

SUPPORTING INFORMATION

A Proposed Timescale for the Evolution of Armophorean Ciliates: Clevelandellids Diversify More Rapidly Than Metopids by Peter Vďačný, Lubomír Rajter, Thorsten Stoeck & Wilhelm Foissner

Table S1. Number of species described in the class Armophorea and proportion of the taxa sampled.

Table S2. Characters, character states and their coding used for ancestral state reconstruction.

Table S3. Parametrization and fitting of constant-rate diversification models to the phylogeny of metopids and clevelandellids.

Table S4. Pagel's test of correlated character evolution performed on the BEAST maximum credibility tree.

Table S1. Number of species described in the class Armophorea and proportion of the taxa sampled.

Family	Total No. of taxa	No. of taxa sampled	Proportion of taxa sampled ^a	Reference ^b
Caenomorphidae	13	2	15%	Kahl (1932)
Metopidae	81	23	28%	Kahl (1932), Biggar and Wenrich (1932), Villeneuve-Brachon (1940), Jankowski (1964a, b), Dragesco (1968), Esteban et al. (1995), Foissner (2016a, b), Omar et al. (2017), Vďačný and Foissner (2017a, b)
Nyctotheridae	186	10	5%	Earl (1972), Albaret (1972, 1973), Lalpotu (1980a, b, c), Shete (1982a, b), Affa'a (1986, 1991), Galavíz-Silva and Jiménez-Guzmán (1986), Kalavati et al. (1991), Grim (1992), Li et al. (1998), Senler and Yildiz (2000), Grim et al. (2002), Li et al. (2002), Xiao et al. (2002), Fokam et al. (2013, 2014)
Clevelandellidae	11	4	35%	Kidder (1937), Uttangi and Desai (1963), Mandal and Nair (1974)

^a Taking into account the 20% synonymy rate in ciliates (Foissner et al. 2008) and the proportion of undescribed and cryptic species in armophoreans, we consider the proportion of sampled taxa as a reasonable estimate for diversification analyses.

^b Kahl (1932) summarized all described free-living metopids known at that time, while Earl (1972) summarized all described endosymbiotic nyctotherids and clevelandellids known at that time.

REFERENCES

- Albaret, J. L. (1972). Description de cinq espèces nouvelles du genre *Nyctotheroides* Grassé, ciliés hétérotriches parasites de batraciens anoures malgaches. *Bulletin du Muséum National d'Histoire Naturelle*, 44, 522–531.
- Albaret, J. L. (1973). Observations sur des nyctothères des genres *Sicuophora* de Puytorac & Grain, *Metasicuophora* gen. n. et *Parasicuophora* Albaret, ciliés parasites de batraciens anoures. *Journal of Protozoology*, 20, 51–57.
- Affa'a, F.-M. (1986). Description de nouveaux Sicuophoridae (ciliés heterotriches) endocommensaux d'anoures Camerounais. *Archiv für Protistenkunde*, 132, 213–230.
- Affa'a, F.-M. (1991). Observations morphologiques sur deux nyctothères (ciliés hétérotriches) commensaux de batraciens anoures du Québec. *Canadian Journal of Zoology*, 69, 2765–2770.
- Biggar, R. B., & Wenrich, D. H. (1932). Studies on ciliates from Bermuda sea urchins. *Journal of Parasitology*, 18, 252–257.

- Dragesco, J. (1968). *Metopus jankowskii* n. sp., *Sonderia sinuate* Kahl, et *Discocephalus minimus* n. sp., ciliés nouveaux ou mal connus. *Annales de la Faculté des Sciences du Cameroun*, 1, 77–88.
- Earl, P. R. (1972). Synopsis of the *Plagiotomoidea*, new superfamily (Protozoa). *Acta Protozoologica*, 9, 247–261.
- Esteban, G., Fenchel, T., & Finlay, B. (1995). Diversity of free-living morphospecies in the ciliate genus *Metopus*. *Archiv für Protistenkunde*, 146, 137–164.
- Foissner, W. (2016a). Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. *Denisia*, 35, 1–912.
- Foissner, W. (2016b). *Heterometopus meisterfeldi* nov. gen., nov. spec. (Protozoa, Ciliophora), a new metopid from Australia. *European Journal of Protistology*, 55, 118–127.
- Foissner, W., Chao, A., & Katz, L. A. (2008). Diversity and geographic distribution of ciliates (Protista: Ciliophora). *Biodiversity and Conservation*, 17, 345–363.
- Fokam, Z., Nana, P. A., Ngassam, P., Bricheux, G., Bouchard, P., Vignes, B., & Sime-Ngando, T. (2013). Description of two new species of Sicuophoridae and Nyctotheridae (Heterotrichina), endocommusal in the rectal ampulla of *Bufo regularis* (Amphibia: Anura) from the northwest of Cameroon. *Protistology*, 8, 16–21.
- Fokam, Z., Nana, P. A., Ngassam, P., Bricheux, G., Bouchard, P., Vignes, B., & Sime-Ngando, T. (2014). Morphological description of four new species of *Nyctotherus* (Ciliophora: Nyctotheridae: Heterotrichida), commusal ciliates of the digestive tract of a terrestrial oligochaete (Megasclecoidea) from the northwest region of Cameroon. *Protistology*, 8, 62–70.
- Galavíz-Silva, L., & Jiménez-Guzmán, F. (1986). *Zelleriella bayonai* n. sp. and *Nyctotherus uscae* n. sp. (Protozoa) from *Leptodeira maculata* (Colubridae) of Guatemala, C.A. *Revista de Biología Tropical*, 34, 237–242.
- Grim, J. N. (1992). Descriptions of two sympatric and phylogenetically diverse ciliated protozoa, *Balantidium zebrascopi* n. sp. and *Paracichlidotherus leeuwenhoekii* n. gen., n. sp., symbionts in the intestines of the surgeonfish, *Zebrasoma scopas*. *Transactions of the American Microscopical Society*, 111, 149–157.
- Grim, J. N., Clements, K. D., & Byfield, T. (2002). New species of *Balantidium* and *Paracichlidotherus* (Ciliophora) inhabiting the intestines of four surgeonfish species from the Tuvalu Islands, Pacific Ocean. *Journal of Eukaryotic Microbiology*, 49, 146–153.
- Jankowski, A. W. (1964a). Morfologiâ i èvolúciâ Ciliophora. I. Novaâ sistema sapropelebiotičeskij Heterotrichida (Morphology and evolution of Ciliophora. I. The new system of sapropelebiotic Heterotrichida). *Zoologičeskij Žurnal*, 43, 503–517. (in Russian).
- Jankowski, A. W. (1964b). Morphology and evolution of Ciliophora. III. Diagnoses and phylogenesis of 53 sapropelebiotics, mainly of the order Heterotrichida. *Archiv für Protistenkunde*, 107, 185–294.
- Kahl, A. (1932). Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Die Tierwelt Deutschlands*, 25, 399–650.
- Kalavati, C., Narasimhamurti, C. C., & Usharani, Y. (1991). Studies on the endocommusal ciliates of anurans of Andhra Pradesh. *Records of the Zoological Survey of India*, 141, 1–65.
- Kidder, G. W. (1937). The intestinal protozoa of the wood-feeding roach *Panesthia*. *Parasitologica*, 29, 163–205.

- Lalpotu, P. A. (1980a). Studies on ciliates of the genus *Nyctotherus* Leidy, 1849 I. Parasites of orthoptera. *Archiv für Protistenkunde*, 123, 31–43.
- Lalpotu, P. A. (1980b). Studies on ciliates of the genus *Nyctotherus* Leidy, 1849 II. Parasites of beetle. *Archiv für Protistenkunde*, 123, 162–165.
- Lalpotu, P. A. (1980c). Studies on ciliates of the genus *Nyctotherus* Leidy, 1849 III. Parasites of millipedes. *Archiv für Protistenkunde*, 123, 261–266.
- Li, L.-X., Wang, J.-G., & Xiao, W.-H. (1998). Taxonomical studies on parasitic nyctotherans from Chinese anura amphibians. I. *Nyctotheroides*. *Acta Hydrobiologica Sinica*, 22 (Suppl.), 187–196.
- Li, L.-X., Wang, J.-G., & Xiao, W.-H. (2002). Taxonomic studies of parasitic nyctotherans from Chinese anura amphibians IV. *Spirocytopharynx* gen. nov. and *Macrocytopharynx* gen. nov. *Zoological Studies*, 41, 77–84.
- Mandal, A. K., & Nair, K. N. (1974). *Clevelandella kidderi* sp. n. (Clevelandellidae) new heterotrichous ciliate from wood-feeding roach (*Panesthia* sp.) of Andaman Islands, India. *Acta Protozoologica*, 12, 351–354.
- Omar, A., Zhang, Q., Zou, S., & Gong, J. (2017). Morphology and phylogeny of the soil ciliate *Metopus yantaiensis* n. sp. (Ciliophora, Metopida), with identification of the intracellular bacteria. *Journal of Eukaryotic Microbiology*, doi:10.1111/jeu.12411
- Şenler, N. G., & Yildiz, I. (2000). The ciliate fauna in the digestive system of *Rana ridibunda* (Amphibia: Anura)—II *Nyctotherus* (Nyctotheridae: Heterotrichida). *Turkish Journal of Zoology*, 24, 245–252.
- Shete, S. G. (1982a). Observations of the rectal ciliate of the genus *Nyctotheroides* Grassé, 1928 from Indian amphibians. 1. Subgenus: *Aduncuperistomatus*. *Archiv für Protistenkunde*, 125, 163–172.
- Shete, S. G. (1982b). Observations of the rectal ciliate of the genus *Nyctotheroides* Grassé, 1928 from Indian amphibians. 2. Subgenus: *Nyctotheroides*. *Archiv für Protistenkunde*, 125, 173–180.
- Uttangi, J. C., & Desai, R. N. (1963). *Metaclevelandella termites*, a new genus and species of heterotrichous ciliate (family Clevelandellidae) found in the Indian termite *Capritermes incola* Wasm. *Parasitologica*, 53, 39–43.
- Vďačný, P., & Foissner, W. (2017a). A huge diversity of metopids (Ciliophora, Armophorea) in soil from the Murray River floodplain, Australia. I. Description of five new species and redescription of *Metopus setosus* Kahl, 1927. *European Journal of Protistology*, 57, 35–76.
- Vďačný, P., & Foissner, W. (2017b). A huge diversity of metopids (Ciliophora, Armophorea) in soil from the Murray River floodplain, Australia. II. Morphology and morphogenesis of *Lepidometopus platycephalus* nov. gen., nov. spec. *Acta Protozoologica*, 56, 39–57.
- Villeneuve-Brachon, S. (1940). Recherches sur les ciliés hétérotiches cinétome, argyrome, myonèmes, formes nouvelles ou peu connues. *Archives de Zoologie Expérimentale et Générale*, 82, 1–180.
- Xiao, W.-H., Wang, J.-G., & Li, L.-X. (2002). Taxonomic studies of parasitic nyctotherans from Chinese anura amphibians III. *Wichtermania*. *Zoological Studies*, 41, 69–76.

Table S2. Characters, character states and their coding used for ancestral state reconstruction.

Taxon	LS ^a	PM ^b	PS ^c	Taxon	LS ^a	PM ^b	PS ^c
<i>Atopospira galeata</i>	0	1	0	<i>Nyctotheroides hubeiensis</i>	1	1	1
<i>Atopospira violacea</i>	0	1	0	<i>Nyctotheroides parvus</i>	1	1	1
<i>Brachonella contorta</i>	0	0	0	<i>Nyctotheroides pyriformis</i>	1	1	1
<i>Caenomorpha medusula</i>	0	1	0	<i>Nyctotheroides</i> sp. AF147882	1	1	1
<i>Caenomorpha uniserialis</i>	0	1	0	<i>Nyctotherus cordiformis</i>	1	1	1
<i>Clevelandella constricta</i>	1	1	1	<i>Nyctotherus ovalis</i>	1	1	1
<i>Clevelandella nipponensis</i>	1	1	1	<i>Nyctotherus</i> sp1. KC139720	1	1	1
<i>Clevelandella panesthiae</i>	1	1	1	<i>Nyctotherus</i> sp2. KC139721	1	1	1
<i>Clevelandella parapanesthiae</i>	1	1	1	<i>Nyctotherus velox</i>	1	1	1
<i>Metopus es</i>	0	0	0	<i>Palmarella lata</i>	0	0	0
<i>Metopus fuscus</i>	0	0	0	<i>Parametopidium circumlabens</i>	1	1	0
<i>Metopus hasei</i>	0	0	0	<i>Urostomides bacillatus</i>	0	0	0
<i>Metopus laminarius</i>	0	0	0	<i>Urostomides caducus</i>	0	0	0
<i>Metopus minor</i>	0	0	0	<i>Urostomides campanula</i>	0	0	0
<i>Metopus palaeformis</i>	0	0	0	<i>Urostomides darwini</i>	0	0	0
<i>Metopus setosus</i>	0	0	0	<i>Urostomides denarius</i>	0	0	0
<i>Metopus</i> sp.	0	0	0	<i>Urostomides pullus</i>	0	0	0
<i>Metopus yantaiensis</i>	0	0	0	<i>Urostomides striatus</i>	0	0	0
<i>Nyctotheroides deslierresae</i>	1	1	1				

^a Lifestyle (LS): free-living coded 0, endosymbiotic coded 1.

^b Paroral membrane (PM): single-rowed coded 0, double-rowed coded 1.

^c Perizonal stripe (PS): present coded 0, absent coded 1.

Table S3. Parametrization and fitting of constant-rate diversification models to the phylogeny of metopids and clevelandellids.

Model	Group	logLik ^a	BF ^b	λ	μ	R	ε
Pure-birth	Armophorea	-285.15		0.0168 [0.0058, 0.0283]	–	0.0168 [0.0058, 0.0283]	–
Birth-death	Armophorea	-273.82	22.66	0.0589 [0.0165, 0.1101]	0.0499 [0.0070, 0.1020]	0.0090 [0.0012, 0.0181]	0.8204 [0.5686, 0.9871]
Group-specific rates under the birth-death model							
	Metopida			0.0207 [0.0050, 0.0421]	0.0127 [6.2e-7, 0.0337]	0.0080 [0.0014, 0.0158]	0.5492 [0.0772, 0.9295]
	Clevelandellida			0.1351 [0.0218, 0.3412]	0.1069 [1.5e-5, 0.3145]	0.0282 [0.0055, 0.0518]	0.6738 [0.1576, 0.9909]

Tabulated are mean rates of speciation (λ) and extinction (μ) as well as net diversification rate (r) and extinction fraction (ε) estimated over 100 trees from the posterior distribution of the BEAST analysis. Square brackets represent the 95% credibility interval.

^alogLik: log likelihoods are marginal likelihoods estimated across the sample of 100 trees in BayesRate using thermodynamic integration.

^bBF: BayesFactor is expressed relative to the model of highest marginal likelihood. $BF = 2(\log Lik_1 - \log Lik_2)$. The higher BayesFactor, the stronger the support for the best model. $BF > 10$ is interpreted as very strong support.

Table S4. Pagel's test of correlated character evolution performed on the BEAST maximum credibility tree.

Character evolution	$\ln\text{Lik}_4$	$\ln\text{Lik}_8$	$\Delta\ln\text{Lik}$	χ^2	p
Lifestyle vs. Paroral membrane	-23.9665	-17.2328	6.73	13.46	< 0.001
Lifestyle vs. Perizonal stripe	-15.4970	-8.9853	6.51	13.02	< 0.001
Perizonal stripe vs. Paroral membrane	-19.8839	-15.6179	4.26	8.52	0.01

$\ln\text{Lik}_4$: log likelihood of four parameter model without correlation, $\ln\text{Lik}_8$: log likelihood of eight parameter model with correlation, $\Delta\ln\text{Lik}$: difference in log likelihoods of four and eight parameter models, χ^2 : chi-square, p : probability.