • | -   |   | •                                    | ŀ           | •   |       | -           | a    | - | - 1 | с.                  |       | 5   | • | • •      | •      | . 1              | :         | . (                              |                | •        |   |                                       | a                                     |   | ŀ | ŀ | •                 | +     |                                       | - | •           | •  | • • |     | • | ÷ |
| - |  |   | • |     | -   |   | •                                    | -           |     | •     | -           |      |   | -   |                     | -     | -   | - | • •      | -      | •                |           |                                  | -              | •        | + | •                                     |                                       |   | - | • | •                 | •     | •                                     | - | •           | •  |     | -   | • | ł |
| - | 3369262 Sepia la   |   | • | • • |     |   | •                                    | ·           | •   | •     | • •         | •    |   |     | • •                 |       | •   | - | • •      | •      | •                |           |                                  | •              | •        | - | -                                     | •                                     | • •   |   | • | •                 | +     | •                                     | - | •           | •  | • • | •   | • | ł |
| - | 3369264 Sepia la   |   | • | •   |     |   | •                                    | •           | -   | •     | -           |      | - |     | • •                 | -     | •   | - | •        | -      | •                | -         | •                                | -              | •        | - | •                                     |                                       |   | • | • | •                 | •     |                                       | - | •           | •  |     |     | • | ł |
| - | AB192338.1 Sepia   |   | • | •   |     | • | •                                    | •           |     | •     | •           |      |   |     | • •                 | -     | -   | • | . (      |        | . 1              | 1         | . (                              | :              | •        |   | -                                     | а                                     |   | ŀ | • | •                 | • •   | •                                     | - | •           | -  | t.  |     |   | ļ |
|   | AB430406.1 Sepia   |   | • | •   |     |   | •                                    | •           | •   |       | • •         | •    | · | - 1 | с.                  |       | •   | • | . (      | :      | . 1              |           | . (                              | :              |          |   |                                       | a                                     | • •   |   | • | •                 | •     |                                       | - |             | -  | t.  |     |   | ļ |
|   | AY185506.1 Sepia   | t   |   |     |     |   |                                      |             |     |       |             | C    |   |     |                     |       |     | - |          | -      | •                |           |                                  |                | •        |   | -                                     | a                                     |   | t | • | •                 | • •   |                                       | t |             | •  | t.  |     |   | l |
| - | -  | 4   |   |     | -   | - | -                                    | _           |     |       |             |      |   |     |                     |       |     |   |          |        |                  |           |                                  |                |          |   |                                       |                                       |   |   |   |                   |       |                                       |   |             |    |     |     |   |   |
| - | KT009663.1 Sepia   |   |   |     |     |   |                                      |             |     |       |             |      |   |     | c .                 |       |     |   | . (      | -      | -                | a .<br>WC | 501-5                            | -              |          |   | •                                     | a                                     |   |   |   |                   |       |                                       |   |             |    | t.  |     |   |   |
| - | -  |   |   |     |     |   |                                      |             |     |       |             |      |   |     | c .                 |       |     |   |          |        | -                |           |                                  | -              |          |   |                                       |                                       |   |   |   |                   |       |                                       |   |             |    |     |     |   |   |
| 1 | KF009663.1 Sepia   |   |   | t ( |     |   | А                                    | T           | c 1 |       |             |      |   | 9   |                     |       | t   | T |          | 1      | vind             |           | 501-5                            | 50             | с (      |   | l a                                   |                                       | <br>т а   | C | c | t 1               | <br>  |                                       | G | c           |    |     |     |   |   |
| 1 | KF009663.1 Sepia   |   |   | t ( | ; ( |   | λ                                    |             |     |       | <br>T 2     |      | T |     |                     | · .   |     |   |          | 1      | vind             | w. !      | 501-5                            | 50             | c (      |   | · .                                   |                                       | <br>T A   | G | C | t (               | <br>  | А                                     | G | C           |    |     |     |   |   |
| 1 | KF009663.1 Sepia   |   | с | t ( | ; ( |   | А                                    | T           | c 1 | <br>  | <br>T 2     |      | T |     |                     | · .   |     |   |          | 1      | vind             | w. !      | 501-5                            | 50             |          |   | · · ·                                 |                                       | T A   | G | C |                   | <br>  |                                       | G | c           |    |     |     | A |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la   |   |   | t ( | ; ( |   | λ                                    | •           | c : |       | <br>T 2<br> | <br> |   |     |                     | · .   | . t |   |          | 1      | vind             | w. !      | 501-5                            | 50<br>50<br>50 | c (<br>a |   | · · ·                                 |                                       | T A   | G | C | <br>              |       | · · · · · · · · · · · · · · · · · · · | C | c           |    |     |     | А |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la   |   |   |     |     |   | λ                                    | •           |     |       | · · ·       |      |   | g   |                     | · · · |     |   |          | 1      | t o              | w. !      | c (                              | 50<br>50<br>50 |          |   | · · · · · · · · · · · · · · · · · · · |                                       | τ λ<br><br>   | G |   | t 1               |       |                                       | G | c           |    |     | t . | А |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la   |   |   |     |     |   | ·<br>·<br>·<br>·                     | •           |     | · · · | · · ·       |      |   | g   |                     | · · · |     |   |          | 1      | t o              | w. !      | c (                              | 50<br>50<br>50 |          |   | · · · · · · · · · · · · · · · · · · · |                                       | τ λ<br><br>   | G |   |                   | G G   | · · · · · · · · · · · · · · · · · · · | G |             |    |     | t . | А |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la   |   |   |     |     |   | ·<br>·<br>·<br>·                     | •           |     |       |             |      |   | g   |                     | · · · | c   |   |          | 1      | vind<br>t (<br>a | w. !      | 501-5<br>c (<br><br>             | 50<br>50<br>50 | a        |   |                                       |                                       | τ λ<br><br><br><br>   | G | C |                   | C C C | · · · · · · · · · · · · · · · · · · · | G |             |    |     | t . | А |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la   | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·   | - |     |     |   | ·<br>·<br>·<br>·<br>·                | •           |     |       |             |      | - | g   |                     |       | c   |   |          | \<br>C | vind<br>t (<br>a |           | 501-5<br>c (<br><br>             | 50<br>c        | a        |   | · • •                                 | T                                     | T A<br><br><br><br>   | G | - | • 1<br>• 1<br>• 1 |       | · · · · · · · · · · · · · · · · · · · | G |             |    |     | t . | A |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369264 Sepia la                     | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>· | - | a . |     |   | ·<br>·<br>·<br>·<br>·                | -           | t . |       |             |      |   | g   | t 1<br><br>         |       | c   |   |          | \<br>C | t (              |           | 501-5<br>c (<br><br><br><br><br> | 50<br>c        | a        |   |                                       | · · · · · · · · · · · · · · · · · · · | <ul> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li> <li>.</li></ul> | G |   |                   |       |                                       | - | •           | a. |     | t t | A |   |
|   | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369264 Sepia la<br>AB192338.1 Sepia | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>· | - | a . |     |   | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>· | ·<br>·<br>· |     |       |             | <br> | - | g   | t 1<br><br><br><br> |       |     |   |          | \<br>C | tind<br>t (      |           | 501-5<br>c (<br><br><br><br><br> | 50<br>C        |          |   |                                       | · · · · · · · · · · · · · · · · · · · | · · · · · · · · · · · · · · · · · · ·   |   |   |                   | · · · |                                       | - | ·<br>·<br>· | a. | A 1 | t t | A |   |

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.

**Acknowledgements**. We would like to appreciate the Ministry of Research and Technology, Directorate General of Higher Education for financially support of this study through University Superior Basic Research Grant 2018. Great gratitude is also given to Mr. Andre Kambey and Ms. Inggrid Akerina for field sampling activities.

#### References

- Alberto F., Raimondi P. T., Reed D. C., Coelho N. C., Leblois R., Whitmer A., Serräo E., 2010 Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. Ecology 91(1):49-56.
- Allen J. J., Mäthger L. M., Barbosa A., Hanlon R. T., 2009 Cuttlefish use visual cues to control three-dimensional skin papillae for camouflage. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioral Physiology 195:547-555.
- Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W., Lipman D. J., 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389-3402.

- Arkley K., Jacklin M. S., Boulter M., Tower J., 1996 The cuttlefish (*Sepia officinalis*): a guide to its exploitation in UK waters. The sea fish Industry Authority, Seafish Report no. SR467, 81 pp.
- Barbosa A., Mäthger L. M., Buresch K. C., Kelly J., Chubb C., Chiao C. C., Hanlon R. T., 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive patterns. Vision Research 48:1242-1253.
- Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2012 Cuttlefish use visual cues to determine arm postures for camouflage. Philosophical Transactions of the Royal Society of London B: Biological Sciences 279:84-90.
- Chiao C. C., Chubb C., Buresch K. C., Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2010 Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. Journal of Experimental Biology 213:187-199.
- Drummond A. J., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowitz S., Moir M., Stones-Havas S., Sturrock S., Thierer T., Wilson A., 2012 Geneious v5.5.6. Available at: http://www.geneious.com.
- Fernandez L. D., Hernandez C. E., Schiaffino R. M., Izaguirre I., Lara E., 2017 Geographical distance and local environmental conditions drive the genetic population structure of a freshwater microalga (Bathycoccaceae; Chlorophyta) in Patagonian lakes. FEMS Microbiology Ecology 93(10):10 pp.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.
- Hanlon R. T., Chiao C. C., Mäthger L. M., Barbosa A., Buresch K. C., Chubb C., 2009 Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Philosophical Transactions of the Royal Society of London B: Biological Sciences 364:429-437.
- Hays G. C., 2017 Ocean currents and marine life. Current Biology 27(11): 470-473.
- IUCN, 2014 The IUCN Red List of Threatened Species. Version 2014.2. Available at: www.iucnredlist.org. Accessed: September, 2014.
- Kelman E. J., Osorio D., Baddeley R. J., 2008 A review of cuttlefish camouflage and object recognition and evidence for depth perception. Journal of Experimental Biology 211:1757-1763.
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K., 2018 MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
- Leopold A., 1933 Game management. Charles Scribner's Sons, New York, 481 pp.
- McManus M. A., Woodson C. B., 2012 Plankton distribution and ocean dispersal. Journal of Experimental Biology 215: 1008-1016.
- Murphy J. M., Balguerias E., Key L. N., Boyle P. R., 2002 Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the Northwest African Coast. Bulletin of Marine Science 71:545-553.
- Neves A., Cabral H., Sequeira V., Figueiredo I., Moura T., Gordo L. S., 2009 Distribution patterns and reproduction of the cuttlefish, *Sepia officinalis* in the Sado estuary (Portugal). Journal of the Marine Biological Association of the United Kingdom 89(3):579-584.
- Pascual M., Palero F., García-Merchán V. H., Macpherson E., Robainas-Barcia A., Mestres F., Roda T., Abelló P., 2016 Temporal and spatial genetic differentiation in the crab *Liocarcinus depurator* across the Atlantic-Mediterranean transition. Scientific Reports 6:29892.
- Pawson M. G., 1995 Bio-geographical identification of English Channel fish and shellfish stocks. CEFAS Fisheries Research Technical Report No 99, 72 pp.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2015 Size at first maturity of cuttlefish, *Sepia latimanus*, from North Sulawesi waters, Indonesia. Marine Science 5(1):6-10.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2016 Mitochondrial CO1 genetic marker-based species diversity of cuttlefish (Cephalopod; Mollusk) in Manado Bay and Lembeh Strait, North Sulawesi, Indonesia. AACL Bioflux 9(6):1345-1354.

- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2017 Egg placement habitat selection of cuttlefish, *Sepia latimanus* (Sepiidae, Cephalopoda, Mollusca) in North Sulawesi waters, Indonesia. AACL Bioflux 10(6):1514-1523.
- Ramachandran S., Deshpande O., Roseman C. C., Rosenberg N. A., Feldman M. W., Cavalli-Sforza L. L., 2005 Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proceedings of the National Academy of Sciences of the USA 102(44):15942-15947.
- Ratnasingham S., Hebert P. D. N., 2007 BOLD: the barcode of life data system. Molecular Ecology Notes 7:355-364.
- Reid A., Jerep P., Roper C. F. E., 2005 Family Sepiidae. In: Cephalopods of the world. An annotated and illustrated catalogue of Cephalopod species known to date. Volume 1. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). Jerep P., Roper C. F. E. (eds), FAO, Rome, pp. 54-152.
- Rozas J., Ferrer-Matta A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., Sánchez-Gracia A., 2017 DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12):3299-3302.
- Shohet A. J., Baddeley R. J., Anderson J. C., Kelman E. J., Osorio D., 2006 Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage. Journal of Experimental Biology 209:4717-4723.
- Tricarico E., Amodio P., Ponte G., Fiorito G., 2014 Cognition and recognition in the cephalopod mollusc *Octopus vulgaris*: coordinating interaction with environment and conspecifics. In: Biocommunication of animals. Witzany G. (ed), Springer, pp. 337-349.

Received: 25 April 2019. Accepted: 31 May 2019. Published online: 22 June 2019. Authors:

Silvester B. Pratasik, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, JI. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: spjong07@yahoo.com

Harjany Sambali, Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, JI. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: hariyanisambali@unsrat.ac.id Lefrand Manoppo, Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, JI. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: lefrandmanoppo@yahoo.com

Frans F. Tilaar, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: fftilaar@unsrat.co.id

Meiske S. Salaki, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: mssalaki@unsrat.co.id

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

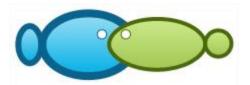
How to cite this article:

Pratasik S. B., Sambali H., Manoppo L., Tilaar F. F., Salaki M. S., 2019 Genetic variations of cuttlefish *Sepia latimanus* (Cephalopoda, Sepiidae) in North Sulawesi waters, Indonesia. AACL Bioflux 12(3):792-803.

### iyahoo 🗙 🕂

| 8  | FINANCE   | SPORTS  | ENTERTAINMENT  | LIFE  | SHOPPING   | YAHOO PLUS  | MORE   |                               |
|--|---|---|--|---|--|---|--|-------------------------------|
| d m  | nessages, doo   | cuments, pho  | tos or people  |   | ~  | Q   |  |                               |
| Bac  | ck 🔦 🔦  | ÷   | Archive  | A Move  | 🖬 Delete   | 😵 Spam \cdots   |  | • •                           |
|  |   |   | 1 1  |   |  | r   |  |                               |
| B  |   | t <b>asik</b> <spjong0<br>aie ionel claudi</spjong0<br>   | )7@yahoo.com><br>u   |   |  | <b>₽</b> % ₩  | /ed, Jul 3, 2019 at                                  | 7:51 AM 🔺                     |
| Reg<br>and<br>199<br>dem   | SHOULD be (<br>7) and BOLDS<br>nonstrates 7 sp  | I form of my an<br>omitted; 2) p.79<br>systems (Ratna<br>becimens; 5) p.  | ticle "Genetic variations<br>94 - For species confirm<br>singham & Hebert 2007<br>801 - It could result fro<br>aad it. | ation, BLAST<br>) were utilized   | (Basic Local A<br>d; 3) have beco  | lignment Search To<br>me SHOULD be ha   | ols) method (Alt<br>s become; 4) Ta                  | schul et al<br>ble 1          |
| Reg<br>and<br>199<br>dem<br>here<br>Sind                                       | parding the fina<br>SHOULD be (<br>7) and BOLDS<br>nonstrates 7 sp  | I form of my ar<br>omitted; 2) p.79<br>systems (Ratna<br>pecimens; 5) p.<br>approval to uplo  | 94 - For species confirm<br>singham & Hebert 2007<br>801 - It could result fro   | ation, BLAST<br>) were utilized   | (Basic Local A<br>d; 3) have beco  | lignment Search To<br>me SHOULD be ha   | ols) method (Alt<br>s become; 4) Ta                  | schul et al<br>ble 1          |
| Reg<br>and<br>199<br>dem<br>here<br>Sinc<br>Silv                               | garding the fina<br>(SHOULD be of<br>(7) and BOLDS<br>nonstrates 7 sp<br>ewith give my a<br>cerely Yours,<br>ester B. Pratas  | I form of my ar<br>omitted; 2) p.79<br>systems (Ratna<br>pecimens; 5) p.<br>approval to uplo<br>sik.  | 94 - For species confirm<br>singham & Hebert 2007<br>801 - It could result fro   | ation, BLAST<br>) were utilized<br>m <b>that</b> this in                          | F (Basic Local A<br>d; 3) have beco<br>dividual belong   | lignment Search To<br>me SHOULD be ha<br>s to species comple  | ols) method (Alt:<br>s become; 4) Ta<br>x Thank you, | schul et al<br>ble 1          |
| Reg<br>and<br>199<br>dem<br>here<br>Sino<br>Silv                               | garding the fina<br>(SHOULD be of<br>(7) and BOLDS<br>nonstrates 7 sp<br>ewith give my a<br>cerely Yours,<br>ester B. Pratas  | I form of my an<br>omitted; 2) p.79<br>systems (Ratna<br>pecimens; 5) p.<br>approval to uplo<br>sik.<br>y 2, 2019 02:54   | 94 - For species confirm<br>singham & Hebert 2007<br>801 - It could result fro<br>bad it.                              | ation, BLAST<br>) were utilized<br>m <b>that</b> this in                          | F (Basic Local A<br>d; 3) have beco<br>dividual belong   | lignment Search To<br>me SHOULD be ha<br>s to species comple  | ols) method (Alt:<br>s become; 4) Ta<br>x Thank you, | schul et al<br>ble 1          |
| Reg<br>and<br>199<br>dem<br>here<br>Sino<br>Silv                               | parding the fina<br>(SHOULD be of<br>(7) and BOLDS<br>nonstrates 7 sp<br>ewith give my a<br>cerely Yours,<br>ester B. Pratas<br>n Tuesday, July<br>ear Professo                                 | I form of my an<br>omitted; 2) p.79<br>systems (Ratna<br>pecimens; 5) p.<br>approval to uplo<br>sik.<br>y 2, 2019 02:54<br>or Pratasik,   | 94 - For species confirm<br>singham & Hebert 2007<br>801 - It could result fro<br>bad it.                              | ation, BLAST<br>) were utilized<br>m <b>that</b> this in                          | F (Basic Local A<br>d; 3) have beco<br>dividual belong<br><ionelclaudiu@< td=""><td>lignment Search To<br/>me SHOULD be ha<br/>s to species comple<br/>yahoo.com&gt; wrote:</td><td>ols) method (Alt:<br/>s become; 4) Ta<br/>x Thank you,</td><td>schul et al<br/>ble 1<br/>and I</td></ionelclaudiu@<> | lignment Search To<br>me SHOULD be ha<br>s to species comple<br>yahoo.com> wrote:                       | ols) method (Alt:<br>s become; 4) Ta<br>x Thank you, | schul et al<br>ble 1<br>and I |
| Reg<br>and<br>199<br>dem<br>here<br>Sinc<br>Silv<br>Or<br>Di<br>Th<br>Ki<br>or | parding the fina<br>(SHOULD be of<br>(7) and BOLDS<br>nonstrates 7 sp<br>ewith give my a<br>cerely Yours,<br>ester B. Pratas<br>n Tuesday, July<br>ear Professo<br>he final form<br>indly check | I form of my ar<br>omitted; 2) p.79<br>systems (Ratna<br>becimens; 5) p.<br>approval to uplo<br>sik.<br>y 2, 2019 02:54<br>or Pratasik,<br>n of your pap<br>it carefully al<br>www.bioflux. | 94 - For species confirm<br>singham & Hebert 2007<br>801 - It could result fro<br>bad it.<br>::10 PM +08, gavriloaie   | ation, BLAST<br>) were utilized<br>in that this in<br>ionel claudiu<br>nade sever | F (Basic Local A<br>d; 3) have beco<br>dividual belong<br><ionelclaudiu@<br>ral correction<br/>ree with the p</ionelclaudiu@<br>   | lignment Search To<br>me SHOULD be ha<br>s to species comple<br>yahoo.com> wrote:<br>ns within the refe | ols) method (Alt:<br>s become; 4) Ta<br>x Thank you, | schul et al<br>ble 1<br>and I |

|                            | FINANCE  | ŝ  | SPORTS   | ENTERT   | AINMENT                   | LIFE                             | SHOPPING     | YAHOO I    | PLUS     | MORE          |         |   |   |
|----------------------------|--|--|--|--|---------------------------|----------------------------------|--------------|------------|----------|---------------|---------|---|---|
| id mes                     | sages, (   | docui  | nents, pho   | itos or peo  | ple                       |                                  | ~            | Q          |          |               |         |   |   |
| Back                       | • •  | *  | •  | j  | M Archive                 | Move                             | 💼 Delete     | 😵 Spam     |          |               |         | Ŧ | × |
| <mark>the</mark> p         | baper v  | with (   | cuttle fish  | has beer   | n publi <mark>sh</mark> e | ed                               |              |            |          | Yaho          | o/Inbox |   |   |
|                            | Profes   | sor F  | itasik<br>Iratasik,                                      |  | stions and                | than Lunk                        | adad the pr  | anor on th | o iouros | l'a wahaita   |         |   |   |
| I have<br>http://<br>We al | Profes<br>e opera<br>/www.b<br>II thank<br>best re | sor F<br>ated t<br><u>pioflu</u><br>c you<br>gard: | ratasik,<br>he necess<br><u>c.com.ro/h</u><br>for publis | sary corrections of the sary correction of th | me-12-3-2                 | l then I uplo<br>2019/.          | paded the pa | aper on th | e journa | al's website: |         |   |   |
| I have<br>http://<br>We al | Profes<br>e opera<br>/www.b<br>Il thank            | sor F<br>ated t<br><u>pioflu</u><br>c you<br>gard: | ratasik,<br>he necess<br><u>c.com.ro/h</u><br>for publis | ome/volui  | me-12-3-2                 | l then I uplo<br>1 <u>019/</u> . | baded the pa | aper on th | e journa | al's website: |         |   |   |



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

<sup>1</sup>Silvester B. Pratasik, <sup>2</sup>Hariyani Sambali, <sup>3</sup>Lefrand. Manoppo, <sup>1</sup>Frans F. Tilaar, <sup>1</sup>Meiske S. Salaki

<sup>1</sup> Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>2</sup> Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia; <sup>3</sup> Study Corresponding author: S. B. Pratasik, spjong07@yahoo.com

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, 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).

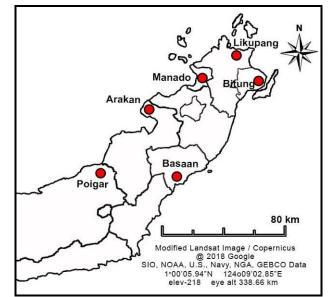


Figure 1. Map of North Sulawesi with sampling points (marked red).

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).

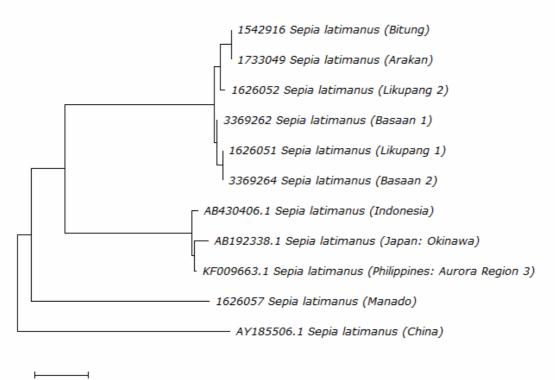
**Results and Discussion**. During the study, 7 individuals of *S. latimanus* were collected, 3 individuals represented population of southern North Sulawesi and 4 did the northern population of North Sulawesi. SCUBA survey (8 dives) encountered only one small-sized cuttlefish (*Sepia* sp.), but not 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 of Basaan waters. On the other hand, both *S. latimanus* samples from Bisaan waters are genetically different as happening for both samples from Likupang waters (Figure 2).

Table 1

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

Genetic distance of cuttlefish Sepia latimanus in North Sulawesi waters



0.010

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.

| _    |  |   |         |                |                        |          | _                                    | _   | _                                     | _                        | _   |                       | _ | _                |  | _                 | _                                     | _  | _ | W          | indo     | w.   | 1-5 | 0   |     | _   | _   | _   | _   | _   | _ | _                               | _  | _   | _      | _       | _     | _   | _   | _  | _  | _  | _   | _   |
|------|--|---|---------|----------------|------------------------|----------|--------------------------------------|---|---------------------------------------|--------------------------|-----|-----------------------|---|------------------|--|-------------------|---------------------------------------|----|---|------------|----------|--|-----|-----|-----|-----|-----|-----|-----|-----|---|---------------------------------|----|-----|--------|---------|-------|---|-----|----|----|----|---|-----|
| _    |  |   |         | 4              | 1                      | -        | -                                    |   |                                       |                          | +   | 1                     | L |                  |  | +                 | 1                                     |    |   | 4          | +        | 1  | Ļ   |     | 4   | 4   | ļ   | +   | Ļ   | ļ   | Ļ |                                 | Ļ  |     |        | 1       | L     | ļ   | Ļ   |    | Ļ  |    |   |     |
| _    |  | _ |         |                |                        |          | +                                    |   |                                       |                          | +   | -                     | - |                  | +  | +                 | -                                     | _  |   | +          | +        |  | -   |     | _   | +   | +   | +   | +   |     | - | -                               | -  |     |        | 4       |       |   |     |    | -  | -  |   |     |
|      | 1542916 Sepia la   | 7 | t       | t C            | a                      | T C      | C a                                  | G   | C                                     | T                        | 1   | · λ                   | T | T                | 9  | G                 | 9                                     | λ  | С | <b>a</b> ' | T C      | À  | t   | T   | λ   | λ ( | 5 1 | 1   | 1   | 2   | A | 1                               | t  | λ   | 1      | t       | C     | G   | t   | λ  | G  | t  | G   | λ   |
|      | 1626051 Sepia la   | * |         |                |                        | • •      |                                      |   | •                                     |                          | •   |                       |   | •                |  |                   | •                                     |    | - | •          |          |  |     |     | •   |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    | a, |   |     |
| -    | 1626052 Sepia la   |   | •       |                |                        | • •      |                                      |   | •                                     | -                        |     |                       |   | -                | •  | •                 | •                                     |    | - | •          | • •      |  |     | •   | •   |     |     |     | •   |     |   |                                 |    |     |        |         |       |   |     |    | •  | -  |   |     |
| 1    | 1626057 Sepia la   |   | a       |                | g                      |          |                                      |   |                                       |                          | •   |                       |   | •                | a  |                   | t                                     |    | - |            |          |  | c   |     | •   |     | . 4 | 1   | : . |     |   |                                 | ,  |     |        | c       |       |   | a   |    |    |    |   |     |
| 5    | 1733049 Sepia la   |   |         |                |                        |          |                                      |   |                                       | -                        |     |                       |   | •                |  |                   | •                                     |    |   |            |          |  |     |     | •   |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    |    |   |     |
| 8    | 3369262 Sepia la   |   |         | • •            |                        |          |                                      |   |                                       | -                        |     |                       |   |                  |  | •                 |                                       |    | - | •          | • •      |  |     | -   | •   |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    |    |   |     |
|      | 3369264 Sepia la   |   |         |                |                        |          |                                      |   |                                       |                          |     |                       |   | •                | •  |                   |                                       |    | - | •          |          |  |     |     | •   |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    |    |   |     |
|      | AB192338.1 Sepia   |   |         |                | g                      |          | . 9                                  |   |                                       |                          |     |                       |   |                  | a  |                   | a                                     |    | - |            |          |  | c   |     |     |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    | a  |   |     |
|      | AB430406.1 Sepia   |   |         |                |                        |          | . 9                                  |   |                                       |                          |     |                       |   |                  | a  |                   | a                                     |    |   |            |          |  | c   |     |     |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    | a  |   |     |
| 10   | AY185506.1 Sepia   |   |         |                |                        |          |                                      |   |                                       |                          |     |                       |   |                  | a  |                   | t                                     |    |   | t          |          |  |     |     |     |     |     |     |     |     |   |                                 | a  |     |        |         |       |   | a   |    |    | a  |   |     |
| 11   | KF009663.1 Sepia   |   |         |                | g                      |          | . 9                                  |   |                                       |                          |     |                       |   |                  | a  |                   | a                                     |    | - |            |          |  | c   |     |     |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    | a  |   |     |
|      |  |   |         |                |                        |          |                                      |   |                                       |                          |     |                       |   |                  |  |                   |                                       |    |   | W          | inde     | NC.  | 51- | 100 |     | _   |     |     |     | _   |   |                                 |    |     |        |         |       |   | _   | _  | _  |    |   |     |
|      |  |   |         |                |                        |          |                                      |   |                                       |                          |     |                       |   |                  |  |                   |                                       |    |   |            |          |  |     |     |     |     |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    |    |   |     |
|      |  |   |         | -              |                        | _        | -                                    |   |                                       |                          |     | 1                     |   |                  |  |                   |                                       |    |   |            |          |  |     |     |     | 4   | 1   |     |     | Ļ   |   | Ļ                               |    |     |        |         |       |   |     |    | Ļ  |    |   |     |
| -    | 1542916 Sepia la   |   | T       | g C            | G                      | t A      | A A                                  | λ   | С                                     | C                        | c C | G                     | T | λ                | C  | a t               | T                                     | a  | t | Ŧ.         | λ )      | A  | t   | G   | A   | t   | 2 2 | 1 0 | C   | 2   | Å | t                               | 1  | λ   | Ŧ      | λ       | t     | A   | λ   | T  | G  | Ŧ  | ¢   | a   |
| _    | 1626051 Sepia la   |   | -       |                | -                      | +-       |                                      | -   |                                       |                          |     |                       |   |                  |  | -                 |                                       |    |   | •          |          |  |     |     | -   |     | +   | -   |     | +   |   |                                 |    |     |        | •       | •     |   |     |    |    |    | •   |     |
|      | 1626052 Sepia la   |   | -       | • •            | $\left  \cdot \right $ |          | +                                    |   |                                       |                          |     | •                     | • |                  |  | •                 | -                                     | •  |   | •          | •        |  |     |     | •   | •   | +   |     |     | +   | - | •                               | ÷  | •   | •      | •       | •     | •   | •   |    | •  |    | t   |     |
|      | 1626057 Sepia la   | · | . 4     | a.             | •                      | • •      | •                                    | -   |                                       | - 4                      | Α.  | ŀ                     | • | •                | •  |                   |                                       | t  | C | •          | •        |  | c   | •   | +   | c   | +   |     |     |     |   | c                               |    | •   | •      | •       | •     | •   | •   |    | •  |    | t   |     |
|      | 1733049 Sepia la   | · | -       | ÷              | +                      | +-       | ÷                                    | -   |                                       | +                        | •   | •                     | • | •                | +  | ÷                 |                                       | •  | + | •          | • •      |  |     | •   | +   | •   | +   | +   | +   | -   |   | ·                               | ŀ  | •   | •      | •       |       | •   | ŀ   | •  | ŀ  | ·  | •   | •   |
| -    | 3369262 Sepia la<br>3369264 Sepia la   |   | -       | +              | ÷                      |          |                                      | *   | •                                     | -                        | •   | ÷                     | • | •                | +  |                   | -                                     | •  | - | +          | •        |  |     | •   | +   | +   | +   | +   | -   | -   |   | ÷                               | ÷  | •   |        | •       | •     | •   | •   | -  | ÷  | -  | t   | •   |
|      | AB192338.1 Sepia   |   |         | а.             |                        | Ŧ        |                                      | 1   | •                                     |                          |     |                       |   |                  |  |                   |                                       |    | • | 1          | 1        |  |     | -   | +   | •   | t   |     |     | ť   |   | •                               | ľ  | •   | -      |         | c     |   | •   |    | 1  |    | ·<br>t  | •   |
| -    | AB430406.1 Sepia   |   |         |                | fil                    | Ť        |                                      |   | Ť                                     | Ť                        |     | ť                     |   |                  | i  |                   | -                                     | i. | c | Ť          | Ť        |  | ľ.  | t   | Ť   | +   | t   |     | +   | t.  | 1 | t                               | t. | i.  | ÷,     | ÷.      | c     | ľ.  | ŕ.  | Ē  | t  | Ť. | t   |     |
|      | AY185506.1 Sepia   |   |         | a .            | i.                     | а.       | T.                                   | j.  |                                       | Ť.                       | Α.  | t.                    |   |                  | j,   | t c               | -                                     | i. | ļ | Ì          |          |  | Ť.  |     | Ĵ,  |     | t   |     | +   |     |   | İ.                              | t. |     | ,      | ÷.      |       |   |     | i. | İ. | İ. | t   | -   |
| -    | KF009663.1 Sepia   |   | -       | а.             | Ĥ                      |          |                                      |   |                                       |                          |     | Ť.                    |   |                  | 1  |                   | -                                     |    | c |            |          |  | Ť.  | İ   | Ť   | 1   | +   |     | -   | T   | + | t                               |    |     |        |         | c     |   |     |    | t  | Ť. | t   |     |
| -    | 1542916 Sepia la   | 1 | C I     | <del>7</del> + | 2                      | C 1      |                                      | c   | c                                     | C 2                      | 1 1 | : G                   | G | t                | 7  |                   | : A                                   | -  | + | 2          |          | 2  | -   | +   | -   | -   |     |     |     |     |   | 1                               | C  | 7   |        | 2       | T     | 2   | C   | 6  |    | A  |   | +   |
|      | 1626051 Sepia la   |   |         |                | -                      |          |                                      |   | •                                     |                          |     | -                     | ~ |                  | 1  |                   |                                       | •  |   | -          |          |  | -   |     |     |     |     | Ŧ   |     | -   |   | -                               |    |     | -      | -       |       | -   |     | -  |    | -  |   | 1   |
|      |  |   | -       |                |                        |          |                                      | -   | •                                     |                          | +   | •                     |   | •                | -  |                   | •                                     | •  | - | •          |          | •  | ÷   | C   | •   | •   | •   | +   |     | -   | • | ŀ                               | -  | •   | •      | •       | ŀ     | •   | •   |    | ŀ  |    | -   | ••  |
|      | 1626052 Sepia la   |   | -       | • • •          | •                      |          |                                      | -   | •                                     |                          |     | -                     | - | •                | -  |                   | -                                     | •  | - | ·          |          | •  |     | C   | •   | •   | •   | +   |     |     | • | ŀ                               | •  | •   | •      | •       | •     |   | •   |    | •  |    | -   | C   |
|      | 1626057 Sepia la   |   | -       | • • •          | •                      | • •      |                                      | -   | •                                     |                          | •   | -                     | - | a                | -  | . 1               |                                       | •  | - | •          |          | •  | ŀ   | -   | •   | •   | •   | +   | C   | : 0 |   | •                               | -  | •   |        | •       | ŀ     | ·   | •   |    | •  |    | -   | ••• |
|      | 1733049 Sepia la   |   | •       |                | •                      | • •      |                                      | •   | •                                     |                          |     |                       |   | •                |  |                   | •                                     |    |   | •          |          |  | ŀ   |     | •   | •   | •   | •   | •   |     |   | •                               | -  | •   | •      | •       | •     | ·   | •   | •  | •  | •  | •   |     |
|      | 3369262 Sepia la   | · | •       | <u>.</u>       | •                      | -        | •                                    | •   |                                       |                          | •   |                       |   |                  | •  |                   | •                                     |    |   |            |          |  |     | C   |     | •   |     |     | •   |     |   |                                 | -  |     |        |         |       |   |     |    |    |    |   |     |
| -    |  |   |         |                |                        |          |                                      |   |                                       |                          |     |                       |   |                  |  |                   |                                       |    |   |            |          |  |     | C   |     | •   |     |     |     |     |   |                                 |    |     |        |         |       |   |     |    |    |    |   |     |
| -    | 3369264 Sepia la   |   |         |                | +                      | • •      | •                                    | •   | •                                     |                          |     |                       | • |                  |  | •                 | •                                     |    | • | •          | -        |  | 11  |     | .   |     | ŧ   |     | c   |     |   | Ι.                              |    |     |        |         |       |   |     |    |    |    |   |     |
|      | 3369264 Sepia la<br>AB192338.1 Sepia   |   |         |                | 11                     |          | +                                    | •   | •                                     |                          |     |                       |   |                  |  | +                 | -                                     | •  |   | +          |          |  | •   | -   | -   |     |     | -   |     |     | - | -                               | _  | 111 | 11     |         |       |   |     |    |    | 1  | 11  |     |
|      | -  |   |         |                | 11                     | -        | +                                    |   | •                                     | •                        |     |                       | • | g                |  | +                 | : .                                   | -  |   | +          | -        |  |     |     |     |     |     |     | c   | -   |   |                                 |    |     |        | diam'r. | 1.000 | 1   | 100 |    |    |    | -   |     |
|      | AB192338.1 Sepia   | • |         | <br><br>. a    |                        | -        |                                      |   | •                                     | · ·                      |     |                       | • | g                |  | . 1               | ; .                                   |    |   | +          | -        |  | •   |     |     |     | t   |     | +   | -   |   |                                 |    | •   | •<br>t |         |       |   |     |    |    |    | -   |     |
| 0    | AB192338.1 Sepia<br>AB430406.1 Sepia   | • |         |                |                        |          |                                      |   |                                       | • •                      |     | -                     |   | g<br>g           | •  |                   |                                       |    |   | +          | -        |  |     |     | •   |     |     |     | +   |     |   |                                 |    |     | t      |         |       | -   |     |    |    |    | -   |     |
| 0    | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia   | • |         | <br>. a        |                        |          |                                      |   | •                                     |                          |     | -                     |   | g<br>g           | •  | . 1               |                                       |    | c | •          | -        |  | 151 | -20 |     |     |     |     |     |     |   |                                 |    |     | t      |         |       |   |     |    |    |    | •   |     |
| .0   | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia   | • |         | <br>. a        |                        |          |                                      |   | •                                     | · ·                      |     | -                     |   | g<br>g           | •  | . 1               |                                       |    | c | •          |          | · · ·  |     | -20 |     |     |     |     |     |     |   |                                 |    |     | t      |         |       | -   |     |    |    |    |   | •   |
| .0   | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia   |   |         | <br>. a.       |                        | <br>     |                                      |   |                                       |                          | -   |                       |   | g<br>g           | -  |                   | · · · · · · · · · · · · · · · · · · · |    | c | W          | <br><br> |  |     |     |     | -   | t   |     | . c |     |   |                                 |    | •   | -      |         |       | •   |     |    |    |    |   | •   |
| .0   | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia   |   |         | <br>. a.       |                        | <br>     |                                      |   |                                       |                          | -   | -<br>-                |   | g                |  |                   | · · · · · · · · · · · · · · · · · · · |    | c |            | <br><br> |  |     |     |     | -   | t   |     | . c |     |   |                                 |    |     | -      |         | G     |   | a   | G  |    |    |   |     |
| .0 . | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la   |   |         | <br>. a.       |                        | <br>     |                                      |   |                                       | · · ·                    | -   |                       |   | g                | -  |                   | · · · · · · · · · · · · · · · · · · · |    | c |            | <br><br> |  |     |     |     | -   | t   |     | . c |     |   |                                 |    |     | -      |         | G     |   |     | G  |    |    | -<br>-<br>-   |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la   |   |         | <br>. a<br>    |                        | <br><br> |                                      |   |                                       |                          | -   | T                     |   | g                | <br><br>   | . t<br>. t<br>. t |                                       |    |   |            | indo     | A  |     | T   | t . | -   | t   |     | . c |     |   |                                 |    |     | -      |         |       |   |     |    |    |    | -<br>-<br>-<br>-  |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la   |   |         | <br>. a.       |                        | <br>     | <br><br>                             | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-                     |                                       | <br><br><br><br><br><br> | -   | -<br>-<br>-<br>-<br>- |   | g<br>g<br>g<br>g | - 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1 |                   |                                       |    | c | W          | <br><br> | A  |     |     |     | -   | t   |     | . c |     |   | -<br>-<br>-<br>-<br>-<br>-<br>- |    |     | -      |         |       |   |     |    |    |    | -<br>-<br>-   |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la   |   |         | <br>. a<br>    |                        | <br><br> |                                      | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |                                       |                          | -   | T                     |   | g<br>g<br>g<br>g | <br><br>   | . t<br>. t<br>. t |                                       |    |   | W          | indo     | A  |     | T   | t . | -   | t   |     | . c |     |   |                                 |    |     | -      |         |       | -<br>-<br>-   |     |    |    |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-   |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>I542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la                     |   |         | <br>. a<br>    |                        | <br><br> |                                      | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |                                       |                          | -   | -<br>-<br>-<br>-<br>- |   | g<br>g<br>g<br>g | - 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1 | . t<br>. t<br>. t |                                       |    |   | W          | indo     | A  |     | T   | t . | -   | t   |     | . c |     |   |                                 |    |     | -      |         |       | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-                |     |    |    |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la   |   | A 2<br> | <br>. a<br>    |                        | <br><br> |                                      | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- | · · · · · · · · · · · · · · · · · · · |                          | -   | -<br>-<br>-<br>-<br>- |   | g<br>g<br>g<br>g | - 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1 | . t<br>. t<br>. t |                                       |    |   | W          | indo     | A  |     | T   | t . | -   | t   |     | . c |     |   |                                 |    |     | -      |         | G     |   |     |    |    |    | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>I542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369264 Sepia la |   | A 2<br> |                |                        | <br><br> | <br><br><br><br><br><br><br><br><br> | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |                                       | <br>t .                  | -   | -<br>-<br>-<br>-<br>- |   | g<br>g<br>g<br>g | - 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1<br>- 1 | . t<br>. t<br>. t | · · · · · · · · · · · · · · · · · · · |    |   | W          | indo     | A<br>A<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4<br>4 | g   | T   | t . | -   | t   |     | . c |     |   |                                 |    | t   | -      |         |       | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-           |     |    |    | t  | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |     |
|      | AB192338.1 Sepia<br>AB430406.1 Sepia<br>AY185506.1 Sepia<br>KF009663.1 Sepia<br>I542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369263 Sepia la |   | A 2<br> |                |                        | <br><br> |                                      | •   |                                       | <br><br>                 |     | -<br>-<br>-<br>-<br>- |   | g<br>g<br>g<br>g |  |                   |                                       |    |   |            |          |  | g   | T   | t   |     |     |     |     |     |   |                                 |    |     | -      |         |       | -<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- |     |    |    |    | -   |     |

|   |  | _ |   |   |   | _   |     |    |   | _    | _   |     |     |    | _  | _ |     | _    |     | _   | 1 | Vin | dow  | : 20 | )1-2 | 50   | _         | _ | _ |     |     | _  |    | _ | _ | _ | _   | _    | _   | _    |     | _   |     | _     |      |
|---|--|---|---|---|---|-----|-----|----|---|------|-----|-----|-----|----|----|---|-----|------|-----|-----|---|-----|------|------|------|------|-----------|---|---|-----|-----|----|----|---|---|---|-----|------|-----|------|-----|-----|-----|-------|------|
|   |  |   | _ | + | + | +   | ╈   | +  |   | +    | +   | +   | ┢   |    | _  | _ | +   | +    | +   | +   | ╞ | -   |      | +    | +    | ł    | $\square$ |   | + | +   | +   | -  |    | _ | + | + | +   | +    | +   | +    | +   | +   | -   | +     | +    |
| 1 | 1542916 Sepia la   | G | A | t | A | 7 2 | ۱ G | С  | t | T    | T 1 | : 0 | : c | a  | С  | G | t i | A 1  | : 2 | A   | A | с   | A    | A    | 7 2  | 1    | λ         | A | G | a 1 | 1   | Ŧ  | 7  | G | a | 7 | T J | A    | c ' | 1    | A ( | c c | : c | : (   | c    |
| 2 | 1626051 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       | T    |
| ) | 1626052 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       |      |
|   | 1626057 Sepia la   |   |   |   |   |     |     |    | a |      |     |     |     |    |    |   | a   |      |     |     |   | t   |      |      |      |      |           |   |   | t   |     |    |    |   |   |   |     | . 1  | t   |      |     |     |     | ι.    |      |
|   | 1733049 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       |      |
|   | 3369262 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     | . , |     |       |      |
|   | 3369264 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     | . , |     |       |      |
|   | AB192338.1 Sepia   |   |   |   |   |     |     |    | a |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     | . 1  | ŧ   |      |     | . , |     |       |      |
|   | AB430406.1 Sepia   |   |   |   |   |     |     |    | a |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     | . 1  | e   |      |     |     |     | Π.    |      |
| 0 | AY185506.1 Sepia   |   |   | c |   |     |     |    | c |      | . ( |     |     | t  |    |   | a   |      |     |     |   | t   |      |      |      |      |           |   |   | ŧ.  |     |    |    |   | g |   |     | . 1  | e   |      |     |     | . t |       |      |
| 1 | KF009663.1 Sepia   |   |   |   |   |     |     |    | a |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     | . 1  | ŧ   |      |     |     |     |       |      |
|   |  |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     | 3 | Win | idov | v: 2 | 51-3 | 00   |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       |      |
|   |  |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     | Ι    |     | Ι   | T   |       |      |
|   |  |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       |      |
|   | 1542916 Sepia la   | a | Ŧ | С | a | 7 1 | 1 2 | a  | С | a    | c   | T a | a c | T  | t  | t | T   | a    | T ( | C c | 1 | C   | a    | 7    | C    | t a  | t         | ī | a | T J | A C | A  | Å  | A | G | a | G   | G    | t   | G    | C   | a ( | G C | 3 1   | t    |
|   | 1626051 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     | _ |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       |      |
|   | 1626052 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     | T     |      |
|   | 1626057 Sepia la   | t | 1 |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     | . a |   |     |      |      |      |      |           |   |   |     |     |    |    |   |   |   |     |      | a   |      |     | t.  |     |       |      |
|   | 1733049 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     | _ |     |      |      |      |      |           |   |   |     |     |    | 1. |   |   |   |     |      |     |      |     | T   |     | T     |      |
|   | 3369262 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    | 1. |   |   |   |     |      |     | T    |     | T   | T   | T     |      |
| - | 3369264 Sepia la   |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     |   |     |      |      |      |      |           |   |   |     |     |    | 1. |   |   |   |     |      |     | T    |     | T   | t   | T     |      |
|   | AB192338.1 Sepia   | t |   |   |   |     | t   | Ϊ. | 1 |      | t   |     | . t | İ. | a  | с |     | t    | t   |     |   | t.  |      |      | Ť    | Ť.   | 1         |   |   | T   |     | Ť. | t. | 1 |   | t |     | 1    | Ţ   | Ť    | t   | Ť   | t   | t     | Ţ    |
| - | AB430406.1 Sepia   | - |   | - |   |     |     | g  |   | -    | t   |     | t   | -  | -  | c | -   | ŧ    |     |     | - | İ   |      |      |      |      |           |   |   |     |     |    |    |   |   | t |     |      | Ì   | Ť    |     |     | T   | T     |      |
| - | AY185506.1 Sepia   |   |   | - | t | -   |     |    |   | t    |     |     | t t | -  | a  | - | -   | -    |     |     | - | -   | c    |      | Ť,   | <br> |           |   | g | ÷   | -   | ŕ  | ŕ  |   |   |   |     |      | ÷   | ÷    | 1   | ŧ.  | Ŧ   |       |      |
| - | KF009663.1 Sepia   | • |   |   | - |     |     |    | - | -    | ŧ   | _   | . t | -  | -  | - | •   |      |     |     | - |     |      |      | -    | . 9  | ÷         | - | 9 | -   |     |    | +  |   | • | t | •   | •    | +   | +    | -   | 6   | +   |       | -    |
| - | Aroosobo.r Septa   | 4 |   |   | • |     |     |    |   | •    | v   |     |     |    | e. | • | •   |      |     |     | - |     |      |      | •    |      |           |   | • | •   |     |    |    |   | • | • | •   | •    | -   |      | •   |     |     |       | -    |
|   |  |   |   |   |   |     |     |    |   |      |     |     |     |    |    |   |     |      |     |     | 1 | Min | dow  | r. 3 | n-3  | 50   |           |   |   |     |     |    |    |   |   |   |     |      |     |      |     |     |     |       |      |
|   |  |   |   |   | _ |     | _   |    |   |      | _   | +   | _   |    |    |   | _   | _    | _   | _   |   |     |      |      | _    | -    |           | _ | _ |     | _   | _  |    |   | _ |   | _   |      | _   | _    |     | _   | _   | ļ     |      |
|   | 1542916 Sepia la   | c | + | 0 | 0 |     |     |    |   | c    |     | c 1 |     | -  | A  |   | c   | c i  | t d |     |   |     |      | 2    |      |      | λ         | e |   | A 2 |     |    |    |   | - | ~ | a   | c .  | λ   | a (  | c 1 | e 1 | E C | c (   | ~    |
| - | 1626051 Sepia la   | - | - | - | 1 |     | T   | -  | - | -    |     | T   | -   |    |    |   | -   |      |     |     | - |     | Ĥ    | -    |      |      | -         |   | 1 |     | -   |    |    | - | - |   |     |      |     |      |     | -   | -   |       | -    |
| - | 1626052 Sepia la   |   |   |   | Ì |     | 1   |    |   |      |     |     |     | ÷  |    |   |     |      |     |     | - |     |      |      |      | -    |           |   | - |     | -   | Ľ. |    |   |   |   |     | Ť    |     | -    | Ť   | -   | +   | -     |      |
| - | 1626057 Sepia la   |   | a | • | Ť |     | +   | ÷  |   |      | -   |     | . a | ÷  |    |   |     |      | a   |     | a |     |      |      |      |      | ÷.        |   |   |     |     |    |    |   |   | - |     | Ì    | •   | t .  | -   |     |     | -     | -    |
| - | 1733049 Sepia la   |   |   |   | - | -   | -   | -  |   | -    | +   |     |     | -  |    |   |     | -    |     | -   |   |     | ŀ    | Ì    |      | +    |           | - |   | -   |     |    | -  |   |   |   | -   | +    | +   | -    | +   | -   | -   | -     | +    |
| - | 3369262 Sepia la   |   |   | - | Ť | -   | -   | ľ  | - | -    | -   |     | -   | f  | -  | 1 | -   |      |     |     | + | -   | i i  | +    |      | -    | -         | 1 | + | -   |     | ľ  | -  |   | - | • |     | 1    | +   | +    | 4   | -   | +   | -     | +    |
| - | 3369264 Sepia la   |   | - |   | Ť |     |     | İ. | • | •    |     |     |     | -  |    | • | -   |      |     |     |   |     | •    | -    |      |      |           | - | Ì |     |     | i  | -  | • |   | • | 1   | Ì    | •   | Ť    | t   | Ť   | Ŧ   | Ť     | Ì    |
| _ | AB192338.1 Sepia   |   |   | - | - |     | +   | +  |   |      |     | -   | . c | -  |    |   | -   | -    | -   | -   | 3 | -   |      | -    |      | t    | -         |   | + | +   |     | -  | -  |   |   |   | +   | -    | +   | t.   |     | ť   |     |       | 1    |
|   | and a second sec |   |   |   | - | -   | 1   | -  | - | -    |     | -   |     | 1  |    |   | _   | -    | -   | _   | - | -   |      | -    | -    | -    |           |   | - | +   | -   | -  | -  | - |   | - | +   | -    | -   | -    | -   | -   | +   | -     |      |
| - | AB430406.1 Sepia   |   |   |   |   |     |     |    |   | . I. |     |     |     |    | 1  | 0 |     | . 14 | 2   |     | 3 |     |      |      |      | 15   |           |   |   |     |     |    |    |   |   |   |     | . I. | . P | t II | цΕ. |     |     | , I., | - P. |
|   | AB430406.1 Sepia<br>AY185506.1 Sepia   | - |   | - | ÷ | • • |     | -  |   |      | -   | •   | -   | -  | -  | с |     | . 4  | -   | _   | a | -   |      | -    | _    | t    | -         | - | - | -   |     | -  | -  | • | • | • | t t | -    | -   | t.   | •   | -   | +   |       | -    |

|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     | 1   | W   | indo | w.  | 351 | 40       | 0  |   |    |      |     |     |    |   |   |   |   |     |     |     |     |     |          |    |   |    |
|----|--------------------------------------|---|----|---|---|---|--------|---|-----|-----|-----|-----|----|----|----|---|---|---|---|----|-----|-----|-----|------|-----|-----|----------|----|---|----|------|-----|-----|----|---|---|---|---|-----|-----|-----|-----|-----|----------|----|---|----|
|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     | _   |     |      |     |     |          |    |   |    |      |     |     |    |   |   |   |   |     |     |     |     |     |          |    |   |    |
| 1  | 1210010 00010 10                     |   |    | _ | - |   |        | G | _   |     | 2 A |     |    | -  |    |   | С |   |   | -  | _   | 7 1 |     |      |     |     | -        | _  | t | _  |      |     |     |    |   | С | _ | _ | _   | _   |     | r c | _   |          | c  | _ | -  |
| 2  | 1542916 Sepia la<br>1626051 Sepia la | 6 | 5  | a | т | 6 | a<br>t | 6 | T   | C 1 |     | 1 1 | C  | Т  | •  | 6 | - | a | • | T  | 5   | T   |     | 0 1  |     | -8  | C        | т  | 5 | 63 | n () | 1 1 | 1   | •  | U | 6 | g | 6 | 6 1 | a - | 1   | r c | T   | 6        | •  | T | -  |
| -  | 1626052 Sepia la                     |   | -  |   |   |   | t      | - |     |     |     |     |    |    | -  | - |   |   | - |    |     |     |     |      |     | -   |          |    |   | -  |      |     |     | -  | • |   |   | - | -   |     |     |     |     | ÷        |    | - | Ì  |
| -  | 1626057 Sepia la                     |   |    | t |   |   |        |   |     | a   |     |     | t  |    | Ē  |   |   | t |   |    | c   |     |     | ε.   | 1.  |     | t.       |    | a |    |      |     |     | İ. |   |   | a |   |     | t a | a . | t   |     | İ.       |    | Ē | Ċ  |
| -  | 1733049 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    | Ī. |   |   |   |   |    |     |     |     |      |     |     | 1.       |    |   |    |      |     |     |    |   |   |   |   |     |     |     | T.  |     |          |    |   | Ì. |
| 6  | 3369262 Sepia la                     |   |    |   |   |   | t      |   |     |     |     |     | ١. |    |    |   |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    |   |   |   |   |     |     |     | Τ.  |     | 1.       |    |   |    |
| 7  | 3369264 Sepia la                     |   |    |   |   |   | t      |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    |   |   |   |   |     |     |     |     |     |          | t. |   |    |
| _  | AB192338.1 Sepia                     |   |    |   |   |   | t      |   |     |     |     |     | t  |    |    |   |   | t |   |    |     |     | . 1 | ŧ .  |     |     |          |    |   |    |      |     |     |    |   | 1 | c |   | . 1 | t 4 | ,   | . 8 |     |          | a  |   |    |
| 9  | AB430406.1 Sepia                     |   |    |   |   |   | t      | 1 |     |     |     |     | t  |    |    |   |   | t | - |    |     |     | . 1 | ε.   |     |     |          |    |   |    | -    |     |     |    |   |   | c |   | . 1 | t 9 | 1.  | . a |     |          | a  |   |    |
| 10 | AY185506.1 Sepia                     |   |    |   |   |   | t      |   |     | a   |     |     | t  |    |    |   |   | t |   |    |     |     | d   | ε.   |     | t   | t        |    | a |    | -    |     |     |    |   |   | t |   | . 1 | t 1 | ;   | . t |     |          |    |   |    |
| 11 | KF009663.1 Sepia                     |   |    |   |   |   | t      |   |     |     |     |     | t  |    | -  | - |   | ŧ | - | •  |     |     | • 1 | ε.   |     |     |          |    |   |    | -    |     |     |    |   |   | c | - | . 1 | t g | g . | . 8 | 1   |          | ð. |   |    |
|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     | W   | ind  | w.  | 40  | 148      | 50 |   |    |      |     |     |    |   |   |   |   |     |     |     |     |     |          |    |   |    |
|    |                                      |   |    |   |   |   |        |   |     | _   |     |     | _  | _  | -  |   |   |   |   |    |     | _   | _   | _    | +   | +   |          |    | _ |    | _    | _   | +   |    |   |   |   |   |     | _   | -   | _   | _   | _        | _  |   |    |
| 1  | 1542916 Sepia la                     | λ | λ  | T | t | T | Ţ      | a | G   | G   | t G | . 0 |    | λ  | Ţ  | t | λ | λ | Ŧ | Ŧ  | T   | t   | A.  | 7 1  | 1 3 |     | a        | λ  | С | a  | λ    | T   | r c | T  | a | λ | λ | Ŧ | λ   | T ( | g ( | C   | ; t | . T      | G  | λ | G  |
| 2  | 1626051 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     | -  |    |    |   |   |   |   |    |     |     | -   |      |     |     |          |    |   |    |      | -   |     |    |   |   |   |   |     | . 1 | a   |     |     |          |    |   |    |
| 3  | 1626052 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     |     |      |     | Τ.  |          |    |   |    |      |     |     |    |   |   |   |   |     |     |     |     |     |          |    |   |    |
| 4  | 1626057 Sepia la                     |   |    |   |   |   |        | g |     |     |     |     | t  |    |    |   |   |   |   |    |     | c   | _   |      |     |     | c        |    |   |    |      | -   | . t |    |   |   |   |   |     |     | a   |     | a   | Π.       |    |   |    |
| 5  | 1733049 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     |     |      |     | Τ.  |          |    |   |    |      |     |     |    |   |   |   |   |     |     |     |     |     |          | Γ. |   |    |
| 6  | 3369262 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    |   |   |   |   |     |     | a   |     |     |          |    |   |    |
| 7  | 3369264 Sepia la                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    |   |   |   |   |     |     | a   |     |     |          | Γ. |   |    |
| 8  | AB192338.1 Sepia                     |   |    |   |   |   |        |   |     |     | с.  |     |    |    |    | c |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    | c |   |   |   |     |     | a   |     |     |          |    |   |    |
| 9  | AB430406.1 Sepia                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    | c |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    | c |   |   |   |     |     | a   |     |     |          |    |   |    |
| 10 | AY185506.1 Sepia                     |   |    |   | с |   |        |   |     |     |     |     | t  |    |    |   |   |   |   |    |     |     |     |      |     |     |          |    |   | t  |      |     | . t |    |   |   |   |   |     |     | a   |     | g   | Π.       | Γ. |   |    |
| 11 | KF009663.1 Sepia                     |   |    |   |   |   |        |   |     |     |     |     |    |    |    |   |   |   |   |    |     |     |     |      |     |     |          |    |   |    |      |     |     |    | c |   |   |   |     |     | a   |     |     |          | Γ. |   |    |
|    |                                      |   |    |   |   |   |        |   |     |     |     |     |    |    | Ī  |   |   |   |   |    |     |     | W   | ndo  | w   | 451 | -50      | 0  |   |    |      |     |     |    |   |   |   |   |     |     | -   |     |     |          |    |   |    |
|    |                                      |   |    |   |   |   |        |   |     |     |     | [   |    |    |    |   |   |   |   |    |     |     | I   | I    |     |     |          |    |   |    | I    | Ι   |     |    |   |   |   | I |     | I   | Ī   | Ι   |     |          |    |   |    |
| 1  | 1542916 Sepia la                     | λ | a  | C | C | 7 | c      | 7 | λ   | c , | L a | λ   | 7  | λ  | C  | λ | λ | с | C | a  | t ' | 7 4 |     | : c  | t   | t   | 7        | λ  | 7 |    | 1 0  | 1   | t   | T  | C | λ | 7 | c | t ( | 3 1 | 1 a | 17  | 7   | t        | λ  | 7 | 7  |
| 2  | 1626051 Sepia la                     |   | Ť. |   |   |   | t      |   |     |     | T.  | T.  |    |    |    |   |   |   |   | a  | Ţ   |     | T.  |      | Ϊ.  |     |          |    |   |    | Ť.   |     |     |    |   |   |   |   |     | T.  | g   | T.  |     |          |    |   |    |
| 3  | 1626052 Sepia la                     |   | ,  |   |   |   | Ť      | Ì | Ì   |     |     | t.  | Ē  | Í. | Ĺ  |   |   | Ť | Ì | a  |     |     | t   |      | ť.  |     |          |    | Ť |    | t    | Ť,  | ť.  |    | - |   | Ť | Ť |     | t   | Ť.  | T.  |     | Í        | Ť, |   | Ĩ  |
| 4  | 1626057 Sepia la                     | - | a  |   | Ì |   | t      |   |     |     | a   | t   |    |    |    |   |   |   | - | a  | -   | t   | t   | ť    | f   |     |          |    | Ť | Ť  | ť    | Ť   | f   |    |   |   | Ť |   |     | t   | 1,  |     | Ĺ   | c        | -  |   |    |
| 5  |                                      |   | -  |   | ÷ | - | -      | • | +   |     | -   | ÷   | *  | ÷  | ÷  | - | * | - | - | -+ | +   | +   | ť   | +    | ť   | ÷   | -        | •  | - |    | +    |     |     |    |   | • | - | 1 |     | ť   | +-  | +-  | +-  | -        | -  |   | *  |
|    | 1733049 Sepia la                     |   |    |   | _ |   | _      |   | _   |     | _   |     | -  | -  | _  |   | _ | _ |   | _  | _   | _   | _   | _    | -   | -   |          | _  | _ | _  |      | _   | -   |    |   | _ | _ | _ | _   | _   | _   | _   | -   |          | _  |   | _  |
|    | 3369262 Sepia la                     |   |    |   |   |   |        |   | - 1 | _   | -   |     | -  |    |    |   |   |   |   |    |     |     | Т   | -    | Т   |     |          |    |   |    | 1    | -   | T   |    |   |   |   |   |     | Т   |     |     | 1   |          |    |   |    |
|    | 3369264 Sepia la                     | - |    |   | _ | - | -      | - | -   | -   |     | Т   | -  | 1  | •  |   |   |   |   | -  | -   | • • | T   | T    | •   | -   | $\vdash$ |    | - | •  | +    | -   | •   |    |   |   | • | + | -   | +   | T   | T   | T   |          | •  |   |    |
| -  | AB192338.1 Sepia                     |   |    |   |   |   |        | _ | -   | •   | _   |     | -  | •  | H  | * |   |   |   | a  | +   | •   | T   | +    | t   | -   | -        |    |   | +  | +    |     | r   | *  | * |   | + | + | +   |     | +   | •   |     |          | •  |   |    |
| -  | AB430406.1 Sepia                     |   | a  |   | • | - | -      |   | •   | •   | a   |     |    |    | •  | × | • | • | - | a  | +   | • • | ÷   | +    | a   | C   | •        | •  | • | •  | +    | •   | 9   |    |   | • | • | + | •   | -   |     |     | •   |          | •  |   |    |
| -  | AY185506.1 Sepia                     | - | -  |   | - | - | t      | - | •   | -   | a   | +-  |    |    | •  | - |   |   | - | a  | +   | . ( | ÷   | +    |     |     |          | •  |   |    | -    |     |     | •  |   |   | • | + | с.  | ÷   |     |     |     | $\vdash$ |    |   |    |
| 11 | KT009663.1 Sepia                     |   | a  |   |   |   | t      |   |     |     | a   |     |    |    |    |   |   |   |   | a  |     |     |     |      | a   | c   |          |    |   |    |      |     | 9   |    |   |   |   |   |     |     |     |     |     |          |    |   |    |

|                                       |  |  |   |     |     |                  |   |   |          |   |                                       |           |   |            |             |       |        |     |          | 1        | Vind             | w.  | 551-4           | 500            |     |   |         |     |       |                  |                       |     |     |     |                       |                       |   |    |     |       |    |   |
|---------------------------------------|--|--|---|-----|-----|------------------|---|---|----------|---|---------------------------------------|-----------|---|------------|-------------|-------|--------|-----|----------|----------|------------------|-----|-----------------|----------------|-----|---|---------|-----|-------|------------------|-----------------------|-----|-----|-----|-----------------------|-----------------------|---|----|-----|-------|----|---|
|                                       |  |  |   |     |     |                  |   |   | _        |   |                                       |           |   |            |             |       |        |     |          |          |                  |     |                 |                |     |   |         |     |       |                  |                       |     |     |     |                       |                       |   |    | _   |       |    | Į |
|                                       | 1542916 Sepia la   | a  | 1 | 7 2 | 17  |                  | ъ | 7 | T 3      |   |                                       |           | G | <b>A</b> 1 | t C         | G     | a      |     | A t      |          | 7.1              | - 3 | A               |                | c.  | 2   | 2 0     |     |       | 7                |                       | 7 5 |     | G   |                       |                       | с | с  | a ) |       |    |   |
| ,                                     | 1626051 Sepia la   | •  | • |     |     |                  | • |   |          |   | 1.0                                   |           |   | ~          |             |       | •      | -   | <u> </u> |          |                  | +   | -               | -              | -   | -   | -       | -   |       |                  | -                     | -   | +   |     | n                     | -                     | - | •  | a / |       | F  | f |
| _                                     | 1626052 Sepia la   | •  | • |     |     | -                | • | • |          | + |                                       | ÷         | ÷ | -          | • •         |       | •      | -   | · · ·    | -        | •                |     | -               |                |     | •   | • •     | +   | -     |                  | -                     |     | +   |     | •                     | -                     | • | •  | -   | +     | •  | - |
| -                                     | 1626057 Sepia la   | ·<br>t   | • | • • |     | -                | • | • |          | + | -                                     | ·<br>a    | ÷ | •          | · ·         |       | t.     | •   | +        |          |                  |     | +++             | <br>c .        | •   | •   | -       |     |       | -                | •                     | -   | -   | +   | ÷                     | -                     | • | •  |     |       | -  | ł |
| -                                     | 1733049 Sepia la   | £.   | • | •   | •   | -                | • | • | • •      | + |                                       | a         | • |            | ¢ .         |       | 5      | •   | • •      | •        | . 1              | 1.  | •               | Ģ .            |     | •   |         |     | • •   |                  |                       | -   |     |     | •                     | •                     | • | •  |     | +     | ÷  | ÷ |
| -                                     | •  |  | • | • • | •   | -                | • | • | • •      | ÷ |                                       | -         |   |            |             | -     | •      | -   | • •      | -        | •                | •   | •               |                | •   | -   |         | +   | -     | -                | -                     |     | -   |     | •                     | -                     | • | •  |     | -     | -  | ł |
| -                                     | 3369262 Sepia la   |  | • | • • | •   | -                | • | • | • •      | + |                                       | •         | • |            |             | •     | •      | •   | • •      | -        | •                | • • | •               | •              |     |   |         | +   | -     |                  | -                     | •   |     | •   | •                     | -                     | • | •  | •   | +     |    | ł |
| _                                     | 3369264 Sepia la   |  | • | • • |     | -                | • |   | • •      |   |                                       |           |   | •          |             | -     | •      | •   | • •      |          | •                | -   | •               | • •            | •   |   |         |     | -     | •                | -                     |     | -   | •   | •                     | -                     | • | •  |     |       | -  | ł |
| -                                     | AB192338.1 Sepia   |  | • | • • |     | -                | • | - |          |   |                                       |           |   | •          |             | -     | •      | •   | . 0      |          | . 1              |     |                 | с.             | •   | •   |         | 8   | i .   | •                | •                     | •   |     | •   | •                     | -                     | • | -  | t.  |       | -  | ļ |
|                                       | AB430406.1 Sepia   |  |   | • • | •   | -                | • |   | • •      | - | •                                     | •         | • | . 1        | c .         | •     | •      | •   | . c      |          | . 1              | -   |                 | с.             |     |   | -       | 3   | ۰.    |                  |                       | •   |     | •   |                       |                       |   | -  | t.  |       | •  | ļ |
|                                       | AY185506.1 Sepia   | t  |   |     |     | -                |   |   |          |   |                                       | С         | - |            |             |       |        | -   |          | -        | •                | -   |                 |                | •   | •   |         | . a | ι.    | -                | t                     | •   |     |     |                       | t                     |   |    | t.  |       | -  | l |
| -                                     | -  |  | • |     | -   | -                | - | _ | _        | _ | _                                     | _         |   |            |             |       |        |     |          |          |                  |     |                 |                |     |   |         |     |       |                  |                       |     |     |     |                       |                       |   |    |     |       |    |   |
| -                                     | KF009663.1 Sepia   |  | • |     |     |                  |   |   |          |   |                                       |           |   | . (        | c .         |       |        |     | . c      | -        | -                | ow: | 501-4           | с<br>550       |     |   |         | a   |       |                  |                       |     |     |     |                       |                       |   |    | t.  | .   . |    |   |
| -                                     | -  |  |   |     |     |                  |   |   |          |   |                                       |           |   |            |             |       |        |     | . c      |          | -                | -   | -               | -              |     |   |         |     |       |                  |                       |     |     |     |                       |                       |   |    |     |       |    |   |
| 1                                     | KF009663.1 Sepia   |  |   | t ( | ; c |                  | λ |   | <br>c 1  |   | · .                                   |           |   | 9          |             |       |        |     |          | 1        | vind             | -   | 501-(           | -              | : c | G   | T a     |     |       | λ                | G                     |     |     | ; c | λ                     | G                     | c |    |     |       |    |   |
| 1                                     | KF009663.1 Sepia   |  |   | t ( | ; c | -                | λ | T | <br>     |   | · .                                   |           |   |            |             |       | t      |     |          | 1        | vind             | wr. | 501-(           | 550            |     | G   | T 2     |     |       | A                | G                     |     | : C | ; c | А                     | G                     | C |    |     |       |    |   |
| 1                                     | KF009663.1 Sepia   |  |   | t ( | ; c | 1                | A | T | c 1      |   | · .                                   |           | T |            |             | · ·   | t      |     |          | 1        | vind             | wr. | 501-(           | 550            |     | G   | <br>T : |     |       | λ.               | G                     |     |     | ; c |                       | C<br>C                | c |    |     |       | λ  |   |
|                                       | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la   |  | • | t ( | ; C | -                | A |   | <br><br> |   | A                                     |           |   |            |             | · ·   | t<br>c |     |          | \<br>' C | vind             | wr. | 501-(           | 550<br>c c     |     | G   | T 2     |     |       | А                | c                     | c ( |     | ; C | A                     | G                     | c |    |     |       | λ. |   |
| 1                                     | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la   |  | • |     |     | -<br>-<br>-<br>- | λ |   |          |   | · A                                   |           |   | g          |             |       |        |     |          | \<br>' C | t o              | wr. | 501-4<br>c      | 550<br>c c     | •   | C<br>C<br>C<br>C  | T 4     |     |       | λ                | G                     | C ( |     | ; G | A                     | G                     | c |    |     | r t   | λ. |   |
| 11<br>2<br>3<br>1                     | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la   |  | • |     | C   | -                | A |   |          |   |                                       |           |   | g          |             | · · · |        |     |          | \<br>' C | t o              | wr. | 501-4<br>c      | 550<br>c c     | •   | G<br>201<br>201<br>201<br>201<br>201<br>201<br>201<br>201<br>201<br>201 | T 4     |     |       | ·<br>·<br>·<br>· | c                     | C ( |     | ; c | ·<br>·<br>·<br>·      | G                     |   |    |     | r t   | λ. |   |
|                                       | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la   |  | • |     |     | -                | A |   |          |   |                                       |           |   | g          |             | · · · |        |     |          | \<br>' C | vind<br>t (<br>a | wr. | 501-3<br>c<br>t | 550<br>C C<br> | •   | •   | T 4<br> |     |       | ·<br>·<br>·<br>· | -<br>C<br>-<br>-<br>- | C ( |     | ; C | A                     | G                     | c |    |     | r t   | λ. |   |
|                                       | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la   | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·  | - |     |     |                  | A | • |          |   |                                       |           |   | g          |             |       | c      |     |          | \<br>C   | vind<br>t (<br>a |     | 501-3<br>c<br>t | 550<br>C C<br> |     | •   |         |     | · · · | λ                | G                     | · · |     | ; C | ·<br>·<br>·<br>·<br>· | -<br>-<br>-<br>-<br>- |   |    |     | r t   | A  |   |
|                                       | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369264 Sepia la                     | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>· | - | a . |     |                  | A | • | t .      |   | · · · · · · · · · · · · · · · · · · · |           | - | g          | t 1<br><br> |       | c      |     |          | \<br>C   | t (              |     | 501.<br>c       | 550            |     | •   |         |     | · · · | •                | -                     | · · |     |     |                       |                       |   | a. |     | - c   | A  |   |
| L1<br>2<br>3<br>5<br>5<br>7<br>8<br>9 | KF009663.1 Sepia<br>1542916 Sepia la<br>1626051 Sepia la<br>1626052 Sepia la<br>1626057 Sepia la<br>1733049 Sepia la<br>3369262 Sepia la<br>3369264 Sepia la<br>AB192338.1 Sepia | ·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>·<br>· | - | a . |     |                  | A | • | t .      |   |                                       | · · · · · | - | g          |             |       |        | T . |          | \<br>C   | tind<br>t (      |     | 501.<br>c       | 550            |     | •   |         |     |       | •                | -                     |     |     |     |                       |                       |   | a. | A 7 | - c   | A  |   |

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.

**Acknowledgements**. We would like to appreciate the Ministry of Research and Technology, Directorate General of Higher Education for financially support of this study through University Superior Basic Research Grant 2018. Great gratitude is also given to Mr. Andre Kambey and Ms. Inggrid Akerina for field sampling activities.

#### References

- Alberto F., Raimondi P. T., Reed D. C., Coelho N. C., Leblois R., Whitmer A., Serräo E., 2010 Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. Ecology 91(1):49-56.
- Allen J. J., Mäthger L. M., Barbosa A., Hanlon R. T., 2009 Cuttlefish use visual cues to control three-dimensional skin papillae for camouflage. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioral Physiology 195:547-555.
- Altschul S. F., Madden T. L., Schaffer A. A., Zhang J., Zhang Z., Miller W., Lipman D. J., 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389-3402.

- Arkley K., Jacklin M. S., Boulter M., Tower J., 1996 The cuttlefish (*Sepia officinalis*): a guide to its exploitation in UK waters. The sea fish Industry Authority, Seafish Report no. SR467, 81 pp.
- Barbosa A., Mäthger L. M., Buresch K. C., Kelly J., Chubb C., Chiao C. C., Hanlon R. T., 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive patterns. Vision Research 48:1242-1253.
- Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2012 Cuttlefish use visual cues to determine arm postures for camouflage. Philosophical Transactions of the Royal Society of London B: Biological Sciences 279:84-90.
- Chiao C. C., Chubb C., Buresch K. C., Barbosa A., Allen J. J., Mäthger L. M., Hanlon R. T., 2010 Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. Journal of Experimental Biology 213:187-199.
- Drummond A. J., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowitz S., Moir M., Stones-Havas S., Sturrock S., Thierer T., Wilson A., 2012 Geneious v5.5.6. Available at: http://www.geneious.com.
- Fernandez L. D., Hernandez C. E., Schiaffino R. M., Izaguirre I., Lara E., 2017 Geographical distance and local environmental conditions drive the genetic population structure of a freshwater microalga (Bathycoccaceae; Chlorophyta) in Patagonian lakes. FEMS Microbiology Ecology 93(10):10 pp.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.
- Hanlon R. T., Chiao C. C., Mäthger L. M., Barbosa A., Buresch K. C., Chubb C., 2009 Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Philosophical Transactions of the Royal Society of London B: Biological Sciences 364:429-437.
- Hays G. C., 2017 Ocean currents and marine life. Current Biology 27(11): 470-473.
- IUCN, 2014 The IUCN Red List of Threatened Species. Version 2014.2. Available at: www.iucnredlist.org. Accessed: September, 2014.
- Kelman E. J., Osorio D., Baddeley R. J., 2008 A review of cuttlefish camouflage and object recognition and evidence for depth perception. Journal of Experimental Biology 211:1757-1763.
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K., 2018 MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
- Leopold A., 1933 Game management. Charles Scribner's Sons, New York, 481 pp.
- McManus M. A., Woodson C. B., 2012 Plankton distribution and ocean dispersal. Journal of Experimental Biology 215: 1008-1016.
- Murphy J. M., Balguerias E., Key L. N., Boyle P. R., 2002 Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the Northwest African Coast. Bulletin of Marine Science 71:545-553.
- Neves A., Cabral H., Sequeira V., Figueiredo I., Moura T., Gordo L. S., 2009 Distribution patterns and reproduction of the cuttlefish, *Sepia officinalis* in the Sado estuary (Portugal). Journal of the Marine Biological Association of the United Kingdom 89(3):579-584.
- Pascual M., Palero F., García-Merchán V. H., Macpherson E., Robainas-Barcia A., Mestres F., Roda T., Abelló P., 2016 Temporal and spatial genetic differentiation in the crab *Liocarcinus depurator* across the Atlantic-Mediterranean transition. Scientific Reports 6:29892.
- Pawson M. G., 1995 Bio-geographical identification of English Channel fish and shellfish stocks. CEFAS Fisheries Research Technical Report No 99, 72 pp.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2015 Size at first maturity of cuttlefish, *Sepia latimanus*, from North Sulawesi waters, Indonesia. Marine Science 5(1):6-10.
- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2016 Mitochondrial CO1 genetic marker-based species diversity of cuttlefish (Cephalopod; Mollusk) in Manado Bay and Lembeh Strait, North Sulawesi, Indonesia. AACL Bioflux 9(6):1345-1354.

- Pratasik S. B., Marsoedi, Arfiati D., Setyohadi D., 2017 Egg placement habitat selection of cuttlefish, *Sepia latimanus* (Sepiidae, Cephalopoda, Mollusca) in North Sulawesi waters, Indonesia. AACL Bioflux 10(6):1514-1523.
- Ramachandran S., Deshpande O., Roseman C. C., Rosenberg N. A., Feldman M. W., Cavalli-Sforza L. L., 2005 Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proceedings of the National Academy of Sciences of the USA 102(44):15942-15947.
- Ratnasingham S., Hebert P. D. N., 2007 BOLD: the barcode of life data system. Molecular Ecology Notes 7:355-364.
- Reid A., Jerep P., Roper C. F. E., 2005 Family Sepiidae. In: Cephalopods of the world. An annotated and illustrated catalogue of Cephalopod species known to date. Volume 1. Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae). Jerep P., Roper C. F. E. (eds), FAO, Rome, pp. 54-152.
- Rozas J., Ferrer-Matta A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., Sánchez-Gracia A., 2017 DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12):3299-3302.
- Shohet A. J., Baddeley R. J., Anderson J. C., Kelman E. J., Osorio D., 2006 Cuttlefish responses to visual orientation of substrates, water flow and a model of motion camouflage. Journal of Experimental Biology 209:4717-4723.
- Tricarico E., Amodio P., Ponte G., Fiorito G., 2014 Cognition and recognition in the cephalopod mollusc *Octopus vulgaris*: coordinating interaction with environment and conspecifics. In: Biocommunication of animals. Witzany G. (ed), Springer, pp. 337-349.

Received: 25 April 2019. Accepted: 31 May 2019. Published online: 22 June 2019. Authors:

Silvester B. Pratasik, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, JI. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: spjong07@yahoo.com

Harjany Sambali, Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Sam Ratulangi University, JI. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: hariyanisambali@unsrat.ac.id Lefrand Manoppo, Study Program of Fisheries Resources Exploitation, Faculty of Fisheries and Marine Science, Sam Ratulangi University, JI. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: lefrandmanoppo@yahoo.com

Frans F. Tilaar, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: fftilaar@unsrat.co.id

Meiske S. Salaki, Study Program of Fisheries Resources Management, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, Manado-95115, North Sulawesi, Indonesia, e-mail: mssalaki@unsrat.co.id

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Pratasik S. B., Sambali H., Manoppo L., Tilaar F. F., Salaki M. S., 2019 Genetic variations of cuttlefish *Sepia latimanus* (Cephalopoda, Sepiidae) in North Sulawesi waters, Indonesia. AACL Bioflux 12(3):792-803.