

SYSTEMATICS OF INDO-WEST PACIFIC LOLIGINIDS

M. Vecchione, E. Shea, S. Bussarawit, F. Anderson, D. Alexeyev, C.-C. Lu, T. Okutani, M. Roeleveld, C. Chotiyaputta, C. Roper, E. Jorgensen and N. Sukramongkol.

Addresses: (MV) NMFS National Systematics Laboratory, National Museum of Natural History, Washington, DC 20013-7012 USA, vecchione.michael@nrmh.si.edu; (ES) Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010 USA, eshea@brynmawr.edu; (SB) Phuket Marine Biological Center, Phuket, Thailand, somchaibussar@hotmail.com; (FA) Dept. of Zoology and Center for Systematic Biology, Southern Illinois University, Carbondale, IL 62901 USA, feander@siu.edu; (DA) Federal Research Institute of Fisheries and Oceanography, Moscow 107140 Russia, alexeyev@vniro.ru; (C-CL) Dept. of Life Sciences, National Chung Hsing University, Taichung 402 Taiwan, cclu@dragon.nchu.edu.tw; (TO) Japan Marine Science & Technology Centre, Yokosuka 237-0061, Japan, okutani@jamstec.go.jp; (MR) Iziko-Museums of Cape Town, Cape Town 8000 South Africa, martina@iziko.org.za; (CC) Dept. of Marine and Coastal Resources, Bangkok, Thailand, cherdch@fisheries.go.th; (CR) Dept. of Systematic Biology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013-7012 USA, gsquidinc@earthlink.net; (EJ) School of Aquatic and Fishery Sciences, Univ. of Washington, Seattle, WA 98195 USA, Elaina.Jorgensen@noaa.gov; (NS) Training Department, Southeast Asian Fisheries Development Center, natinee43@hotmail.com.

INTRODUCTION

The charge to this group was discussion of the taxonomy and systematics of the family Loliginidae in Indo-West Pacific waters. However, problems of this family in the region are representative of broader problems and should be considered on a worldwide basis. The major problems of loliginid systematics can be divided into two general categories: (1) species-level problems, including determination of cryptic species and the possibility of natural hybridization among these closely related species, and (2) higher-level systematic problems, such as morphological character homology and stabilization of higher-level taxonomy (subgenus, genus, and subfamily).

Some species are termed “cryptic” because of very close morphological similarity to previously described species. Many examples of cryptic loliginid species have been discovered during the past two decades (*e.g.*, Cohen, 1976; Izuka *et al.*, 1994; 1996; Yeatman and Benzie, 1993). Some of these species complexes include pairs, or larger groups, of species that appear to be sympatric. In order for molecular methods to be used to test hypotheses of speciation, specimens must be identified based on morphological characters. Therefore, proper determination of species within species complexes requires careful examination of

morphology. In particular, the amount of morphological variability both within and between species must be determined. This goal could not be accomplished within the scope of the current workshop. Instead, we recommend below a research plan to examine the nature of morphological variability in loliginid species, which may lead, in turn, to better understanding of how to distinguish cryptic loliginid species, as well as possible morphological indications of hybridization.

The 1988 CIAC workshop on loliginid systematics and biogeography attempted, with limited success, to resolve several conflicting schemes of higher-level systematics of the Loliginidae. The published workshop proceedings (Vecchione *et al.*, 1998) described a provisional generic classification of the family, but this classification has only been adopted in part by the cephalopod research community. Two impediments to the success of the previous workshop were (1) lack of consensus on homology of some character states in some species, and (2) lack of cladistic analyses of loliginid phylogeny. Since the previous workshop, additional morphological characters have been described and evaluated (Alexeyev, 1989; 1991) and phylogenetic hypotheses have been proposed, based on both morphological and molecular characters (Anderson, 1996; 2000a; 2000b). We therefore re-examined the provisional

classification and recommend below a classification more in agreement with the expanded information now available.

The workshop began with four presentations, two applicable to the higher-level systematics problem, one an example of the species-level problems in the family, and one illustrating that our understanding of the diversity of myopsid squids is far from complete. The first, by Anderson, summarized his recently published cladistic studies of morphology, 16S and COI gene sequences, analyzed both separately and in various combinations. Then Alexeyev presented his detailed morphological studies, focusing on, but not limited to, characters of the gladius. Roeleveld described a newly recognized photophore-bearing loliginid species and Lu described a new family of myopsid squids that he currently believes may be most closely related to Loliginidae (both published in the present volume).

Workshop participants also visited the commercial landing dock in Phuket and collected fresh specimens of several species and tissues for future molecular analyses.

Cryptic Species.

As Okutani pointed out, in diagnosing new species, it is important to focus on the characters that are essential to distinguish the new species from morphologically similar species previously described, rather than trying to describe and illustrate the states of all possible characters. If possible, mature specimens of both sexes should be included. Among the most important diagnostic indications are the suites of characters associated with the hectocotylus, tentacular clubs, and gladius. Egg size is also important, as are arm sucker dentition, presence of photophores, suckers on the buccal membranes, fin shape and size, and spermatophore structure. Location of spermathecae may be important. Other characters that may be diagnostic include morphology of the beaks and statoliths and, possibly, structure of the photophores. Behavior, when it can be observed comprehensively, can offer important indications of species differences, as can color of fresh specimens.

A lack of understanding of the amount of variability in a single loliginid species is a major impediment to describing cryptic species and distinguishing species complexes from broadly variable individual species. To alleviate this problem, species that are presently considered to be taxonomically well-established and stable (Table 1) should be examined from throughout the geographic range of the species, including all life-history stages. Detailed examination of these species should be designed to address the following topics: (1) How can we rigorously quantify morphological characters? Which characters are appropriately used for either meristic or morphometric analyses? Can color standards be used rigorously? (2) How much character variability is expected within a species? A baseline of expected variability in species whose identification is relatively certain would allow proposal of testable hypotheses about the delimitation of problematic species. Once the amount of variability is known, adequate sample size can be determined to test for significant differences among subgroups of problematic species.

After the morphological variability in taxonomically stable species has been assessed, this information can be used to help delineate species within species complexes (*e.g.*, *Sepioteuthis lessoniana*, *Uroteuthis duvaucelii*). Patterns of variability in the data within a species complex can be described using methods such as cluster analysis and ordination. Then differences in mean measurements can be tested for statistical significance using analysis of variance, followed by *post-hoc* pairwise comparisons.

Table 1. Species recommended for within-species analysis of morphological variability.

Doryteuthis opalescens
Lolliguncula brevis
Sepioteuthis sepioidea
Afrololigo mercatoris
Loligo vulgaris
Lolliguncula diomedea
Loliolus japonica
Uroteuthis noctiluca
Heterololigo bleekeri

Systematics of Indo-West Pacific Loliginids

The workshop participants concluded that the best way to accomplish these goals is to establish an international network of collaboration. This would allow collection of sufficient specimens of both taxonomically stable species to determine variability and putative species complexes to test for significant differences. This network could also contribute to a tissue bank for DNA analyses to test further the hypotheses supported by morphological information. DNA methods could further be used to address questions of possible hybridization among closely related loliginid species.

Phylogenetic taxonomy of Loliginidae.

Table 2 presents the group's consensus on generic and sub-generic taxonomy of the family, based primarily on the phylogenetic research of Anderson (1996; 2000a; 2000b) and Alexeyev (1989; 1991). Both of those authors built upon the previous workshop proceedings (Vecchione *et al.*, 1998). The most noteworthy differences from the classification of Vecchione *et al.* (1998) include

the following: (1) removal of *mercatoris* from *Lolliguncula* based primarily on gladius characters, DNA sequence data and biogeography, and recognition of *Afrololigo* Brakoniecki as a valid genus for this species. (2) elevation of *Alloteuthis* Wulker from sub-generic to full generic status based on gladius structure and DNA sequence data. (3) removal of American species from the genus *Loligo* because DNA sequence analyses indicate that *Loligo* sensu Vecchione *et al.* is probably paraphyletic. The generic name with priority for the American species is *Doryteuthis* Naef. This genus further comprises two natural subgroups based on differences in gladius structure; these subgroups are considered here to be the subgenera *Doryteuthis* Naef and *Amerigo* Brakoniecki. *Doryteuthis sanpaulensis* does not belong in either of these subgenera and is therefore considered to be the sole recognized species in an undescribed subgenus. (4) removal of *bleekeri* from *Loligo* and recognition of *Heterololigo* Natsukari as a valid genus based on DNA sequence analysis. (5)

Table 2. Revised generic and sub-generic classification of the Loliginidae.

Genus	Subgenus	species included
<i>Loligo</i>		<i>vulgaris, reynaudii, forbesii</i>
<i>Afrololigo</i>		<i>mercatoris</i>
<i>Alloteuthis</i>		<i>media, africanus, subulata</i>
<i>Doryteuthis</i>	<i>Doryteuthis</i>	<i>plei, roperi</i>
	<i>Amerigo</i>	<i>pealeii, surinamensis, gahi,</i> <i>opalescens, ocula</i>
	subgenus undescribed	<i>sanpaulensis</i>
<i>Heterololigo</i>		<i>bleekeri</i>
<i>Loliolus</i>	<i>Loliolus</i>	<i>hardwickei, affinis</i>
	<i>Nipponloligo</i>	<i>japonica, beka, sumatrensis,</i> <i>uyii</i>
<i>Lolliguncula</i>	<i>Lolliguncula</i>	<i>brevis, panamensis, argus</i>
	<i>Loliolopsis</i>	<i>diomedae</i>
<i>Pickfordiateuthis</i>		<i>pulchella, vossi, bayeri</i>
<i>Sepioteuthis</i>		<i>sepioidea, australis,</i> <i>lessoniana</i>
<i>Uroteuthis</i>	<i>Uroteuthis</i>	<i>bartschi</i>
<i>Photloligo</i>	<i>Photloligo</i>	<i>edulis, abulati, arabica, bengalensis,</i> <i>chinensis, duvaucelii, robsoni, singhalensis, vossi</i>
	<i>Aestuariolus</i>	<i>noctiluca</i>
	subgenus undetermined	<i>pickfordae, reesi</i>

removal of *noctiluca* from subgenus *Uroteuthis* of genus *Uroteuthis* and recognition of *Aestuariolus* Alexeyev as a valid monotypic subgenus of *Uroteuthis* primarily because of differences in photophore structure from the rest of the genus. The workshop participants did not reach consensus on subgeneric assignments *Uroteuthis reesi* and *U. pickfordae*.

REFERENCES

- Anderson, F.E. 1996. Preliminary Cladistic Analyses of Relationships Among Loliginid Squids (Cephalopoda: Myopsida) Based on Morphological Data. *American Malacological Bulletin*. **12**: 113–128.
- Anderson, F.E. 2000a. Phylogeny and Historical Biogeography of the Loliginid Squids (Mollusca: Cephalopoda) Based on Mitochondrial DNA Sequence Data. *Molecular Phylogenetics and Evolution*. **15**: 191–214.
- Anderson, F.E. 2000b. Phylogenetic relationships among loliginid squids (Cephalopoda: Myopsida) based on analyses of multiple data sets. *Zoological Journal of the Linnean Society*. **130**: 603–633.
- Alexeyev, D.O. 1989. Advantages and limitations of using the gladius in diagnosis of species and genera of the family Loliginidae (Cephalopoda). *Zoologichesky Zhurnal*. **68**(6): 36–42.
- Alexeyev, D.O. 1991. Systematics, phylogeny, propagation, biology and business perspectives of the squid Myopsida. Dissertation, Academy of Science USSR, P.P. Shirshov Institute of Oceanology.
- Cohen, A.C. 1976. The systematics and distribution of *Loligo* (Cephalopoda, Myopsida) in the western North Atlantic, with descriptions of two new species. *Malacologia*. **15**(2): 299–367.
- Izuka, T., S. Segawa, T. Okutani and K. Numachi. 1994. Evidence on the existence of three species in the oval squid *Sepioteuthis lessoniana* complex in Ishigaki Island, Okinawa, southwestern Japan, by isozyme analyses. *Venus* **53**(3): 217–228.
- Izuka, T., S. Segawa and T. Okutani. 1996. Identification of three species in oval squid, *Sepioteuthis lessoniana* complex by chromatophore arrangements on the funnel. *Venus*, **55**(2): 139–142.
- Vecchione, M., T.F. Brakoniecki, Y. Natsukari, and R.T. Hanlon. 1998. A provisional generic classification of the Loliginidae. **In**: N.A. Voss, *et al.* (eds.). *Systematics and Biogeography of Cephalopods*. Smithsonian. Contr. Zool. **586**: 215–222.
- Yeatman, J. and J.A.H. Benzie. 1993. Cryptic speciation in *Loligo* from northern Australia. **In**: T. Okutani, R.K. O'Dor and T. Kubodera (eds.). *Recent Advances in Cephalopod Fisheries Biology*. Tokai Univ. Press, Tokyo. pp. 641–652.